ETHNOMEDICINAL STUDIES OF FLORA OF SOUTHERN PUNJAB AND ISOLATION OF BIOLOGICALLY ACTIVE PRINCIPLES

DEPARTMENT OF CHEMISTRY
LAHORE COLLEGE FOR WOMEN UNIVERSITY,
LAHORE, PAKISTAN

ETHNOMEDICINAL STUDIES OF FLORA OF SOUTHERN PUNJAB AND ISOLATION OF BIOLOGICALLY ACTIVE PRINCIPLES

A THESIS SUBMITTED TO
LAHORE COLLEGE FOR WOMEN UNIVERSITY, LAHORE
IN THE PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN CHEMISTRY (PHYTO)

By

TAHIRA AZIZ MUGHAL

DEPARTMENT OF CHEMISTRY
LAHORE COLLEGE FOR WOMEN UNIVERSITY,
LAHORE, PAKISTAN
2008

DEPARTMENT OF CHEMISTRY
LAHORE COLLEGE FOR WOMEN UNIVERSITY, LAHORE, PAKISTAN

It is certified that the thesis entitled "Ethnomedicinal Studies of Floraof Southern Punjab and Isolation of Biologically Active Principles" submitted by Ms. Tahira Aziz Mughal to the Department of Chemistry, Lahore College for Women University, Lahore, PAKISTAN is her own work and is not submitted previously, in whole on in parts, in respect of any other academic award.

Supervisor .....
Prof. Dr. Ismat Naeem

Chairperson of
the Department .....
Prof. Dr. Raskhshanda Nawaz

ACKNOWLEDGEMENT

First of all I bow my head before Almighty Allah with humble thanks that have enabled me to complete my research work. My all loves for Hazrat Mohammad (PBUH), a torch of guidance for all mankind for ever.

My special gratitude goes to our ever active Vice Chancellor Prof Dr Bushra Mateen for providing me excellent research facilitations and patronage during the whole course of this research.

I deem it a real privilege and source of pleasure to express my profound and cordial gratitude to my supervisor Prof Dr Ismat Naeem for her keen and continuous interest, ideas, suggestions, guidance, encouragement and support throughout the period of this research work. I also want to offer my gratitude to her for generously helping and allowing the facilities and supplies from her HEC National Grant project which provided me resources for establishment of Prem Madan Herbarium by visiting various locations in Pakistan.

I am grateful to Prof .Miss Shaista Vine, Prof Dr Rukhshanda Nawaz, Dr Surrya Ahmed, Mrs Azra Niamat, Mrs. Zafar Hussain and other colleagues for their moral assistance during the course of this study.

I am thank full to Dr Khadija Ismail for helping me in antibacterial studies .I am thank ful to Dr Rukhsana Bajwa for helping me in antifungal studies. Special thanks are due to Dr Samina Shahid for helping me in the diagnosis of cancerous studies.

I am most grateful to all of my family members, especially my husband Muhammad Saqib Pasha, my kids Saud, Ayesha, Saad, and Saadan and my sisters and brothers for their encouragement, constant care, moral support and love. Special thanks are due to Mr. Abdul Shakoor Dogar for his special cooperation and moral support. Special thanks are due for Rani for taking care of household affairs.

I am thankful to all the technical and non-technical staff and all of my colleagues at Lahore College for Women University, Lahore for their help, especially Mr. Talib Mahmood who helped me in GC MS analysis.

Special thanks are devoted to Mrs Alya Anwaar, Miss Zeb Saddiqe, Miss Uzma Nauman, Miss Marium Jalal, Abida Teskeen, Shahnaz Bakhtawar and Hifsa Mubeen for their cooperation and support, throughout this study.

I dedicate this effort to

My respected (late) parents-in-law

Muhammad Shafi Butt &
Ghulam Fatima

and

My respected parents

Abdul Aziz Mughal & Hameeda Begum

For their prayers and well wishes

ABSTRACT

This thesis is divided into three sections, Section A, Section B and Section C. Section A deals with ethnomedicinal studies of flora of Southern Punjab, Pakistan and establishment of a Herbarium in Lahore College for Women University. Section B consists of biological activity (antimicrobial and anticancer) of extracts of some of the plants collected from South Punjab and Section C describes the isolation and characterization of bioactive compounds by chromatographic and spectroscopic techniques.

It is for the first time that the flora of Southern Punjab has been searched and reported. Complete ethno medicinal studies of 187 plants belonging to 52 families found in South Punjab is documented and also categorized therapeutically for the first time from this region.

A first world class herbarium has been established in Lahore College for Women University by categorizing and preserving 186 voucher specimens of plants collected from all over Pakistan. The herbarium is named after the renowned teacher and pioneer Botanist Miss Prem Madan in dedication to her services in the field of Botany in Lahore College for Women University, Lahore.

For biological activity seven popular ethnomedicinal plants collected from Southern Punjab namely *Capparis decidua* (Capparidaceae), *Coronopus* didymus (Brassicaeae), *Heliotropium strigosum* (Boraginaceae), *Salsola kali* (Chenopodiaceae), *Salvadora oleoides* (Salvadoraceae), *Tamarix aphylla* (Tamaricaceae) and *Withania coagulans* (Solanaceae) were selected for antimicrobial and anticancer activity.

Topical anti tumor activity of these seven ethnomedicinal plants have been investigated in detail for the first time and all extracts of the *Coronopus didymus, Salsola kali, Salvadora oleoides* and *Tamarix aphylla* showed anti tumor activity and provided a new source of further exploration in this respect. While methanol extracts of *Withania coagulans, Capparis decidua* and *Heliotropium stirgosum* had been shown to possess best potential against the topical tumor for the first time. The Pet ether, methanolic and

dichloromethane extracts of *Withania coagulans, Capparis decidua* and *Heliotropium strigosum* reduced the malignancy and cured the fibrous hyperplasia.

The screening of these selected medicinal plants for antibacterial activity against six bacterial strains namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Bacillus subtilus* and *Sarcina lutae* after fractionation in different solvents (methanol, pet ether, dichloromethane) by agar well diffusion method showed the methanol extracts to be more potent then pet ether and dichloromethane extracts. The antibiotic properties of these seven strains were studied against *Sarcina lutae, Streptococcus pneumoniae and Pseudomonas aeruginosa* bacterial strains for the first time.

Methanol extracts of *Capparis decidua* and *Withania coagulans* were found to be best bactericidal against all the tested bacterial strains. While methanolic extract of *Heliotropium strigosum* was very active against all the bacterial strains except *Streptococcus pneumoniae.*

Synergistically *Withania coagulans* in combination with *Pinus wallichiana, Capparis decidua, Hypericum perforatum, Heliotropium strigosum, Coronopus didymus* and *Salvadora oleoides* showed best activity against *Staphylococcus aureus* (MIC > 0.1µg/ml) only.

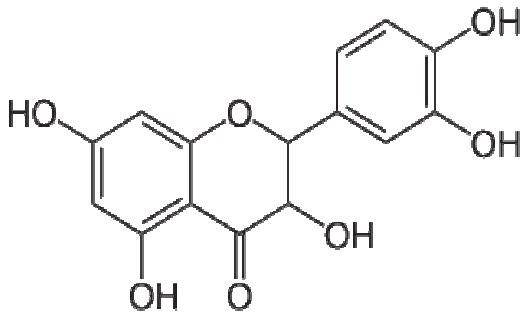
The methanolic extract of *Salsola kali* in equal amounts with *Senecio chrysthenoides* synergistically inhibited the growth of all bacterial strains except *Sarcina lutae.*

The screening of pet ether, methanolic and dichloromethane extracts of *Coronopus didymus, Withania coagulans, Capparis decidua, Salsola kali, Heliotropium strigosum, Salvadora oleoides,* and *Tamarix aphylla* was performed against seven fungal strains namely *Trichoderma viridis*, *Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Candida albicans, Trichophyton mentogrophytes* and *Microsporum canis* . By using ANOVA critical value $F.05_{(6, 36)} = 2.38$ the plant extracts were compared with other antifungal drugs and it was found that extracts of *Capparis decidua, Withania*

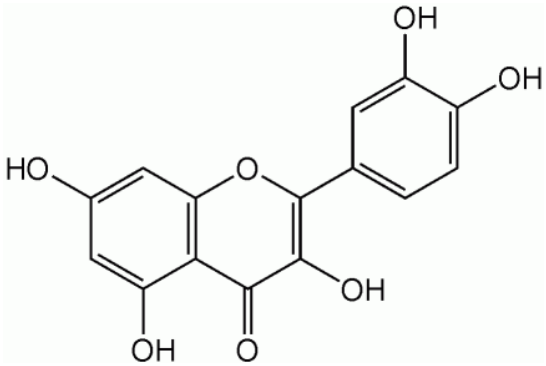
coagulans and Heliotropium strigosum showed best antifungal activity against all the fungal strains.(F-Table value< F-calculated value).

On the basis of ethnomedicinal studies and for showing best biological activity three medicinal plants namely *Heliotropium strigosum*, *Withania coagulans* and *Calotropis procera* were selected for isolation and identification of bioactive principles and some other useful applications so as to explore the potential of these plants on commercial basis. Due to its reported biological activityl, abundance and wild nature and use as heavy metal ion remover *Calotropis procera* was also selected for the first time for identification of its organic phytochemicals having functional groups responsible of removing Cr (III) by spectroscopic techniques so as to put it to some commercial use.

Two Flavonoid aglycones Taxifolin **(1)** and quercetin **(2)** had been isolated from *Heliotropium stirgosum* for the first time and identified by comparison of their spectral data with that given in the literature.



Taxifolin **(1)**



Quercetin **(2)**

GC MS studies had resulted in identification of the following compounds in *Withania coagulans* essential oil for the first time,

1. Cyclohexane **(3)** (C_6H_6)
2. Borane carbonyl **(4)** (CH_3BO)
3. 3-methyl, hexane **(5)** **(**C_7H_8)
4. Heptane **(6)** (C_7H_{16})
5. Hexanoic acid **(7)** ($C_6H_{12}O_2$)

6. Nonanoic acid **(8)** ($C_9H_{18}O_2$)

While *n*-hexane fraction of *Calotropis procera* was subjected to GC MS analysis following open-chain carboxylic acids and their methyl esters were identified,

1. *n*-Heptanoic acid methyl ester **(9)** ($C_8H_{16}O_2$)
2. *n*-Decenoic acid **(10)**: ($C_{10}H_{18}O_2$)
3. *n*-Nonanoic acid methyl ester **(11)** ($C_{10}H_{20}O_2$)
4. *n*-Decenoic acid methyl ester **(12) (**$C_{11}H_{20}O_2$)

The FT-IR spectra before and after adsorption of *Calotropis procera* roots indicated that bonded –OH groups and /or –NH and carboxyl groups especially played a major role in chromium (III) biosorption which was confirmed by GC MS analysis of *Calotropis procera* showing the presence of carboxylic acids for the first time.

TABLE OF CONTENTS

Page #

Certificate ..... (ii)

Acknowledgement ..... (iii)

Abstract ..... (vi)

Section A

ETHNOMEDICINAL STUDIES OF SOUTHERN PUNJAB AND ESTABLISHMENT OF PREM MADAN HERBARIUM

Chapter 1 Ethnomedicinal Studies of Southern Punjab and Establishment of Prem Madan Herbarium 1

1.1 Ethnomedicinal Studies of Southern Punjab 2

1.1.1 General Introduction and Historical Review 3

1.1.2 Ethnomedicinal studies in Asia 5

1.1.3 Ethnomedicinal studies in Pakistan 8

1.1.4 Geography of Southern Punjab 11

1.1.4.1 Physical features 11

1.1.4.2 Climate 12

1.1.5 Biodiversity 12

1.2 Establishment of Herbarium in LCWU 14

1.2.1 Historical background 14

Chapter 2 Experimental 15

2.1 Ethnomedicinal Study 16

2.1.1 Material and Methods 16

2.2 Establishment of Herbarium 24

2.2.1 Material and Methods 24

2.2.2 Identification of Plants 24

Chapter 3 Results and Discussion 31

3.1 Description of Ethnomedicinal plants 32

3.1.1 Collection of data from local people and Hakims 86

3.2 Establishment of Prem Madan Herbarium 87

Section B

BIOLOGICAL ACTIVITY

Chapter 4 Biological Activity 89

4.1 Introduction and Historical review 90
4.1.1 Anticancer Activity 90
4.1.2 Antimicrobial Activity 90
4.1.3 Synergistic Activity 92

4.2 Selection of Plants for Biological Activity 93

4.3 General Extraction Procedure 93

Chapter 5 Experimental 94

5.1 Anticancer Activity 95
5.1.1 Extraction procedure 95
5.1.2 Preparation of Stock Solution for Biological activity 95
5.1.3 Food routine and Hygiene 95
5.1.4 Preparation of animals for carcinogens 96
5.1.5 Method (Route) of dose administration 96
5.1.6 Solvents used as dilutors 97
5.1.7 Record of animals 97
5.1.8 Particulars of lesions recorded 98
5.1.9 Anesthesia 98
5.1.10 Specimen collection and preservations 98
5.1.11 Staining procedure (Haematoxyline and Eosin stain) 99
5.1.12 Experimental Plan 100

5.2 Antibacterial Activity 102
5.2.1 Sterilization and preparation of Culture Media 102
5.2.2 Minimum Inhibitory Concentration (MIC) 103

5.3 Synergistic bacterial activity 103

5.4 Antifungal Activity 104
5.4.1 Fungal Strains 104
5.4.2 Fungal Bioassay 104
5.4.3 Statistical data 105

Chapter 6 Tables 106

6.1 Anticancer 107

6.2 Antibacterial Activity 141

6.3 Antifungal Activity 151

Chapter 7 Discussion 155

7.1 Anticancer activity 156

7.2 Antibacterial Activity 160

7.3 Synergistic bacterial activity 162

7.4 Antifungal Activity 164

Section C

ISOLATION AND CHARACTERIZATION OF BIOACTIVE PRINCIPLES

Chapter 8 Isolation of Biologically Active Compounds 168

8.1 Introduction and Historical Review 168

8.2 Secondary Metabolites 169

8.3 Bio Flavonoids 172

8.4 Selection of Plants 173

8.4.1 *Heliotropium strigosum* (Boraginaceae) 173

8.4.2 *Withania Coagulans* (Solonaceae) 176

8.4.3 *Calotropis procera* (Asclepiadaceae) 176

Chapter 9 Experimental 178

9.1 Material and Methods 179

9.1.1 Extraction and Separation 179

9.1.2 Extraction of Essential Oils 180

9.1.3 Acid Hydrolysis 180

9.1.4 HPLC analysis 180

9.1.5 GC-MS Analysis 181

9.1.6 Phytochemical absorption of Cr (III) on *Calotropis procera* 181

9.1.7 Fourier Transform Infrared Analysis 181

9.2 Isolation of Compounds of *Heliotropium strigosum* 182

9.2.1 Taxifolin (1) 182

9.2.2 Quercetin (2) 182

9.3 Identification of Compounds of *Withania coagulans* (Essential oil) by GC MS 183

9.4 Identification of Compounds of *Calotropis procera* by GC MS 184

9.4.1 GC-MS Spectral Data 184
9.4.1.1 *n*-Heptanoic acid methyl ester (**9**) 184
9.4.1.2 *n*-Decenoic acid (**10**) 184
9.4.1.3 *n*-Nonanoic acid methyl ester (**11**) 184
9.4.1.4 *n*-Decenoic acid methyl ester (**12**) 185

Chapter 10 Discussion 187

10.1 Compounds Isolated from *Heliotropium stirgosum* 188
10.1.1 Taxifolin (Dihydro quercein) 188
10.1.2 Quercetin (**2**) 189

10.2 Compounds identified from *Withania coagulans* 190
10.2.1 Cyclohexane (**3**) 190
10.2.2 Borane carbonyl (**4**) 190
10.2.3 Hexane 3-methyl (**5**) 191
10.2.4 Heptane (**6**) 191
10.2.5 Hexanoic acid (**7**) 192
10.2.6. Nonanoic acid (**8**) 192

10.3 Compounds identified from *Calotropis procera* 193
10.3.1 *n*-Heptanoic acid methyl ester (**9**) 193
10.3.2 *n*-Decanoic acid (**10**) 193
10.3.3 *n*-Nonanic acid methyl ester (**11**) 194
10.3.4 *n*-Decenoic acid methyl ester (**12**) 194

10.4 Organic functions responsible for Phytoabsorption of Lead (CrIII) by *Calotropis procera* 194

Chapter 11 References 195

LIST OF PUBLICATIONS 207

APPENDIX I 208

Map of South Punjab 209

Questionnaire 210

APPENDIX II 211

1. A view of Sulaiman Ranges (The Fort Munore) Punjab South Region . 212
2. A view of Tribal Area Dera Ghazi Khan 212
3. A view of Cholistan Desert 213
4. Road side view along Multan to Muzaffargarh 213
5. A view of *Desmotachya bipinnata* near river Chenab 214
6. Author collecting the *Tamarix aphylla* near Sakhi Sarwar 214
7. Author interviewing the local people 215
8. Ulcered albino rat 215
9. Normal skin Albino rat 216
10. Abnormal mitotic stages of cancer in the of Albino rat 216
11. Carcinoma insitu in the skin of Albino rat 217
12. The vacuolated skin of Albino rat 217

LIST OF TABLES

2.1 Categorization of plants on the basis of therapeutic profile 17

2.2 Names and addresses of some Hakims and local persons who filled the Questionnaire 23

2.3 List of collected plants (May 2007) for Prem Madan Herbarium 25

3.1 The Description of Plants belonging to Southern Punjab 33

4.1 Medicinal plants selected for isolation and biological activity 93

6.1 Distribution of Animals in different experimental groups on the basis of administration of controls and drug (plant extract) 107

6.1.2 Solvent (3) extracts of Medicinal plants (7) collected from different localities of Southern Punjab for Biological Activity 108

6.1.3 Medicinal plants (8) from Northern areas of Pakistan used to enhance the antibacterial activity of Experimental Medicinal Plants (7) of Southern Punjab. 109

6.1.4 The division of animals and the dose schedule of chemical carcinogens and Plant extracts. 110

6.1.5 Particulars of animals of control (Group A) 111

6.1.6 Lesions obtained in animals of control (Group A) 112

6.1.7 Experimental Protocol for Animals of group B- M 113

6.1.8 Lesions observed in animals (rats) of control (Group B) after applying experimental protocol 114

6.1.9 Lesions observed in animals (rats) of control (Group C) in acetone after applying experimental protocol 115

6.1.10 Lesions observed in animals (rats) of control (Group D) in Pet ether after applying experimental protocol 116

6.1.11 Lesions observed in animals (rats) of control (Group E) in Methanol after applying experimental protocol 117

6.1.12 Lesions observed in animals (rats) of control (Group F) in Dichloromethane after applying experimental protocol 118

6.1.13 Lesions observed in experimental animals treated with Pet ether extract of *Coronopus didyma* (Group G1) after applying experimental protocol 119

6.1.14 Lesions observed in experimental animals treated with methanolic extract of *Coronopus didyma* (Group G2) after applying experimental protocol 120

6.1.15 Lesions observed in experimental animals treated with dichloromethane extract of *Coronopus didymus* (Group G3) after applying experimental protocol 121

6.1.16 Lesions observed in experimental animals treated with Pet ether extract of *Salsola kali* (Group H1) after applying experimental protocol 122

6.1.17 Lesions observed in experimental animals treated with methanolic extract of *Salsola kali* (Group H2) after applying experimental protocol 123

6.1.18 Lesions observed in experimental animals treated with dichloromethane extract of *Salsola kali* (Group H3) after applying experimental protocol 124

6.1.19 Lesions observed in experimental animals treated with Pet ether extract of *Capparis decidua* (Group I-1) after applying experimental protocol 125

6.1.20 Lesions observed in experimental animals treated with methanolic extract of *Capparis decidua* (I-2) after applying experimental protocol 126

6.1.21 Lesions observed in experimental animals treated with dichloromethane extract of *Capparis decidua* (Group I-3)) after applying experimental protocol 127

6.1.22 Lesions observed in experimental animals treated with Pet ether extract of *Salvadora oleoides* (Group J1) after applying experimental protocol 128

6.1.23 Lesions observed in experimental animals treated with methanolic extract of *Salvadora oleoides (*Group J2) after applying experimental protocol 129

6.1.24 Lesions observed in experimental animals treated with dichloromethane extract of *Salvadora oleoides* (Group J3) after applying experimental protocol 130

6.1.25 Lesions observed in experimental animals treated with pet ether extract of *Withania coagulans* (Group K1) after applying experimental protocol 131

6.1.26 Lesions observed in experimental animals treated with Methanolic extract of *Withania coagulans* (Group K2) after applying experimental protocol 132

6.1.27 Lesions observed in experimental animals treated with dichloromethane extract of *Withania coagulans* (Group K3) after applying experimental protocol 133

6.1.28 Lesions observed in experimental animals treated with pet ether extract of *Heliotropium strigosum* (Group L1) after applying experimental protocol 134

6.1.29 Lesions observed in experimental animals treated with methanolic extract of *Heliotropium strigosum* (Group L2) after applying experimental protocol 135

6.1.30 Lesions observed in experimental animals treated with dichloromethane of *Heliotropium strigosum* (Group L3) after applying experimental protocol 136

6.1.31 Lesions observed in experimental animals treated with pet ether extract of *Tamarix aphylla* (Group M1) after applying experimental protocol 137

6.1.32 Lesions observed in experimental animals treated with methanolic of *Tamarix aphylla* (Group M2) after applying experimental protocol 138

6.1.33 Lesions observed in experimental animals treated with dichloromethane of *Tamarix aphylla* (Group M3) after applying experimental protocol 139

6.1.34 Percentage composition of synthetic diet with normal amount of vitamins and minerals administered to experimental animals (rats) during the study 140

6.2.1 Zone of inhibition of different antibiotics (Controls) 141

6.2.2 MIC values of methanol extracts of experimental medicinal plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali, Heliotropium strigosum* and *Coronopus didymus*) 142

6.2.3 MIC values of pet ether extracts of experimental medicinal plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali, Heliotropium strigosum* and *Coronopus didymus*) 143

6.2.4 MIC values of dichloromethane extracts of experimental medicinal plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali, Heliotropium strigosum* and *Coronopus didymus*) 144

6.2.5 MIC values of synergistic activity of methanolic extract of *Withania coagulans* with other plants 145

6.2.6	MIC values of synergistic activity of methanolic extract of *Salsola kali* with other plants	146
6.2.7	MIC values of synergistic activity of methanolic extract of *Capparis decidua* with other plants	147
6.2.8	MIC values of synergistic activity of methanolic extract of *Heliotropium strigosum* with other plants	148
6.2.9	MIC values of synergistic activity of methanolic extract of *Salvadora oleoides* with other plants	149
6.2.10	MIC values of synergistic activity of methanolic extract of *Coronopus didymus* with other plants	150
6.3.1	In vitro antifungal activity of pet ether extracts of ethno medicinal ethnomedicinal plants	151
6.3.2	In vitro antifungal activity of Methanolic extracts of ethno-medicinal plants	152
6.3.4	In vitro antifungal activity of Dichloromethane extracts of ethno-medicinal plants	153
6.3.5	Ethnomedicinal Plant extracts are compared with other antifungal drugs using ANOVA (Critical value F.05 (6, 36) = 2.38)	154
8.1	Some medicinal plants and their uses	171
8.2	Reported Flavonoids of Boraginaceae (1992-2003)	182
9.1	Compounds (%age) found in *Withania coagulans* by GC-MS	183
9.2	Compounds (%age) found in *Calotropis procera* by GC-MS	184
9.3	Difference between absorption bands (cm^{-1}) of *Calotropis procera* roots before and after absorption of Cr (III) on it	186
10.1	^{13}C-NMR Spectral data of Taxifolin	189

LIST OF ILLUSTRATIONS

Fig. 9.1	(a) Infrared spectrum of Calotropis procera roots before absorption of Cr (III) on it	185
Fi.g. 9.1	(b) Infrared spectrum of Calotropis procera roots after absorption of Cr (III) on it	185
Fig. 10.1	Mass chromatogram of Cyclohexane (**3**)	190
Fig. 10.2	Mass chromatogram of Borane carbonyl (**4**)	190
Fig. 10.3	Mass chromatogram of Hexane 3-methyl (**5**)	191
Fig. 10.4	Mass chromatogram of Heptane (**6**)	191
Fig. 10.5	Mass chromatogram of Hexanoic acid (**7**)	192
Fig. 10.6	Mass chromatogram of Nonanoic acid (**8**)	192

SECTION A

1. Ethnomedicinal Studies of Southern Punjab

and

Establishment of P.M. Herbarium

1. ETHNOMEDICINAL STUDIES OF SOUTHERN PUNJAB AND ESTABLISHMENT OF HERBARIUM

1.1 Ethnomedicinal Studies of Southern Punjab

1.1.1 General Introduction and Historical Review

Majority of the people living in the developing world is struggling to increase the standard of living and to improve the health care delivery in the face of increasing poverty and growing population. It has been estimated that 70% - 80% of population in the developing countries have only their traditional herbal remedies for their ailments as the costly pharmaceuticals are out of their reach.

Keeping in view the above fact it can be inferred that by careful collection of data and experimentation, medicine of much higher value and low cost can be isolated from the plants, to fulfill the requirement of the major portion of the world population specially that of developing world. Therefore, importance, necessity, and potentiality of medicinal plants cannot be overlooked.

Ethno botany, the study of how people in traditional societies use plants, has great potential to provide new and useful plant products for the benefits of the world. The practice of ethno botany in itself being modified to ensure that the rights of traditional people benefit from any commercial discoveries made from their knowledge. [Veilleux, C. and King, S.R. 2002]

This science has emerged as an interdisciplinary study which can involve in addition to botany and ethnology, area of archeology, sociology, folklore, mythology, linguistic, forestry, ecology, agriculture, literature, medicinal science, economics, phytochemistry, pharmacology and veterinary medicine etc. The multidisciplinary nature of ethno botany, occasionally leads to some confusion in definition of its objectives. Though variety of

subjects has internationally or even accidentally contributed to the objectives of ethno botany, yet this science has remained primarily an applied discipline of botany.

Medicinal ethno botany is the sub-discipline of ethno botany which refers to the study of traditional uses of plants and folk knowledge concerning plants and human health care, including prevention and caring of human illness using plants. Ethno medicinal/botanical information on medicinal plants and their uses by indigenous cultures is useful not only in the conservation of traditional cultures and biodiversity, but also for community health care and drug development. This information is utilized as a guide for drug development under the assumption that a plant that has been used by indigenous people over a long period of time may have an allopathic application. [Farnsworth, N.R.O., 1993]

Ethno botanical documentation and inventories are scientifically organized with local and scientific name, medical use, cultural interpretation and information on the ecology, botany, harvesting, distribution, management and conservation of medicinal plants. This information is acquired from local herbal practitioners, community healers and herbal traders at various local markets as well as from community members who had the knowledge. This knowledge often has rich diverse and reliable local experience in prevention, curing and maintaining the health of the people in the local environments. The inventory and documentation can be usefully incorporated into community resource management programs and biodiversity conservation at the local level. It can be used as information to guide the selection of plants and collection of samples for laboratory identification and pharmacological testing in drug development. Thus the documentation and inventory can be considered an information bank of traditional medicines.

The local communities of different areas of Pakistan have the knowledge of centuries old traditional uses of most of the plants of this area. This indigenous knowledge of plants is transferred generation after generation by their ancestors.

Unani, Ayurvedic and Homeopathic health care systems are entirely based on the medicinal properties of these plants. It is feared that the precious wealth of indigenous knowledge will not be known to the future generation if not documented.

The use of medicinal herbs for various health disorders for human and live stock is the common practice in rural areas. Main reason for using traditional medicinal is their economic condition. The number of medicinal plants collectors has increased giving rise to the comprehensive trade. The medicinal plants are exported to the other countries of the world as well.

1.1.2 Ethnomedicinal Studies in Asia

Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance. [Diallo *et.al*.1999] The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. [Edeoga, H.O.*et al.* 2005]

Rural communities, in particular Paliyar tribes, depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries. [Sandhu, D.S. and Heinrich, M., 2005] [Gupta *et.al*. 2005]

Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections. [Rojas, *et.al.* 2006]

Similarly ethno medicinal knowledge in Chinese culture can be traced back to very ancient times through as literature on Chinese Material Meteria and Chinese works of agriculture and Horticulture. [Pei, S., 1995] [Manandhar, N.P., 1987]

Pakistan is rich in plant resources, particularly the medicinal plants. [Ali *et. al*. 2001]

More than 6,000 species of higher plants and 4,000 spices of fungi has been recognized and established in the Peshawar region. At least 1,000 medicinal plants species were reported from Pakistan [Baquar, 1989] of which 500 species were commonly used in health care practices and 350 were traded for billion of Rupees to the national and international markets. Most of the medicinal plants available in the market or supplied directly to the pharmaceutical industries are extracted from the rural forest. [Amin, M., 1961] [Chaudhari, I.I, 1958] [Chaudhari, I.I., 1961] [Ikram, M., and Hussain, S.F., 1978] [Zaman, *et.al*.1972]

So far only a few papers have been published on the medicinal plants species in Pakistan. Chaudhari (1961) documented more than 1500 medicinal plants species in Pakistan. Some other workers had also contributed in this regard. [Hocking, G.M., 1962] [Paris, R. and Dillemann.G, 1960] [Khan, A.K., 1962] [Malik, A.R., 1958] [Dastur, H., 1952] [Shinwari, Z.K., and Khan, M.A., 1998]

Medicinal plants have provided a source of inspiration for novel drug compound as plant derived medicine has made significant contribution to world human health. [Eisenberg, *et.al*. 1998]

The important necessity and patentability of medicinal plants in practice of medicine today is well established and cannot be over looked. The use of alternative medical therapy increased the interest of pharmacologists and herbalists over the past decade. Plant medicine had become a topic of global importance. A lot of interest has been taken in investigation of medicinal source. A large number of these medicinal plants are used in the form of powder, decoction and infusion for treatment of various diseases including the infection caused by microbes with per amount of success by Hakims and local people. [Hussain, M.A., and Gorsi, M.S., 2004]

An ethnobotanical survey of tribal area of southern Rajasthan was carried out during the year 2001-2002 for ethno sexicological herbal medicines. The information on ethno sexicological herbs was based on the exhaustive interview with local medicine-men and -women, birth attendants and other knowledgeable persons who prescribed their own herbal preparation to check birth control, including abortion at initial stages, preventing

conception or by making either member of the couple sterile and to cure various sexual diseases like leucorrhoea, gonorrhea, menorrhagia, to regularize menses/periods and syphilis in both the sexes. During ethno botanical survey, 53 plants belonging to 33 families had been reported from the study area, which were used to cure sexual diseases, and for family planning. [Jain, *et. al*. 2004]

Fakim, A.G., [1999] reported 197 species of plants of medicinal properties from Mauritius. He listed their local names, part used and uses.

Hamid, A. and Sitepus,D., [1990] reported 7500 species of medicinal plants from Indonesia, out of these only 187 were used in traditional system of medicine. They described the local name and uses for each species.

Medicinal components from plants play an important role in conventional Western medicine. In 1984, at least 25% of the prescription drugs issued in the USA and Canada were derived from or modeled after plant natural products. [Farnsworth, N. R. O., 1985] [Balick, M.J., 1996]

Coelho, *et. al.* [2004] described the four-stage process of documentation and evaluation of the medicinal plants.

(a) Ethno botanical studies;

(b) Analysis of traditional uses;

(c) Literature survey on phytochemical and pharmacological data;

(d) Microbiological screening of selected plants.

Jeric, *et.al,* (2007) reported the ethno botanical survey carried out on the territory of the highest mountain in Central Serbia, Kopaonik. In total, 83 wild species from 41 families and 96 preparations for use in human therapy were recorded. Most commonly used plants for medicinal purposes *are Hypericum perforatum* L., *Urtica dioica* L., *Achillea millefolium* L., *Matricaria chamomilla* L., *Sambucus nigra* L., and *Thymus serpyllum* L.. The most frequently reported medicinal uses were for treating gastrointestinal ailments (50%), skin injuries and problems (25.6%), followed by respiratory, urinary-genital and

cardiovascular problems Plants with unusual phytotherapeutic uses were *Gallium verum* L. and *Eupatorium cannabinum* L. while plants with interesting but lesser-known properties include *Daphne laureola* L. and *Ficaria vernahuds*. In addition, 10 wild species used in veterinary medicine, as well as 25 herbs used for human nourishment were noted.

1.1.3 Ethnomedicine in Pakistan

In Pakistan the medicinal plants have immense potential, but unfortunately very little were known about the actual production size and potential of plant species, their conservation status, actual trade and production areas.

Haq, I. and Hussain, M. [1987] described 55 medicinal plants of Mansehra, each with its family, vernacular name, botanical name, synonyms, part used, distribution, constituents and uses. Chaudhri and Qureshi [1991] stated that as many as 709 species of vascular plants of Pakistan, constituting about one fourth of vascular flora were in danger of being gradually wiped out or exterminated altogether.

Goodman, S. M. and Ghafoor, A., [1992] surveyed the Baluchistan province and collected 114 species with local ethno medicinal plants. For some plants, source area and market price were also given. Haq [1993] published a list of 53 wild and 17 cultivated medicinal plants from Mansehra district, with botanical, English and vernacular names, family names, part used, distribution, constituents, medicinal and local uses.

Khan, *et. al,* [1994] investigated 5 tree species belonging to family Papilionaceae, 2 to family Caesalpiniaceae and 6 to family Mimosaceae, along with their description, macroscopic characters and medicinal uses of their bark. He studied the thorn forest area of Punjab and its decline due to overgrazing, wind erosion, desertification, water logging and salinity. He referred to *Salvadora oleoides* for its great ecological and ethno medicinal importance. The local uses of the traditional plants in the treatment of the various ailments in Peshawar region were reported by Haq and Shah [Haq. A.H and

Shah, S.A. [1986] Sher [2001] worked on the ecology of alpine and sub-alpine medicinal and other economic plants of district Swat and Chitral Pakistan.

Haq, I. and Hussain, M. [1995] conducted a survey of medicinal plants of Palandary District Poonch (Azad Kashmir). They revealed 47 medicinal plants used to cure various diseases in the area by the local in the traditional system of health care. They also described the local names in Pahari, Urdu, Pashto, and Punjab.

Shinwari, Z.K. and Khan, M.A. [1995] worked on traditional uses of plants in Kaghan Valley and reported that out of 48 medicinal plants the local people used only 26 species. In these 21 species were used as animal fodder, while some woody plants were used for tool making purposes.

Sadaqat, M., [1995] discussed 10 medicinal plants of Cucurbitaceae, which were *Benincasa hispida, Citrullus spp, Corallocarpus epiglous, Cucumis melo, C. sativus, Luffa acutangula, L.echinata, Momardica dioca, Trichosanthus cucumerica* and *T. dioca*. Sultana, *et. al*. [1996] reported total of fifty-six edible species of mushrooms from Pakistan including four from Baluchistan, three from Sindh, five from the Punjab and forty-four from NWFP and Azad Kashmir. Some of the species that were being commercially exploited in the world include *Agaricus bisporus, Auricularia spp. Coprinus comatus, Flammulina vellutipes, Lentinus edodes*, *Phellorina inquinanes, Pleurotus ostreatus, Stropharia rugusoannulata and Volvariella volvacea*.

Shinwari, Z.K. and Shah, M. [1996] studied the ethno botany of Kharan valley (Mansehra), Pakistan and reported the important medicinal, food, poisonous and ornamental plants with their common and botanical names and uses.

Marwat, Q. and Shinwari, Z.K., [1996] worked out the ethno botany of upper Siren (Mansehra), Pakistan and reported 79 species of plants belonging to 48 families. They described the local uses of these plants, such as medicinal, fodder, food, shelter, etc.

Badshah, *et.al*. [1996] documented ethno botanical information of 83 species from Pirgarh Hills, South Waziristan Agency. Hussain, F. and Khaliq, A. [1996] reported that 125 species had various local uses in Dabargai Mills District Swat. They were classified

as fodder (76 spp.), medicinal (69 spp.), fuel wood (18 spp.), timber wood (13 spp.), mud supporter (6 spp.), fence and hedge plants (45 spp.) and snuff making powder plants (2 spp).

Shinwari, Z.K. [1996] discussed the present status of ethno botany in Pakistan. He emphasized on the need of investigation, documentation, explanation and application of traditional knowledge in the use of natural resources. Ajab, M. and Ilyas, I. [1999] reported brief account of ethno botanical information of Malam Jaba area of district Swat for varied purposes like medicines, food, tool making, fuel, timber wood, etc.

Arshad, M. and Akram, S. [1999] described the medicinal plants of Arid Agriculture areas of Rawalpindi along with their botanical names, local names, part used and method of recipe preparation. Shinwari, Z.K. and Khan, M.A. [1999] discussed the dependence of the inhabitants of Margalla Hills National Park, Islamabad, on surrounding plant resources for their food, shelter, fodder, health care and other cultural purposes. They recorded 50 species of herbs used medicinally by the inhabitants of the Park. They found *Aspharagus adescendens* and *Viola chinescence* vulnerable to harvesting. Sher, *et.al*. [2000] worked on market survey of medicinal plants in major cities of Pakistan. They reported that more than 300 items of plant origin were traded in herbal market of the country. They also showed the export and import of these species in the country.

Qureshi, R. (2002) conducted an ethno botanical survey on Rohri Hills in Sindh and recorded 78 species belonging to 30 angiospermic families during year 1998-2000. The local people used these plants to meet their daily requirements of medicines, food, fodder, agricultural equipments and shelter. Shinwari, Z.K. and Gilani, S.S. (2003) conducted ethno botanical study of 33 plants being used as medicinal by the local people of Bulashhar valley, Astore district Diamer. They also reported the occurrence, general distribution and abundance in the investigated area.

The review of literature showed that ethno medicinal studies of plants of southern, Punjab have not been carried out till now. The present endeavor was therefore, carried out to document the ethno medicinal information of this area.

1.1.4 Geography of South Punjab

South Punjab is the part of the Punjab province with diversity of climate and geographic location. The administrative boundaries of the area has boarder simultaneously with the rest of the three provinces of Pakistan. The area boundary is marked by D.I. Khan (N.W.F.P) in the north, Indus River in the East, Jacobabad and Sibbi District (Sind and Baluchistan province respectively) in the South and Loralai district (Baluchistan Province) in the west (Appendix-1). Very little has been known and written about this area. The peoples of the study area are very simple and illiterate. They totally depend on agriculture, livestock, hand made wooden articles and embroidery. The 80% population of the area depends on the traditional health care system. Due to diversity in the geographical nature the area is rich in diversity of plant resources.

1.1.4.1 Physical Feature

The area under study is of an irregular square shape and extended within latitude 29°.12′ to 31°.15′ north and longitude 71°.27′ to 73°.15′ east. The total area is 1373 squares kilometer. The area includes Sahaiwal, Multan, Dera Ghazi Khan, Ranjanpur and Tribal area of Dera Ghazi Khan on north, Vahari, Bahawalnagar on south west, Bahawalpur and Cholistan desert on west and Rahim yar Khan and Sadiqabad on north-west. The elevation varies from 118-168 m (Established in 1926-1962). The whole area is diversified in to

- Cultivated Plains
- Piedmont Plains
- River Plain
- Flooded Plain
- Salt range
- Desert
- Mountain

1.1.4.2 Climate

The climate area under study is arid and semi arid. During summer, it is very hot in the day time but a bit cool at night and the temperature rises above 42-45°C while in winter temperature drop to 2°C. The monsoon starts from July to August .The post monsoon starts from October last till the end of November. The average rain fall is from 100-180 mm. About 50% of the total rain fall is received during the month of July and August. Because of the low humidity, the scarcity of vegetation is expected due to extreme of temperatures both diurnal and seasonal. The area represents one of the hottest places of the country. May, June and July are the hottest months with a mean maximum temperature 108°F. January is the coldest month with a mean minimum temperature about 38°F. In a narrow Western South most belt of the area, however, some moderating effects are expected in temperature and humidity due to nearness of river and some other climatic factors. [Pakistan Metrological department, Jail Road, Lahore, 2007]

1.1.5 Biodiversity

Much of the native vegetation of southern Punjab has been replaced by new introduced species or eliminated by cultivation, overgrazing and felling. Some natural vegetation still remains on foot hills, terraces, piedmont basins, plains, sandy deserts and unreclaimed areas occupied by saline-alkali soils .The irrigated parts support a variety of food and fodder crops. The mountainous regions are barren except for some vegetation that survives along waterways.

The terraces and foot hills supports similar vegetal cover, restricted to low sites where moisture is occasionally available. *Salvadora oleoides, Prosopis glandulosa, Acacia arabica, Acacia nilotica* and *Capparis aphylla* are the trees that grow in this region. *Alhagi camelorum, Suaeda fruiticosa, Calligonum polygonoides, Rhazya stricta, Peganum hermala, Cassia italica, Coronopus didymus, Sophora millis, and Withania coagulans* are herbs and shrubs of medicinal value and are used by local farmers and

Ethnomedicinal practitioners. The chief palatable grasses are *Eclionurus hirsutue, Eleusine flagellifera* and *Cynodon dactylon*.

The young sandy piedmont plains support, *Kaur tumma*, *Gul kandiari*, *Calotropis gigenta, Heliotropium strigosum willd., Salsola kali, Aerva javanica*, and some vegetation similar to the terraces and foot hills.

The sub-recent flood plains are dominantly irrigated, with the exception of saline– alkali area or high lying sandy ridges and are mostly cleared of the natural vegetation. The natural vegetation on saline- alkali patches consists of a scrub of salt tolerant species such as *Tamarix articulate, Alhagi banghlensis* and a thick cover of *Demostachya bipinnata*. The sandy ridges support *Saccharum munja* and some other grasses.

The irrigated parts support *Phonix dactylifera* and some introduced species like *Dalbergia sisso*, *Acacia Arabica* and *Mangifora indica*. Reserved forests at the junction of the sub recent and active flood plains support *Tamarix articulate* etc.

In active plains, fresh plants and grasses show up on freshly deposited sediments, soon after the floods. These plants continue growing throughout the year and provide poor grazing till they are washed away by the flood during the next year.

1.2 Establishment of Herbarium in LCWU

1.2.1 Historical background

The glorious academic values of this oldest premier post-graduate female institution have been shaped by its institutional history, which is spread over a span of 85 years. Established in May 1922 as an Intermediate residential college, it was housed in a building on Hall Road, Lahore with strength of 60 students, 25 of whom were boarders and 13 staff members. Since 1922 L.C.W. has proved its worth as the highest seat of learning for science subjects. F.Sc. classes started right from the day, Lahore College for Women was founded.

Botany as one of the science subjects was taught by Hindu, Sikh or Christian female teachers. After independence, some of the science classes had to be run with the help of male teachers. When the college shifted to the present building, in 1951, Mrs. Dilara Maqeet was the head of the Botany Department B.Sc. classes. (Botany and Zoology) started in 1956. After Mrs. Maqeet, Miss. Prem Madan was appointed as the Head of the Department. She worked throughout her whole career with missionary zeal for the promotion of the department. Mrs. Khatoon Zahoor was another prominent teacher of that era, who succeeded Miss. Prem Madan, as the Head of the Botany Deptt. She was a woman of substance. She put on commendable efforts in starting post graduate classes in the Deptt. M.Sc. Botany Classes started in 1993. (Reitereived from URL: http//www.lcwu.edu.pk)

Miss Prem Madan took initiative in collection of plants and in a way started the categorization of plants. Though there was a considerable data of plants but that needed a expansion and world class scientific approach with proper cataloging. About 200 plants were added and cataloged in shape of proper Herbarium. The Herbarium was named after Miss Prem Madan to eugilize her services to the Department of Botany of Lahore College for Women University and properly inaugurate.

2. EXPERIMENTAL

2. EXPERIMENTAL

2.1 Ethnomedicinal Study

2.1.1 Material and Methods

The study was conducted during 2005- 2007.Servaral visits were made on seasonal basis to the different parts of the South Punjab. The study was based on direct communication with the local inhabitants and local Hakims of the area. This information was then compared with available literature and found to be authentic.

Material and other necessary information was collected from the council offices, Forestry, Wild life and Fisheries Department of the concerned area.

The study area was divided into different parts on geographical basis. These were as follows:

- Sahiwal
- Muzzafarabad
- Multan
- Sadiqabad (Rahim yar Khan)
- Bahawalpur (Cohlistan)
- Dera Ghazi khan
- Rajanpur (Kot Mithan)

Table 2.1: Categorization of Plants on the Basis of Therapeutic Profile

Sr. #	Botanical Name	Local name	Family	Part used	Categorization
1.	*Ocimum americanum* Linn	Kali niazboo	Lamiaceae	Young shoot	DD
2.	*Phyllanthus amarus*	Kilanelli	Euphorbiaceae	Leaves	LD
3.	*Acacia farhesiana* Linn	Phali	Mimosaceae	Gum	MSD
4.	*Adhatoda vasica (Justicia adhatoda)*	Baikar	Acanthaceae	Whole Plant	GI, RI, VD, LD, DD, GI, MSD, Dd
5.	*Ajuga bracteosa*	Darkan booti	Lamiaceae	Shoot	GI
6.	*Allium ascalonicum*	Gandanaa	Alliaceae	Whole Plant	VD
7.	*Anisomeles indica*	Gandi booti	Lamiaceae	Whole Plant	RI
8.	*Asparagus adscendens*	Khairuwa	Lillaceae	Root, Tuber	SD, DD
9.	*Asparagus capitatus*	Dusa	Lillaceae	Root	VD
10.	*Berberis lycium*	Kashmal	Berberidaceae	Root, Bark	MSD, SD, ED, GD
11.	*Buxus papillosa*	Papper	Euphorbiaceae	Whole Plant	MSD, VD, CD
12.	*Cannabis sativa*	Bhang	Cannabinaceae	Shoot	GI
13.	*Capparis spinosa* Linn	Kakri/ Kobra	Capparidaceae	Root, Leaves, Fruit	RI
14.	*Caralluma edulis*	Pippu	Asclepidiaceae	Whole Plant	SD, DD
15.	*Gallium aparine*	Banosha	Rubiaceae	Sape	RD
16.	*Geranium ocellalum*	Bhanda	Geraniaceae	Whole Plant	MSD, RD
17.	*Geranium rotunifolium*	Bhanda	Gerniaceae	Root	MSD, RD
18.	*Hyoscyamus insanus*	Dewana bhang	Solanaceae	Whole Plant	RI,SD, DD
19.	*Jasminum officinale*	Chambely	Oleaceae	Whole Plant	GI, SD, AF
20.	*Lallemantic royleana*	Tukhumbalanga	Lamiaceae	Seed	GI, SD
21.	*Litsea monopetala*	Maida lakri	Lauraceae	Bark	MSD, SD
22.	*Malva neglecta* Wall	Khubasi	Malvaceae	Whole Plant	RI, GI, SD
23.	*Malvastrium coromendelianum*	Jhar	Malvaceae	Leaves, Flowers	VD, GI
24.	*Martynia annua*	Hathjoy	Pedaliaceae	Fruit, Shoot	GI, ENT, SD
25.	*Cassia fistula* Linn	Amaltas	Caesalpiniaceae	Whole Plant	GI,DD,SD,GD
26.	*Cassia occidentals*	Kaswandi	Caesalpiniaceae	Root, Leaves, Seed	SD,DD,RI,GD

27.	*Cassia Obtusifolia*	Chakunda	Caesalpiniaceae	Root, Leaves	DD, AF, SD, RI, CD, RD, ED, Dd
28.	*Cassia angustifolia* Linn	Senna	Caesalpiniaceae	Leaves, Seed	DD, SD, AC
29.	*Cassia tora*	Pamad	Caesalpiniaceae	Leaves, Seed	SD, DD, AF
30.	*Tamarindus indica*	Imali	Caesalpiniaceae	Leaves, Flowers, Bark	GI, RD, MSD, ED, CD
31.	*Bauhinia veriegata*	Kachnar	Caesalpiniaceae	Root, Bud, Bark	LD, ENT, GI, ED, SD
32.	*Prosopis spicigera*	Jundh	Mimosaceae	Bark, Leaves, Flowers	SD, DD, ENT, RI,
33.	*Acacia arabica*	Babool	Mimosaceae	Bark, Leaves, Gum	RI, DD, SD, ED, MSD, VD
34.	*Acacia rugata*	Ritha	Mimosaceae	Seed, Leaves, Pods	SD, CD, GD, RD
35.	*Rosa gallica*	Chota Gulab	Rosaceae	Petals	MSD, ED
36.	*Rosa alba*	Gulab	Rosaceae	Flowers	CD, DD, GI
37.	*Rosa indica* linn	Gulab	Rosaceae	Fruit	MSD, GI
38.	*Optuntia monacantha*	Danda Thuar	Cactaceae	Stem, Fruit	SD, GI
39.	*Opuntia stricta*	Thur	Cactaceae	Fruit	GI
40.	*Opintia dillenii*	Kunda thur	Cactaceae	Fruit, Leaves	GI, AC, RI, VD, SD
41.	*Bupleurum falcatum*	Spili	Apiaceae	Root	VD, GI, LD
42.	*Bupleuram jucundum*	Amurland	Apiaceae	Root	LD
43.	*Foeniculum capillacerm*	Sonf	Apiaceae	Leaves, Seed, Root	VD, SD, ED, GI, RI
44.	*Angelica glauca*	Chora	Apiaceae	Leaves	CD, SD, GI
45.	*Vernonia cinerea*	Gandhavaki	Asteraceae	Seed, Flowers	SD, RD
46.	*Ageratum conzeoides*	Ageera	Asteraceae	Root, Stem, Leaves	SD, GI, DD
47.	*Pulchea indica*	Mandar	Asteraceae	Leaves, Root	MSD, GI, SD
48.	*Eclipta alba*	Tikka	Asteraceae	Leaves, Root	RI, CD, LD, VD, RD
49.	*Helianthus annuus*	Surajmuki	Asteraceae	Leaves, Root, Flowers, Seed	DD, ENT, MSD, GD, SD, RI
50.	*Achillea millefolium*	Biranjassfa	Asteraceae	Flowers	RI,RD,LD,DD
51.	*Cotula anthemoides*	Babuna	Asteraceae	Leaves, Root	MSD, ED, GI, SD
52.	*Cotula aurea*	Babni	Asteraceae	Flowers	SD, VD
53.	*Artemisia scoparia*	Biur	Asteraceae	Whole Plant	SD, GI, ENT

54.	*Artemisia maritime*	Kirmala	Asteraceae	Whole Plant, Seed	SD, GI, RI
55.	*Artemisia vulgaris*	Baniru	Asteraceae	Leaves	RI, ND
56.	*Echinops echinatus*	Kantalu	Asteraceae	Root	SD, ND, MSD, GI, VD
57.	*Sonchus oleraceus*	Sadi	Asteraceae	Root, Leaves, Stem	LD, RI, GI
58.	*Thevetia aperuviana*	Pali Kanar	Apocynaceae	Seed, Bark	DD, ED, SD
59.	*Abrus precatorius*	Rati	Papilionaceae	Root, Leaves	DD, SD, VD, ND, RI, CD
60.	*Frankenia pulverulenta*	Khareeya	Frankeniaceae	Whole Plant	DD
61.	*Indigofera articulata*	Surmaii	Papilionaceae	Leaves, Root, Seed	SD
62.	*Melilotus alba*	Aspurk	Papilionaceae	Whole Plant	DD, RI
63.	*Portulaca tuberose*	Lunuk	Portulacaceae	Leaves	SD
64.	*Dicoma tomentosa*	Dayii	Asteraceae	Whole Plant	GD
65.	*Geisekia pharnacoides*	Aluka	Ficoidaceae	Whole Plant	VD, CD, GI, SD, RD
66.	*Rhazya stricta*	Ishwarg	Apocynaceae	Leaves, Fruit	GI,ENT,RI,DD
67.	*Sonchus arvensis*	MaliBoti	Asteraceae	Root	RD,LD
68.	*Aerva tomentosa*	Buikallan	Amaranthaceae	Leaves, Root	GD,SD,GI
69.	*Amaranthus spinosus*	Cholai	Amaranthaceae	Root, Leaves	GD,SD,GI
70.	*Mallotus philippnensis*	Kambal	Euphorbiaceae	Leaves	SD,RD,GI,DD
71.	*Callicarpa macrophylla*	Daya	Verbenaceae	Root, Leaves	SD,MSD
72.	*Calligonum polygonoides*	Phogalli	Polygonaceae	Root	ENT
73.	*Convolvulus glomeratus*	Loaralli	Convolvulaceae	Whole Plant	SD
74.	*Euphorbia helioscopia*	Gandabuti	Euphorbiaceae	Plant juice, Seed	SD, GI, DD
75.	*Flueggea leucopyrus*	karan	Euphorbiaceae	Leaves	GI
76.	*Glossonema varians*	khurram	Asclepiadaceae	Fruit	GI
77.	*Lycium barbarum*	Chirchitta	Solanaceae	Leaves	DD,ENT,ED
78.	*Tecomella undulata*	Luar	Bignoniaceae	Whole Plant	GI
79.	*Coldenia procumbens*	Tripunki	Boraginaceae	Bark	SD,DD,VD
80.	*Acacia jacquemontii*	Kikari	Mimosaceae	Stem	GI
81.	*Calligonum polygonoides*	Phog	Asclepiadaceae	Roots	GI
82.	*Citrulus colocyntus*	Tumba	Cucurbitaceae	Fruit and root	LD, GI, RD, MSD
83.	*Haloxylon salicornicum*	Safed lanra	Chenopodiacae	Whole Plant	For washing
84.	*Kochia indica*	Bui	Chenopodiaceae	Whole plant	CD

85.	*Sophora millis*	Lathia	Papillionaceae	Whole Plant	GI
86.	*Eleusine flagellifera*	Chhimbe	Poaceae	Whole Plant	GI
87.	*Solanum xanthocarpum*	Katilla	Solanaceae	Whole Plant	GI,RI
88.	*Phoenix dactylifera*	Pend	Palmaceae	Whole Plant	GI, VD, RI, DD, GD
89.	*Salsola kali*	Lanan	Chenopodiaceae	Whole Plant	GI, DD
90.	*Coronopus didyma*	Charini boti	Brassicaceae	Whole Plant	GI, RI
91.	*Heliotropium strigosum*	Gorakhpamo	Boraginaceae	Whole Plant	GD, RD, MSD
92.	*Ficus racemosa*	Gular roomul	Moraceae	Bark, Fruit	MSD, RI
93.	*Ehretia obtusifolia*	Chamror	Boraginaceae	Root	VD, GI
94.	*Dodonaea viscose*	Sanath	Sapindaceae	Leaves, Bark	MSD
95.	*Dioscorea deltoids*	Kanis	*Dodonaea viscose Linn.*	Rhizome	SD, GI
96.	*Dicleptera roxburghiana*	Kirich	Acanthaceae	Shoots	RI
97.	*Desmodium gangeticum*	Salpan	Papilionaceae	Root	RI, RD
98.	*Datura metel*	Datoora	Solanaceae	Seed, Leaves	RI
99.	*Cichorium intyblus*	Karni	Asteraceae	Whole Plant	RI, SD
100.	*Chrozophora tinctoria*	Nilkhanti	Euphorbiaceae	Whole Plant	SD, GI
101.	*Chenopodium botrys*	Jausag	Chenopodiaceae	Whole Plant	RI, SD
102.	*Chenopodium ambrosioides*	Lunak	Chenopodiaceae	Whole Plant	RI, SD
103.	*Carissa opaca*	Garanda	Apocyanacea	Root	SD, VD
104.	*Caralluma tuberculata*	Choungan	Asclepediaceae	Whole Plant	RI
105.	*Rosa indica*	Gulab	Rosaceae	Petals	SD, GI, ED,DD
106.	*Ocimum basilicum*	Naywee thulasi	Lamiaceae	Flowers, Leaves	GI
107.	*Eclipta alba*	Karichalai Bhangea	Asteraceae	Leaves, Shoot	LD, ENT, RI
108.	*Cassia auriculata*	Avarai	Caesalpiniaceae	Flowers, Leaves	GI
109.	*Cadaba fruiticosa*	Vizliin	Capparidaceae	Leaves	GI
110.	*Datura innoxia*	Batoora	Solanaceae	Whole Plant	DD, GI
111.	*Capsicum annuum*	Mirch	Solanceae	Fruit, Seed	GI, ENT
112.	*Portulaca oleracea*	Kulfa	Portulaceae	Aerial part	RD, RI
113.	*Rumex dentatus*	Jangli Palak	Polygonaceace	Whole Plant	RD
114.	*Blepheris maderaspatensis*	Vaychivettu thalai	Acanthaceae	Leaves	GI
115.	*Curcuma domestica*	Halhard	Zingiberaceae	Rhizome	GI

116.	*Azadirachta indica*	Neem	Meliaceae	Leaves, Flowers, Seed	GI, ENT, AB, AF, AP
117.	*Aerva lanata*	Kooripoo	Amaranthaceae	Leaves	GI
118.	*Peganum hermala*	Hermal	Zygophyllaceae	Whole Plant	ND
119.	*Viola stacksii*	Banafsha	Violaceae	Whole Plant	RI
120.	*Withania coagulans*	Paneer dodi	Solanceae	Whole Plant, Fruit	GI, DD, SD, CD, Dd
121.	*Psidium guajava*	Amrood	Myrtaceae	Root, Fruit	SD
122.	*Eucalyptus globulus*	Sufaida	Myrtaceae	Seed, Leaves, Oil	AB, RD, ENT, RI
123.	*Ficus benghalensis*	Bohr	Moraceae	Aerial Part, Latex	Dd, GI
124.	*Ficus religeosa*	Peppal	Moraceae	Seed, Fruit, Bark	RD, SD, GI, RI
125.	*Albizzia yrocera*	Chhita sirin	Mimosaceae	Bark, Leaves, Flowers, Pods	SD, DD
126.	*Albizzia lebbek*	Kala sirin	Mimosaceae	Bark, Leaves, Flowers, Pods	DD, GI, SD
127.	*Tamarix diocica*	Lei	Tamaricaceae	Leaves, Branches	VD, SD, GI
128.	*Lathyrus aphaca*	Jangli matter	Papillionaceae	Seed	GI
129.	*Dalbergia sissio*	Tahli	Papillionaceae	Leaves	SD
130.	*Morus alba*	Shahtoot	Moraceae	Fruit, Leaves, Seed	SD, GI ,DD ,RI
131.	*Acacia modesta*	Phulai	Mimosaceae	Bark	MSD, VD
132.	*Tribulus terrestris*	Bakhro-Bhust	Zygophyllaceae	Leaves	CD, MSD, GI, RD
133.	*Tribulus longipetalus*	Bakhro Bhust	Zygophyllaceae	Whole Plant	RD, VD
134.	*Fagonia indica*	Damaho	Zygophyllaceac	Whole Plant	GD, DD
135.	*Withania somnifera*	KoreePaneer	Solanaceae	Whole Plant	GD, MSD, GI, DD, AC, SD
136.	*Ziziphus nummularia*	Desi Baeer	Rhamnaceae	Leaves, Fruit	LD, SD, GI
137.	*Polygonum plebejum*	Kheera wal	Polygonaceae	Root, Whole Plant	SD, GI
138.	*Boerhavia procumbens*	Dakhri / Satti	Nyctaginaceae	Root	GD, ENT
139.	*Boerhavia diffusa*	Dakhari	Nyctaginaceae	Whole Plant	CD, RI, LD
140.	*Prosopis cineraria*	Babul/Jal	Mimosaceae	Pods	ENT
141.	*Acacia senegal*	Angrezi Kiker	Mimosaceae	Leaves, Gum, Branches	DD, AF, ENT
142.	*Abutilon indicum*	Pattear	Malvaceae	Leaves	DD, ENT, GI, SD

143.	*Fumaria indica*	Shahtroo	Fumariaceae	Whole Plant	CD, DD
144.	*Indigofera oblongifolia*	Jhil	Papilionaceae	Branches, Twigs	ENT
145.	*Crotalaria burhia*	Chagg	Resedaceae	Root	GI
146.	*Cordia dichotoma.*	Lasora	Boraginaceae	Whole Plant	SD, GI
147.	*Alhagi maurorum*	Jowan	Papilionaceae	Whole Plant	DD
148.	*Convolvulus arvensis*	Naaro	Convolvulaceae	Whole Plant	SD
149.	*Saueda fruticosa*	Koori lani	Chenopodiaceae	Whole Plant	ED, SD
150.	*Salsola baryosma*	Looran Lani	Chenopodiaceae	Whole Plant	GI
151.	*Haloxylon recuvrum*	Zeekhann/Khar	Chenopodiaceae	Whole Plant	SD
152.	*Chenopodium album*	Chullibathu	Chenopodiaceae	Whole Plant	SD, LD
153.	*Cloeome brachycarpa*	Dhanar khathoori	Capparidaceae	Whole Plant	MSD
154.	*Capparis decidua*	Karir	Capparidaceae	Whole Plant	MSD, SD, GI, LD
155.	*Cassia italica*	Ghorawal	Caesalpiniaceae	Leaves	GI
156.	*Trichodesma indicum*	Gaozaban	Boraginaceae	Whole Plant, Flowers	ED, GD, MSD, GI, RD
157.	*Heliotropium crispum*	Karsan	Boraginaceae	Whole Plant	DD
158.	*Pluchea lanceolata*	Phar buti	Asteraceae	Whole Plant	MSD, GI
159.	*Launea procumbe*		Asteraceae	Whole Plant	SD, RD
160.	*Conyza Canadensis*	Giddar buti	Asteraceae	Whole Plant	ENT
161.	*Leptadenia pyrotechnica*	Khippa	Asclepiadaceae	Whole Plant, latex	DD, GD, MSD
162.	*Calotropis gigantean*	Wadha Ak	Asclepiadaceae	Whole Plant, latex	GI, MSD, SD
163.	*Calotropis procera Linn*	Ak	Asclepiadaceae	Whole Plant, latex	GI, ENT, DD, RI
164.	*Amaranthus virdis Linn*	Choli	Amaranthaceae	Whole Plant	GI
165.	*Aerva javanica*	Kanderi	Amaranthaceae	Leaves, Stem	AA, GI, GD
166.	*Achyranthes aspera Linn.*	Putkanda	Amaranthaceae	Whole Plant	CD, GI
167.	*Zaleya pentandra*	Wasanh	Solanaceae	Leaves, Root	SD, GD,GI
168.	*Desmotachya bipinnate*	Drab	Poaceae	Leaves, Root	RI, LD, CD
169.	*Cynodon dactylon*	Khabbal	Poaceae	Leaves, Root	GI
170.	*Cymbopogon jawaracusa*	Bur/ Khawi	Poaceae	Root,Leaves,Flower	DD, RI, GI
171.	*Phoenix acaulis*	Pend	Palmaceae	Whole Plant	GI, RD

Key to the medical terms

ND	Neurological disorder	CD	Cardiovascular disorder
DD	Dermatological disorder	LD	Hepatic disorder
RD	Renal disorder	AA	Allergies
MSD	Muscular/Skeleton disorder	ED	Eye Problem
ENT	ENT Problem	GI	General Infection
GD	Gynecological disorder	RI	Respiratory Infections
AC	Anti cancer	AF	Antifungal
AB	Anti bacterial	AP	Antiprotozoal
AV	Antiviral	VD	Vernal disease
Dd	Diabetes	SD	Skin disorder

Table 2.2: Names and Addresses of Some Hakims and Local Persons who Filled the Questionnaire

Sr. #	Name of Hakim/ Local People	Address
1-	Hakim Ajmal Khan	Chowk Ghanta Ghar ,Multan
2-	Hakim Arshad	Chowk Ghanta ,Multan
3-	Hakim Shuja Ahmed Tahir	Block No 15 Dera Ghazi Khan
4-	Hakim Abdual Rehman	Jampur Road ,D.G.Khan
5-	Hakim Ibraheem Laghari	Block No 15 Dera Ghazi Khan
6-	Hakim Atta Muhammad	Block No 04 Dera Ghazi Khan
7-	Hakim Siraj Ahmed	Block No 04 Dera Ghazi Khan
8-	Hakim Musa Bansaar	Block No 12 Dera Ghazi Khan
9-	Prof.Mumtaz	Jampur Road, D.G.khan
10-	Mrs.Sahib Jan	Block No 16 Dera Ghazi Khan
11-	MrsZanib Qadir Buksh	Sarwar Wali Road ,Chanab ,Muzzafar Ghar
12-	Mr.Sarwer Baloch	Raki Monh, Sakih Sarwer.
13-	Mr.Sabbir Sadoozi	Raki Monh, Sakih Sarwer.
14-	Mr.Mian Muhammad	Model Town, Bahawalpur.
15-	Mrs.Safia Babi	Bahawalpur Road Lodhran.
16-	Mrs.Ameer Mahi	Jampur Road Choti.
17-	Mrs.Karim Baloch	Saki Sarwer Road DeraGhazi Khan
18-	Mr.Abdul Rahim	Gidder wali ,D.G.Khan
19-	Mr.Abdul Rehman	Gidder wali ,D.G.Khan

2.2 Establishment of Herbarium

2.2.1 Material and Method

Establishment of herbarium in Lahore College for Women University was the part of the research. For the establishment of the herbarium various materials were designed and purchased.

- Special steel and wooden cupboards
- Fixed and mobile presser
- Herbarium sheets (16 × 11 inches)
- Herbarium sheets envelopes
- Magnifying glasses
- Vasculam
- Printed registers (Printed from Toor press center)
- Chemicals (Preservative chemicals for plants)
- Various books
- Flora of Pakistan
- Reference material /printed material concerned with ethno botanical studies
- Notebook, Pencil, Paper, Polythene bags, Blotting paper, Top Sheets, knife, map (Appendix-02) and plant presser.

The standard procedure was applied for making herbarium sheets and cataloging.

2.2.2 Identification of Plants

Plant specimens were collected from different areas .They were dried and pressed properly and voucher specimen deposited in Prem Madan Herbarium (PMH) of Lahore College for Women University (LCWU), Lahore.

The selected ethno medicinal plant specimens were identified by Prof. Dr Zaheer-ud-Din, Department of Botany, Government College University, Lahore, Pakistan and Prof. Dr. Mir Ajab Khan, Department of botany, Quaid-e-Azam University, Islamabad. They were also identified through available literature [Nasir, E. and Ali, S.I., 1970].

Table 2.3: List of Collected Plants (May 2007) for Prem Madan Herbarium

S. #	Ethno medicinal Plants	Family	Voucher #
1.	*Abrus precatorius*	Papilionaceae	42
2.	*Abutilon indicum*	Malvaceae	9
3.	*Acacia ampliceps*	Mimosaceae	17
4.	*Acacia farnelsiana* L	Mimosaceae	21
5.	*Acacia holosoreceae*	Mimosaceae	20
6.	*Acacia modesta* wale	Mimosaceae	7
7.	*Acacia nilotica* (Linn)	Mimosaceae	22
8.	*Acacia Senegal* (Linn) Willd	Mimosaceae	191
9.	*Acacia stenophylla*	Mimosaceae	19
10.	*Acacia Victoria*	Mimosaceae	18
11.	*Achyranthes aspera*(Linn)	Amaranthaceae	11
12.	*Aerva javanica*	Amaranthaceae	5
13.	*Ajuga bracteosa* Wall	Apiaceae	44
14.	*Albizzia lebbek* L.Benth.in.Hook	Mimosaceae	133
15.	*Albizzia yrocera* L.Roxb	Mimosaceae	128
16.	*Alhagi maurorum* Medic	Papilionaceae	12
17.	*Allium Cepa*	Liliaceae	159
18.	*Allium sativam*	Liliaceae	150
19.	*Aloe barbadensis*	Liliaceae	45
20.	*Alternanthrus caudatus*	Amaranthaceae	46
21.	*Amaranthus virdis* (Linn)	Amaranthaceae	10
22.	*Amarantus spinosa*	Amaranthaceae	129
23.	*Anethum sowa* Linn.	Apiaceae	384
24.	*Artimisia vulgaris*	Asteraceae	97
25.	*Azaderichta indica*	Meliaceae	303
26.	*Blepheris Linaraefolia*	Acanthaceae	47
27.	*Blepheris maderaspatensis*	Acanthaceae	135
28.	*Boerhavia procumbens* .Bank ex Roxb	Nyctaginaceae	110

S. #	Ethno medicinal Plants	Family	Voucher #
29.	*Boerhavia diffusa*	Nyctaginaceae	108
30.	*Cadaba farinose*	Capparidaceae	48
31.	*Callicarpa macrophylla*	Polygonaceae	49
32.	*Calligonum polygonoides* L.	Asclepiadaceae	27
33.	*Calotropis giganta* (Willd.)R.Br	Asclepiadaceae	111
34.	*Calotropis procera* (Willd.)R.Br	Asclepiadaceae	31
35.	*Capparis decidua* (Forssk) Edgew	Capparidaceae	02
36.	*Capparis spinosa* Linn	Capparidaceae	227
37.	*Capparis zeylanica*	Capparidaceae	51
38.	*Capsicum annum*	Solanaceae	188
39.	*Cassia auriculata* L.	Caesalpiniaceae	137
40.	*Cassia angustifolia*	Caesalpiniaceae	52
41.	*Cassia italica*	Caesalpiniaceae	54
42.	*Cassia tora*	Caesalpiniaceae	53
43.	*Cenchrus ciliaris*	Poaceae	23
44.	*Chenopodium album* Linn	Chenopodiaceae	218
45.	*Chenopodium botrys*	Chenopodiaceae	57
46.	*Chenopodium karoi*	Chenopodiaceae	230
47.	*Chenopodium murale*	Chenopodiaceae	444
48.	*Citrulus colocynthis* Schred.	Cucurbitaceae	33
49.	*Cloeome brachycarpa* .Vahl	Capparidaceae	34
50.	*Coldenia procumbens*	Boraginaceae	59
51.	*Commiphora wighiti*	Boraginaceae	60
52.	*Convolvulus arvensis* (Linn)	Convolvulaceae	427
53.	*Convolvulus glomeralus*	Convolvulaceae	61
54.	*Conyza Canadensis*(linn)	Asteraceae	222
55.	*Cordea gharaf*	Asteraceae	62
56.	*Cordia dichotoma*	Asteraceae	23
57.	*Cotula hemispherica* Wall	Asteraceae	106
58.	*Crotalaria burhia* Ham.ex Benth	Resedaceae	319
59.	*Cucumis propheratum*	Curcurbitaceae	63
60.	*Cymbopogon jawaracusa*	Poaceae	140
61.	*Cynandropsis gynandra*	Poaceae	56
62.	*Cynodon dactylon*	Poaceae	201
63.	*Dalbergia sissio*	Papilionaceae	263

S. #	Ethno medicinal Plants	Family	Voucher #
64.	*Datura stramonium* L.	Solanaceae	270
65.	*Daucus Corato* L.	Apiaceae	225
66.	*Desmotachya bipinnate* (L) Stapf.(Dabh)	Poaceae	01
67.	*Dicoma tomentose*	Asteraceae	64
68.	*Dodonaea viscose*	Sapindaceae	334
69.	*Ecicostemma hyssopofolium*		66
70.	*Eclipta prostrate*	Asteraceae	143
71.	*Eichhornia crassipes*	Poaceae	224
72.	*Eleusine* sp	Poaceae	116
73.	*Eragrotis curvula*	Poaceae	16
74.	*Eucalyptus globules*	Myrtaceae	322
75.	*Euphorbia prostrate*	Euphorbiaceae	113
76.	*Fagonia indica* Burm.f.,F.I.	Zygophyllaceae	145
77.	*Ficus benghalensis* L.	Moraceae	267
78.	*Ficus religeosa* L.	Moraceae	272
79.	*Echinopis echinatus*	Asteraceae	69
80.	*Foeniculum vulgar* L.	Apiaceae	277
81.	*Frankenia pulverulenta*	Frankeniaceae	70
82.	*Fumaria indica* (Hwsskn.) Pugsley	Fumariacea	221
83.	*Gisekia pharnaceoides*	Ficoidaceae	71
84.	*Glossoema varians*	Asclepiadaceae	72
85.	*Haloxylon recurvum* (Moq) Bunge ex Boiss	Chenopodiaceae	123
86.	*Haloxylon salicornicum*	Chenopodiaceae	67
87.	*Heliotropium bacciferum*	Boraginaceae	68
88.	*Heliotropium crispum* Desf	Boraginaceae	125
89.	*Hypericum oblongifolium* Choisy	Hypericaceae	107
90.	*Hypericum perforatum* Linn	Hypericaceae	131
91.	*Iberis ammara*	Apiaceae	117
92.	*Indigofera articulate*	Papilionaceae	73
93.	*Indigofera oblongifolia* Forssk	Papilionaceae	122
94.	*Ipomoea biloba*	Convolvulaceae	74
95.	*Justicia adhatoda*	Acanthaceae	130
96.	*Lathyrus aphaca* L.	Papilionaceae	189
97.	*Launaea residifolia*	Asteraceae	75
98.	*Launea procumbenx*	Asteraceae	146

S. #	Ethno medicinal Plants	Family	Voucher #
99.	*Leptadenia pyrotechnica*	Asclepiadaceae	153
100.	*Leucaeana leucocephala*	Mimosaceae	39
101.	*Lycium edgeworthii*	Solanaceae	76
102.	*Malvastrum coromendelianum*	Malvaceae	77
103.	*Melia azedarach* L.	Meliaceae	304
104.	*Melilotus alba*	Papilionaceae	177
105.	*Mallotus philippensis*	Euphorbiaceae	193
106.	*Menipermum hirsutum*	Mimosaceae	58
107.	*Mimosa himalyana*	Mimosaceae	78
108.	*Morus alba* L.	Moraceae	197
109.	*Ocimum americanum*	Lamiaceae	141
110.	*Ocimum basilicum* L.	Lamiaceae	142
111.	*Olea ferruginea*	Oleaceae	144
112.	*Oxalis cornculata* L.	Oxalidaceae	138
113.	*Pancium antidotal*	Poaceae	25
114.	*Pavonia odorata*	Malvaceae	79
115.	*Pedalium murex*	Malvaceae	80
116.	*Peganum hermala* L.	Zygophyllaceae	08
117.	*Pennisetum perpurum*	Poaceae	32
118.	*Pennisteum lanatum*	Poaceae	126
119.	*Phoenix dactylifera*	Palmaceae	115
120.	*Phyllanthus multiflorus*	Euphorbiaceae	81
121.	*Pluchea lanceolata*	Asteraceae	139
122.	*Poa annua*	Poaceae	109
123.	*Polygonum plebejum* R.Br	Polygonaceae	152
124.	*Portulaca oleracea* L.	Portulaceae	99
125.	*Portulaca tuberose*	Portulaceae	82
126.	*Prosopis cineraria* (Linn)	Mimosaceae	132
127.	*Pteropyrum olivierii*	Polygonaceae	83
128.	*Rhazya stricta*	Apocyanacea	84
129.	*Rose indica.*L	Rosaceae	120
130.	*Rumax dentatis* L.	Polygonaceae	293
131.	*Rumax hastatus*	Polygonaceae	100
132.	*Salsola boryosma*	Chenopodiaceae	85
133.	*Salsola kali*	Chenopodiaceae	86

S. #	Ethno medicinal Plants	Family	Voucher #
134.	*Salsola vermiculata*	Chenopodiaceae	15
135.	*Salvadora persica* Dcne	Salvadoraceae	29
136.	*Salvadora oleoides* Linn	Salvadoraceae	28
137.	*Sarcococca saligna*	Buxiaceae	154
138.	*Senecio chrysanthemoides*	Asteraceae	185
139.	*Sesbania sesban*	Papilionaceae	38
140.	*Sida cordifolia*	Malvaceae	88
141.	*Solanum nigrum* Linn	Solanaceae	147
142.	*Solanum obicaula*	Solanaceae	89
143.	*Solanum surattense* Burm	Solanaceae	151
144.	*Suaeda fruticosa* Linn Forssk	Chenopodiaceae	136
145.	*Sueda mononia*	Chenopodiaceae	90
146.	*Tamarix aphylla*	Tamaricaceae	03
147.	*Tamarix diocica*	Tamaricaceae	91
148.	*Terminelia chebula*	Combretaceae	92
149.	*Tribulus longipetalus.*	Zygophyllaceae	158
150.	*Tribulus terrestris* Linn.	Zygophyllaceae	013
151.	*Trichoderma indium* Linn.	Boraginaceae	94
152.	*Trigonella occulata*	Papilionaceae	95
153.	*Vinca major* Linn	Apocyanacea	379
154.	*Withania coagulans* L.	Solanaceae	04
155.	*Withania somnifera*	Solanaceae	186
156.	*Zaleya Pentandra*(L)Jaffrey	Solanaceae	93
157.	*Zizyphus nummularia* L.	Rhamnaceae	96
158.	*Solanum xanthocarpum.*	Solanaceae	445
159.	*Thevetia* Sp	Apocyanaceae	07
160.	*Amranthus viridis*	Amarantaceae	10
161.	*Coronopus didymus*	Brassicaeae	105
162.	*Cotula hemispherica*	Asteraceae	106
163.	*Poa annua*	Poaceae	109
164.	*Pinus wallichiana*	Pinaceae	101
165.	*Pinus roxburgii*	Pinaceae	102
166.	*Impatietens walleriana*	Balsamaceae	103
167.	*Hypericumoblongifolia*	Hypericaceae	107
168.	*Alhagi maurorum*	Papilionaceae	112

S. #	Ethno medicinal Plants	Family	Voucher #
169.	*Euphorbia prostrate*	Euphorbiaceae	113
170.	*Iberis ammara*	Brassicaceae	117
171.	*Anethum graveolens*	Apiaceae	119
172.	*Ferula asafetida*	Apiaceae	121
173.	*Penisetum lanatum*	Poaceae	126
174.	*Amarantus spinosa*	Amarantaceae	129
175.	*Hypericum perforatum*	Hypericaceae	131
176.	*Suaeda fruiticosa*	Chenopodiaceae	136
177.	*Sarcococca saligna*	Buxiaceae	154
178.	*Rununnculus muricatus*	Ranunculaceae	155
179.	*Ricinus communis*	Euphorbiaceae	168
180.	*Melilotus alba*	Papillionaceae	177
181.	*Ranunnculus sceleratus*	Ranunnculaceae	178
182.	*Senecio chrysanthemoides*	Asteraceae	185
183.	*Euphorbia splendens*	Euphorbiaceae	190
184.	*Euphorbia pilulifera*	Euphorbiaceae	192
185.	*Foeniculum vulgare*	Apiaceae	195
186.	*Brassica compesteris*	Brssicaceae	200
187.	*Chenopodium album*	Chenopodiaceae	218
188.	*Eichhornia crassipes*	Pontederiaceae	224
189.	*Capparis spinosa*	Capparidaceae	227
190.	*Hibiscus rosa-sinesis*	Malvaceae	243
191.	*Murraya exotica*	Rutaceae	245
192.	*Fumaria parviflora*	Fumariaceae	257
193.	*Stellaria media*	Caryophyllaceae	260
194.	*Argemone Mexicana*	Papaveraceae	249
195.	*Ocimum basillium*	Lamiaceae	268
196.	*Urtica dioica*	Urticaceae	266
197.	*Salvia officinalis*	Lamiaceae	271
198.	*Rumex dentatus*	Polygonaceae	293
199.	*Cannabis sativa*	Cannabinaceae	297
200.	*Mazus rugosus*	Scrophulariaceae	376
201.	*Vinca major*	Apocyanaceae	379

3. Results and Discussion

3. RESULTS AND DISCUSSION

3.1 Description of Ethnomedicinal plants:

Southern Punjab due to its topographic diversity is abundant in plants especially medicinal plants used by the local population for herbal tea, applications and decoctions. No study on *Ethnomedicinal* plants was ever carried out in this region hence it was selected for this study and plants were categorized on the basis of information provided by the local population and Hakims practicing ethano-medicine (**Table 2.2**).

Results of ethno medicinal studies were based on 189 plant species belonging to 52 families collected from different areas of Southern Punjab (**Table 3.1**). Among them were 3 families of Monocots while the remaining 49 families represented the Dicots. The families well represented were Asteraceae (20), Acanthaceae (03), Cactaceae (03), Papilionaceae (09), Lamiaceae (05), Poaceae (04) Rosaceae (04), Amaranthaceae (06), Solanaceae (11), Apiaceae (06), Euphorbiaceae (05), Chenopodiaceae(08), Asclepiadaceae (07), Malvaceae (03), Zygophyllaceae (04), Portulaceae (02), Polygonaceae (04), Mimosaceae (11), Nyctaginaceae (02), Geraniaceae (02), Boraginaceae (06), Salvadoraceae (02), Caesalpiniaceae (09), Convolvulaceae (02), Liliaceae (06), Meliaceae (02), Tamaricaceae (02), Moraceae (04), Myrtaceae (02), Apocynaceae (03), Capparidaceae (04), Cactaceae (02), Palmaceae (03), Tamaricaceae (02), Brassicaceae, Rubiaceae, Sapindaceae, Violaceae, Zingiberaceae, Pedaliaceae, Lauraceae, Rhamnaceae, Resedaceae, Fumariaceae, Oxalidaceae, Ficoidaceae, Sapindaceae, Aizoaceae, Bignoniaceae, Cannabinaceae, Oleaceae, Frankeniaceae, Verbenaceae, Berberidaceae and Cucurbitaceae by one species each. For each species botanical name, family, local name, part used, method of preparation, dose administration and ailments treated are mentioned in the description.

A plant species have single or multiple medicinal uses. Among such plants *Coronopus didymus, Withania coagulans, Capparis decidua, Salsola kali, Heliotropium strigosum, Salvadora oleoides* and *Tamarix aphylla* were the most common medicinal plants used against the various ailments locally. Medicinal plants used in the local community were about 76% of the total number of species reported.

Most of the ethno medicinal plants (172) of the South Punjab were also categorized on the basis of their therapeutic profile (**Table 2.1**).

Table 3.1 Description of Plants Belonging to Southern Punjab

1- Botanical Name: *Cymbopogon jawaracusa* (Jones) SchultzMant
Vernacular Name: Bur/Khawi
Family: Poaceae
Part Used: Leaves, flowers, root.

Ethnomedicinal Applications:

- The plant is crushed in water and made into poultice which is applied over painful joints and inflamed parts of the body.
- The infusion of the plant is used as a gargle for toothache and strengthening of the gums.
- The decoction of the leaves, flowers and roots given in cough and chronic rheumatism.
- The paste of leaves used in burning sensation and in leprosy.

2- Botanical Name: *Cynodon dactylon* (Linn).Pers.
Vernacular Name: Khabbal
Family: Poaceae
Part Used: Leaves, Root

Ethnomedicinal Applications:

- A paste is made which is applied on cuts and wounds
- The roots crushed and mixed with curd are used in cases of chronic gleets.
- A cold infusion often stops bleeding from piles

3- Botanical Name: *Desmostachya bipinnata*
Vernacular Name: Drab
Family: Poaceae
Part Used: Leaves, Root

Ethnomedicinal Applications:

- The decocation of the root and leaves are used to control the vomiting and vaginal discharges.
- It was used in asthma, jaundice and high blood pressure.

4- Botanical Name: *Zaleya pentandra*
Vernacular Name: Wasanh
Family: Aizoaceae
Part Used: Leaves, Root

Ethnomedicinal Applications:

- The Juice of plant is used as a purgative and diuretic.
- The decoction of the plant is given in amenorrhea.

5- Botanical Name: *Achyranthes aspera* Linn.
Vernacular Name: Puthkand
Family: Amaranthaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The paste of leaves applied on insect bites for relief.
- It is used as diuretic, purgative and astringent by the local peoples.

6- Botanical Name: *Aerva javanica* (Burn.f.) juss.
Vernacular Name: Kanderi /Bue
Family: Amaranthaceae
Part Used: Stem, Leaves

Ethnomedicinal Applications:

- The decoction of the root is used to relieve skin infection of animals.
- The poultice is made from the leaves and is used to relieve pain and inflammation.
- The decoction of the plant is given to cattle to expel the abdominal worms.

7- Botanical Name: *Amaranthus virdis* Linn.
Vernacular Name: Choli
Family: Amaranthaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The plant is used as a laxative for chronic constipation.
- It is used by the local people in anemic condition.

8- Botanical Name: *Calotropis procera* Linn
Vernacular Name: AK
Family: Asclepiadaceae
Part Used: Whole plant, Latex

Ethnomedicinal Applications:

- The dry plant is used for asthma, cough, indigestion and joint pain.
- The stem is force fully administered orally in cattles against colic and indigestion.
- The fresh yellow leaves are slightly warm over fire and juice extracted. This juice is poured into ears for the relief of pain.
- The smoke of the plant keeps mosquitoes away from the house.
- The latex applied to the necks of bulls for regeneration of hairs.
- The latex applied on the skin to remove the warts.

9- Botanical Name: *Calotropis giganta*
Vernacular Name: Wadha AK
Family: Asclepiadaceae
Part Used: Whole plant, Latex

Ethnomedicinal Applications:

- All parts of the plant dried and taken with milk act as a good tonic, expectorant, and antihelminthic.
- The leaves are applied to paralyzed parts, painful joints, scabies, and ringworm of the scar.
- The root, bark, and latex of this plant are used in medicine for their purgative properties.
- The latex is commonly used for skin diseases.

10- Botanical Name: *Leptadenia pyrotechnica*
Vernacular Name: Khippa
Family: Asclepiadaceae
Part Used: Whole plant, Latex

Ethnomedicinal Applications:

- The watery juice is externally applied to ring worm for relief.
- The plant is boiled in water and given to cattles after parturition for the expulsion of placenta.
- Young twigs are grounded and poultice is made which is applied externally to relieve pain and inflammation.

11- Botanical Name: *Conyza canadensis*
Vernacular Name: Giddar buti
Family: Asteraceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The plant boiled in water (decoction) is used to relieve sore throat.

12- Botanical Name: *Launea procumben*
Vernacular Name:
Family: Asteraceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The plant is crushed in water and given in painful urination and gonorrhea.

13- Botanical Name: *Pluchea lanceolata*
Vernacular Name: Phar buti
Family: Asteraceae
Part Used: Whole plant
Ethnomedicinal Applications:

- Whole plant is crushed in water and used as a cooling agent.
- The leaves are astringent.
- Grounded leaves are applied as paste by women on hair for keeping them healthy.

14- Botanical Name: *Heliotropium crispum* Desf.
Vernacular Name: Karsan
Family: Boraginaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- An infusion made from plant is used in skin disorder.

15- Botanical Name: *Trichodesma indicum* (L.) R.Br, Prodr.
Local Name: Gaozaban
Family: Boraginaceae
Part Used: Leaves

Ethnomedicinal Applications:

- The plant is beneficial in the disease of the eye.
- A cold infusion of the leaves helps in the expulsion of the dead fetus.
- The root is powdered and made into a paste, is applied to reduce swellings, particularly of the joints.
- The plant is considered as a cure for fever.
- The poultice made up of leaves is useful against infection and snake bite.
- The infusion of leaves and flowers is used as diuretic and cooling agent.
- Poultice made up of leaves is applied over wounds for relief.

16- Botanical Name: *Cassia italica* (Mill.)Lam.
Vernacular Name: Ghorawal
Family: Caesalpiniaceae
Part Used: Leave

Ethnomedicinal Applications:

- Leaves boiled in tea are given for body pain.
- For lactagogue given to cattles.

17- Botanical Name: *Capparis decidua* (Forsk) Edgew
Vernacular Name: Karir
Family: Capparidaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The ash of the bark mixed in seasamum oil and mustard (Sarsoon) oil is externally applied in muscular injuries and on ulcer.
- The coal of the old plant mixed with honey is internally used for asthma, cough and chronic pain in joints.
- The young branches are chewed to relieve toothache.
- The tender leaves wrapped in wet cloth and put in fire of cow dung, when upper part of cloth burns, then these warm leaves are put on the painful joints and wrapped tightly with the help of any bandage. The colour of the leaves turns yellow on next day. Repeat it daily until the leaves retain their green colour.
- The decoction of the aerial parts is given to cattles for stomach complaints.
- Flower, bud and unripe fruits are boiled and cooked as vegetable for rheumatic pains
- The unripe fruits are made into pickles and used as a natural appetizer, also in liver and stomach complaints and in rheumatism.
- The whole plant is used for anemia.
- The decoction used as expectorant and febrifuge.

18- Botanical Name: *Cloeome brachycarpa* Linn.
Vernacular Name: Dhanar khathoori
Family: Capparidaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The whole plant is boiled with sesamum oil, which is applied over joint for pain and inflammation.

19- Botanical Name: *Chenopodium album* Linn.
Vernacular Name: Chulli
Family: Chenopodiaceae
Part Used: Whole plant

Ethnomedicinal Applications

- Leaves are cooked with *Lens culinaris* (Masur) used as vegetable and are regarded as mild laxative.
- Its juice is given in spleen disorder.
- Used in hepatic disorder.

20- Botanical Name *Haloxylon recuvrum* (Sensu.Bunge) L.
Vernacular Name: Zeekhann/Khar
Family Chenopodiaceae
Part Used: Whole plant

Ethnomedicinal Applications

- Clothes are washed with ash.
- Forage for camel in stomach disorder.
- The decocation of the plant is used to remove the kidney stone.

21- Botanical Name *Salsola baryosma* Schult.
Vernacular Name Looran Lani
Family Chenopodiaceae
Part Used Whole plant

Ethnomedicinal Applications

- The ash of the plant is used for washing cloth.

22- Botanical Name *Saueda fruticosa*
Vernacular Name Koori lani
Family Chenopodiaceae
Part Used Whole plant

Ethnomedicinal Applications

- Ash of the plant is used for washing clothes.
- The infusion of leaves is used as an emetic.
- Poultice made from leaves is applied in ophthalmic for relief.

23- Botanical Name *Convolvulus arvensis*
Vernacular Name Naaro
Family Convolvulaceae
Part Used Whole plant

Ethnomedicinal Applications

- The powder of the plant is given in chronic constipation.

24- Botanical Name *Alhagi maurorum* Medic.
Vernacular Name Jowan
Family Papilionaceae
Part Used Whole plant

Ethnomedicinal Applications

- Bath taken from the decoction of this plant is effective against skin eruption.

25- Botanical Name: *Cordia dichotoma* Forster.
Vernacular Name Lasora
Family Boraginaceae
Part Used Tree

Ethnomedicinal Applications

- It is used as febrifuge and expectorant.

26- Botanical Name *Crotalaria burhia*
Vernacular Name Chagg
Family Resedaceae
Part Used Root

Ethnomedicinal Applications

- Roots soaked in water in a new earthen pot for whole night, the following morning the roots are crushed and juice/infusion obtained is given three times a day to stop bleeding from nose.
- The infusion acts as styptic and cooling.

27- Botanical Name: *Indigofera oblongifolia* Forssk.
Vernacular Name: Jhil
Family: Papilionaceae
Part Used: Branches, Twigs

Ethnomedicinal Applications

- Twigs are used for brushing teeth.

28- Botanical Name: *Fumaria indica* (Hausskn.)Pugsely
Vernacular Name: Shahtroo
Family: Fumariaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The dried plant along with *Piper nigrum* is given for the relief of ague.
- The decoction of the plant is prescribed in ailment due to blood disorders and generally acts as an antipyretic and blood purifier.
- The infusion of plant is externally applied in skin diseases.

29- Botanical Name: *Abutilon indicum* Linn.
Vernacular Name: Pattear
Family: Malvaceae
Part Used: Leaves

Ethnomedicinal Applications

- Poultice is made from the leaves, which is used to treat boils.
- It acts as analgesic, to cure diarrhea, bleeding piles and toothache.

30- Botanical Name: *Acacia senegal*
Vernacular Name: Angrezi Kiker
Family: Mimosaceae
Part Used: Leaves, gum, branches.

Ethnomedicinal Applications

- The Poultice of leaves is applied on ring worm.
- The sticks are used for toothache.

31- Botanical Name: *Prosopis cineraria* (Linn)
Vernacular Name: Babul/Jal
Family: Mimosaceae
Part Used: Pod

Ethnomedicinal Applications

- Unripe pods are used as vegetables.

32- Botanical Name *Boerhavia diffusa*
Vernacular Name: Dakhari
Family: Nyctaginaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The infusion of the plant used for anemia, as expectorant and as febrifuge.
- The decocation of the plant used in jaundice.

33- Botanical Name: *Boerhavia procumbens* Roxb.
Vernacular Name: Dakhri / Satti
Family Nyctaginaceae
Part Used: Root

Ethnomedicinal Applications

- The decoction of the root is prescribed to treat amenorrhea and painful periods/menses.
- The powder of root with honey is given in cough and asthma.
- Roots are crushed and mixed with milk, and then a bandage is made which is applied for external ulcer

34- Botanical Name: *Polygonum plebejum*
Vernacular Name: Kheera wal
Family: Polygonaceae
Part Used: Root, Whole plant

Ethnomedicinal Applications

- Powder of the root is used in diarrhea.
- Decoction of the plant is given in pneumonia.

35- Botanical Name: *Ziziphus nummularia*
Vernacular Name: Desi Baeer
Family: Rhamnaceae
Part Used: Leaves, Fruit

Ethnomedicinal Applications

- The dried fruit is soaked in water at night with *Ficus carica* L. and this infusion is given for jaundice.

- Used in scabies and constipation.
- The infusion of the leaves used as hair cleaner.

36- Botanical Name: *Salvadora oleoides* Decne.
Vernacular Name: Peelu /Jal (Yellow seeded)
Family: Salvadoraceae
Part Used: Stem, Root, Oil, Seed, Leaves, Bark

Ethnomedicinal Applications

- The decoction of root and stem is used in fever and to regulate the menstrual periods.
- The decoction of the leaves is given in cough.
- Fruit is chewed as a carminative and purgative and is prescribed in rheumatism.
- The seed, oil and bark specially used in cough and in rheumatism.

37- Botanical Name: *Salvadora persica* Linn
Vernacular Name: Peelu (Red seeded)
Family: Salvadoraceae
Part Used: Stem, Root, Oil, Seed, Leaves, Bark

Ethnomedicinal Applications

- The decoction of root and stem is used in fever and to regulate the menstrual periods.
- The decoction of the leaves is given in cough.
- Fruit is chewed as a carminative and purgative and is prescribed in rheumatism.
- The oil of seed and bark specially used in cough and in rheumatism.
- The fruit is sweet; aphrodisiac; stomachic; improves appetite; useful in biliousness.
- The oil is used in digestion disorders.
- The leaves are bitter and used as astringent

38- Botanical Name: *Solanum nigrum* Linn
Vernacular Name: Mako
Family: Solanaceae
Part Used: Leaves, Fruits and leaves.

Ethnomedicinal Applications

- The juice of the leaves is dropped in eyes for improving the eye sight.
- Leaves are chewed for the treatment of phthisis.
- The leaves are cooked as vegetable for jaundice, enlargement of spleen and dropsy.
- Juice of the leaves is used as gargle for sore throat and taken for laryngitis.
- Fruit is given for relief in dropsy.
- Fruit is used in liver disease, diabetes, rheumatism, diarrhea and constipation.
- Fruit is also used in skin disorder and heart disease.
- The juice of fruit used as expectorant and sedative.
- The boiled juice of root, leaves and fruit are used as carminative, analgesic and febrifuge.

39- Botanical Name: *Solanum surattense* Burm.f.
Vernacular Name: Kanderi, Mahukeri
Family: Solanaceae
Part Used: Leaves, Fruits and leaves.

Ethnomedicinal Applications

- The powder of the dry fruits is given for relief in cough, asthma and rheumatic pain in recommended doses.
- The whole plant is expectorant, stomachic and diuretic. It is used in fever and chest pain.
- Used against cough bronchitis, respiratory trouble abdominal pain and for blood purification.

40- Botanical Name: *Withania somnifera* (L) Dunal.
Vernacular Name: Ashwaghanda/ Kora paneer
Family: Solanaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The powder of the root is given with milk in spermatorehoea, leucorrhoea and as nerving tonic
- It is also given in rheumatic pain, loss of memory and general ability.
- Fresh roots are grinded and applied over ulcer and painful swellings.
- A paste made up of root in milk is locally applied over breast for beauty.
- A poultice made up of leaves is also applied to sub side tumors and tubercular glands.
- The leaves are applied to tumors.
- The roots are regarded as useful in rheumatism and dyspepsia.

41- Botanical Name: *Fagonia indica* Burm.f.,I.
Vernacular Name: Damaho
Family: Zygophyllaceac
Part Used: Whole plant

Ethnomedicinal Applications

- The decoction of the whole plant mixed with sugar is administered for the control of periods/menses.
- The decoction of the plant is used as bath to check (some) skin diseases.
- The powder made from the whole plant is dusted in boils and skin eruptions for relief.

42- Botanical Name: *Tribulus longipetalus* (Presl) Hadidi.
Vernacular Name: Bakhro Bhust
Family: Zygophyllaceae
Part Used: Fruit / Whole plant

Ethnomedicinal Applications

- The fruit grounded in water is regarded as a cooling agent. It is given in painful urination.
- The whole plant is crushed in water or milk and given for the treatment of spermatorrhoea /impotence.

43- Botanical Name: *Tribulus terrestris* linn.
Vernacular Name: Bakhro-Bhust
Family: Zygophyllaceae
Part Used: Leaves

Ethnomedicinal Applications

- It has cooling effect. It is diuretic, demulcent astringent.
- It is used to cure heart diseases.
- For chest Pain: The leaf juice 100 ml per day taken internally for three days.
- It is used to prevent bachache.

44- Botanical Name: *Acacia modesta* Wale
Vernacular Name: Phulai
Family: Mimosaceac
Part Used: Bark

Ethnomedicinal Applications

- The extract from the bark is used as tonic and stimulant.
- It is used as sexual tonic.
- The infusion of the bark is given in pastorate lumbago.

45- Botanical Name: *Morus alba* Linn.
Vernacular Name: Shahtoot
Family: Moraceae
Part Used: Fruits, Leaves, Seed

Ethnomedicinal Applications

- Fruits are eaten both fresh and dry, they are said to be laxative.
- Leaves are used as emolliating agent.
- It acts as a refrigerant and anthelmintic.
- Its seeds are used as a remedy for sore throat.

46- Botanical Name: *Dalbergia sissio*
Vernacular Name: Tahli
Family: Papillionaceae
Part Used: Leaves,

Ethnomedicinal Applications

- The leaves in combination with seeds are said to be used in stomach disorder.

47- Botanical Name: *Lathyrus aphaca* L.
Vernacular Name: Jangli mater
Family: Papilionaceae
Part used: Seed

Ethnomedicinal Applications

- Ripened seeds are said to be narcotic and have soothing effect.

48- Botanical Name: *Rosa indica* L
Vernacular Name: Gulab
Family: Rosaceae
Part used: Petals

Ethnomedicinal Applications

- Gulkhand is made by mixing petals with sugar for letting some days which is given in stomach disorders and in lingcod fever also.
- For eye: Dip the petal in water for whole night and in morning put that water in eye give cooling effect.

- Arq Gulab given to the new born babies for stomach disorder.

49- Botanical Name *Datura stramonium* L.
Vernacular Name: Dahtoora
Family: Solanaceae
Part used: Seeds and leaves

Ethnomedicinal Applications

- The seeds have an acrid and bitter taste used as tonic, febrifuge.
- The leaves after roasting used to relive the body pain.

50- Botanical Name *Daucus carota* Linn
Vernacular Name: Gajar
Family: Apiaceae
Part Used: Stem, root, carrot

Ethnomedicinal Applications

- The rhizome used as appetite, carminative; cures leprosy, piles, pains, burning sensation and tumors
- The use of rhizome is good for inflammation, asthma, good for liver and used in bronchitis.
- Seeds are given in uterine pain.
- A poultice made of the roots is used to correct the discharge from ill-conditioned scores.

51- Botanical Name: *Foeniculum vulgare* Linn.
Vernacular Name: sonf
Family: Apiaceae
Part used: Seed

Ethnomedicinal Applications

- Fresh leaves are used for stomachic.
- It is used for indigestion and gastritis.
- Seeds are used for strengthening the eye sight.
- Oil of seed is used for lengthen the hairs.

52- Botanical Name: *Allium sativam*
Vernacular Name: Thoom
Family: Liliaceae
Part used: Leaves, Bulb

Ethnomedicinal Applications

- The bulbs are used in cardiac diseases, hysteria and flatulence.
- It is used as very effective antiseptic.
- It is used for hypertension, leprosy and in the respiratory disease.
- The infusion is used for whooping cough and cold for children.
- Garlic mixed with sesame oil is used in epilepsy.
- Useful in disease of the eye and the heart, low fevers, inflammation piles and in leucoderma.

53- Botanical Name: *Allium cepa*
Vernacular Name: Wasal
Family: Liliaceae
Part used: Bulb

Ethnomedicinal Applications

- It is used for gastric problems.
- Burn the whole bulb the in fire, the upper burned leaves remove, while the inner hot leaves with turmeric are helpful in ejecting the spine from the skin.
- The infusion of the seeds is useful in caries of the teeth and urinary discharge.
- Local women's used the onion tea, will often give relieve to the sleepless and irritable children when opium and other narcotics have failed.
- The centre portion of a bulb heated and put the juice in to the ear for earache.
- As an external application onions are used in scorpion-stings and to allay irritation in skin disease.

54- Botanical Name: *Melia azedarach* L.
Vernacular Name: Bakain
Family: Meliaceae
Part Used: Young branches, leaves and fruits

Ethnomedicinal Applications

- Young branches, leaves and fruits are used as carminative for cattle.
- The juice of the leaves is used as anthelmintic and seeds in reheutism.

55- Botanical Name: *Accacia niloctica* Wall
Vernacular Name: Kikar
Family: Mimosaceae
Part Used: Leaves fruit, gum

Ethnomedicinal Applications

- The fruit is useful for constipation, diarrhea, dysentery and throat diseases.
- The gum and fruit are used for restorative lumbago.

56- Botanical Name: *Tamarix diocica*
Vernacular Name: Lei
Family: Tamaricaceae
Part Used: Leaves, Branches

Ethnomedicinal Applications

- The decocation used for dysentery and old chronic diarrhea.
- The decocation used as gargles for gums.
- The steam of cooled leaves is used for piles and ulcer.

57- Botanical Name: *Albizzia lebbek* Linn.
Vernacular Name: Kala sirin
Family: Mimosaceae
Part Used: Bark, flower, seeds, pods

Ethnomedicinal Applications

- Bark and seeds are used as restorative in piles, diarrhea, and dysentery.
- Flowers are used in skin disease.

58- Botanical Name: *Albizzia yrocera* Linn.
Vernacular Name: Chhita sirin
Family: Mimosaceae
Part Used: Bark, flowers, seeds

Ethnomedicinal Applications

- The infusion of bark and seeds are used as restorative diarrhea and dysentery.
- Flowers are used in skin disease.

59- Botanical Name: *Ficus religiosa* Linn.
Vernacular Name: Peppal
Family: Moraceae
Part Used: Bark of tree, fruits, seeds

Ethnomedicinal Applications

- Bark of tree and fruits are used against asthma, weakness of urinary bladder and constipation.
- Decoction of bark is used for vomiting.

60- Botanical Name: *Ficus benghalensis* L.
Vernacular Name: Bohr
Family: Moraceae
Part Used: Aerial Parts, Latex

Ethnomedicinal Applications

- Aerial roots are used to treat diarrhea.
- It is used to control rise of blood sugar among diabetic patient.
- The paste of the latex mixed with honey used for emission of piles.

61- Botanical Name: *Eucalyptus globulus*
Vernacular Name: Sufaida
Family: Myrtaceae
Part Used: Seeds, oil of leaves.

Ethnomedicinal Applications

- It is used as antiseptic, antibacterial and diuretic.
- It is used in cold and cough for the remedies throat, lozenges, malaria and toothache.

62- Botanical Name: *Psidium guajava* Linn.
Vernacular Name: Amrood
Family: Myrtaceae
Part Used: Fruit and root bark

Ethnomedicinal Applications

- The fruit is used as laxative.
- The bark of the root is given in the diarrhea of children.

63- Botanical Name: *Oxalis corniculala* Linn.
Vernacular Name: Khatti mithi booti
Family: Oxalideaceae
Part Used: Whole plant (Shoot)

Ethnomedicinal Applications

- Decoction of the roots is given for warms.
- The decocation used in scurvy.

64- Botanical Name: *Rumex dentatus* Linn.
Vernacular Name: Jangli Palak
Family: Polygonaceace
Part Used: Whole plant

Ethnomedicinal Applications

- It is used to cure inflammation of urinary system.
- It is used for bladder cleaner and removal of kidney stone.

65- Botanical Name: *Portulaca oleracea* L.
Vernacular Name: Kulfa
Family: Portulaceae
Part Used: Aerial Part of Plant

Ethnomedicinal Applications

- Hot water extract of dried aerial part is taken orally as diuretic.
- Water extract of plant is taken for asthma.

66- Botanical Name: *Capsicum annuum* Linn.
Vernacular Name: Mirch
Family: Solanaceae
Part Used: Fruit and seed
Ethnomedicinal Applications

- It acts as a stimulant and antiseptic.
- It is used as blood circulator and stimulant for cattle.
- Also used as condiments and spices.
- *Cilius medica L, Cleome gynandra L and Capsicum frutescens L*. leaves mixed in the form of paste and applied on the effected area for toothache.

67- Botanical Name: *Datura innoxia* Mill.
Vernacular Name: Batoora
Family: Solanaceae
Part Used: Whole plants

Ethnomedicinal Applications

- Seeds are very poisonous, antipyretic and narcotic.
- Leaves are applied to lumbago and swelling of limbs.
- It is also helpful in headache, toothache and epilepsy.
- Over dose of seeds causes vomiting, coma and even death.
- Fruits are sedative and intoxicating.

68 - Botanical Name: *Withania coagulans* (Stocks) Dunal.
Vernacular Name: Paneer dodi
Family: Solanceae
Part Used: Whole plant, Fruits

Ethnomedicinal Applications

- It is used for digestive disorders, gastritis and blood purification.
- Seeds are used for diabetes.
- Local women soak the seed in the water overnight and drink the water early in the morning to reduce the obesity.

69 - Botanical Name: *Viola stacksii* L.
Vernacular Name: Banafsha
Family: Violaceae
Part Used: Whole plant

Ethnomedicinal Applications

- Used in cold, cough and fever.

70- Botanical Name: *Peganum hermala* L.
Vernacular Name: Hermal
Family: Zygophyllaceae
Part Used: Whole plant

Ethnomedicinal Applications

- Plant used as insecticidal and is used as brain tonic.
- The decocation of the plant used as aphrodisiac, emmangague hypotonic and antispasmodic.

71- Botanical Name: *Aerva lanata* (L) Juss.
Vernacular Name: Kooripoo
Family: Amaranthaceae
Part Used: Leaves

Ethnomedicinal Applications

- Leaf juice is applied externally on bitten area of cobra bite.

72 - Botanical Name: *Azadirachta indica*
Vernacular Name: Neem
Family : Meliaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The leaves are boiled in mustard oil for half hour .This oil is applied externally for chicken pox in children, on burned skin and for achene on the face.
- Decoction of leaves is antiseptic and used for ulcer and wounds
- Seed oil is used as an antiseptic.
- Dry flowers used as tonic.
- The gum bark leaves, and seeds are used in snake bite.

73- Botanical Name: *Curcuma domestica*
Vernacular Name: Halhard
Family: Zingiberaceae
Part used: Rhizome

Ethnomedicinal Applications

- 50gm of Rhizome part mixed with small amount of lime applied on clean cloth and allowed to wrap upon fire. Small amount of this ash is mixed with 25 ml of water and taken internally, three times for chicken pox in children.

- Mix the powder of rhizome with milk to relive the body pain.

74- Botanical Name: *Blepheris maderaspatensis* L.
Vernacular Name: Vaychivettu thalai
Family Acanthaceae
Part Used: Leaves:

Ethnomedicinal Applications

- Handful of leaves, mixed with onion bulb, made into paste and applied externally for cut and wounds.

75- Botanical Name: *Cadaba fruiticosa* L. Druce
Vernacular Name: Vizliin
Family: Capparidaceae
Part Used: Leaves

Ethnomedicinal Applications

- Used for snake bite and fracture.

- Leaf juice boiled in caster oil applied for snake bite and fracture.

76- Botanical Name: *Cassia auriculata* L.
Vernacular Name: Avarai
Family: Caesalpiniaceae
Part Used: Flowers and leaves

Ethnomedicinal Applications

- Handful of leaves made into juice, is taken internally to reduce body heat.

- Paste of flower with small amount of lime used for cuts.

77 - Botanical Name: *Eclipta prostrata*
Vernacular Name: Karichalai Bhangea
Family: Asteraceae
Part Used: Leaves, Shoots

Ethnomedicinal Applications

- The juice of leaf is taken internally for jaundice.
- The paste of leaf applied on the effected area for toothache.
- It is used as anti asthmatic

78- Botanical Name: *Ocimum basilicum* Linn.
Vernacular Name: Naywee thulasi
Family: Lamiaceae
Part Used: Flower, Leaves

Ethnomedicinal Applications

- The infusion of the leaves is used to reduce fits.

79- Botanical Name: *Ocimum americanum* Linn.
Vernacular Name Kali niazboo
Family: Lamiaceae
Part used: Young shoot

Ethnomedicinal Applications

- Paste applied on infected skin.

80- Botanical Name: *Phyllanthus amarus*
Vernacular Name: Kilanelli
Family: Euphorbiaceae
Part Used: Leaves

Ethnomedicinal Applications

- The leaf paste mixed with root of *Calotropis procera* (AK) in water, 200 ml per day for three days, taken internally to cure jaundice.

81- Botanical Name: *Acacia farhesiana*
Vernacular Name: Phali
Family: Mimosaceae
Part Used: Gum

Ethnomedicinal Applications

- It is used for the restorative lumbago.

82- Botanical Name: *Adhatoda vasica*
Vernacular Name: Baikar
Family: Acanthaceae
Part Used: Whole plant

Ethnomedicinal Applications

- It is useful in bronchitis, leprosy, blood impurities, heart troubles, thirst, asthma, fever, vomiting, leucoderma, jaundice, tumors, and diseases of the mouth.
- The root facilitates the expulsion of the fetus.
- The leaves and the root of this plant are considered a very efficacious remedy for all sorts of cough, being administered along with ginger.
- The leaves are also used for rheumatism.

83- Botanical Name: *Ajuga bracteosa* Wall.
Vernacular Name; Darkan booti
Family: Lamiaceae
Parts Used: Shoot

Ethnomedicinal Applications

- The decocation of root used as blood purifier, in hypertension and epilepsy
- Used as astringent given in the treatment of fevers.
- Local women are used to kill lice and are regarded as depurative.

84- Botanical Name: *Allium ascalonicum*
Vernacular Name Gandanaa
Family Liliaceae
Part used: Whole plant

Ethnomedicinal Applications

- Used for aphrodisiac

85- Botanical Name: *Anisomeles indica* Linn.
Vernacular Name:
Family: Lamiaceae
Part Used:

Ethnomedicinal Applications

- Used as astringent.
- Used as carminative.

86- Botanical Name: *Asparagus adscendens* Roxb
Vernacular Name: Khairuwa
Family: Liliaceae
Part Used: Root and tubers

Ethnomedicinal Applications

- Used in diarrhea, dysentery and glactogogue
- The tuberous roots are used as demulcent tonic.

87- Botanical Name: *Asparagus capitatus*
Vernacular Name: Dusa
Family: Liliaceae
Part Used: Roots

Ethnomedicinal Applications

- Used as tonic and in dysentery and aphrodisiac

88 - Botanical Name: *Berberis lycium*
Vernacular Name: Kashmal
Family: Berberidaceae
Part Used: Root, Bark

Ethnomedicinal Applications

- Root used in spleen troubles
- Used as tonic, intestinal astringent; good for cough, chest and throat troubles, piles and monorehagia.
- Useful in chronic diarrhea.
- The root is highly esteemed as a febrifuge and as a local application in eye diseases.

89 - Botanical Name: *Buxus papillosa*
Vernacular Name: Pepper
Family: Euphorbiaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- The whole is used as diaphoretic, purgative and ant rheumatic

90- Botanical Name: *Cannabis sativa* Linn.
Vernacular Name: Bhang
Family: Cannabinaceae
Part used: Flower and leaves

Ethnomedicinal Applications

- Leaves are analgesic, narcotic, anodyne and antispasmodic

91- Botanical Name: *Capparis spinosa* Linn.
Vernacular Name: Kakri/Kobra
Family: Capparidaceae
Part used: Whole plant

Ethnomedicinal Applications

- Condiment, edible fruit analgesic, tonic, expectorant.
- The fruit is used as vegetable.

92- Botanical Name: *Caralluma edulis*
Vernacular Name: Pippu
Family: Asclepiadaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The whole plant is used as anthelmintic.
- It used for leprosy.
- The decocation causes constipation.

93- Botanical Name: *Caralluma tuberculata*
Vernacular Name: Choungan
Family: Asclepediaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- A costly vegetable, tonic, febrifuge and carminative.

94- Botanical Name: *Carissa opaca*
Vernacular Name: Garanda
Family: Apocyanacea
Part Used: Roots

Ethnomedicinal Applications

- Used in veterinary as purgative

95- Botanical Name: *Chenopodium ambrosioides* Linn.
Vernacular Name: Lunak
Family: Chenopodiaceae
Part Used: Whole plant

Ethnomedicinal Applications

- The infusion of the plant used as wormicide and carminative.

96- Botanical Name: *Chenopodium botrys* Linn.
Vernacular Name: Jausag
Family: Chenopodiaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- It is used in catarrh and hum oral asthma.

97- Botanical Name: *Chrozophora tinctoria*
Vernacular Name: Nilkhanti
Family Euphorbiaceae
Part used: Whole Plant

Ethnomedicinal Applications

- Emetic and cathartic

98- Botanical Name: *Cichorium intyblus*
Vernacular Name: Karni
Family: Asteraceae
Part used: Whole Plant

Ethnomedicinal Applications

- Tonic, pot herb and febrifuge.

99- Botanical Name: *Datura metel* Linn.
Vernacular Name: Datoora
Family: Solanaceae
Part Used: Seeds, leaves

Ethnomedicinal Applications

- Analgesic, antiseptic and expectorant

100- Botanical Name: *Desmodium gangeticum* Linn.
Vernacular Name: Salpan
Family: Papilionaceae
Part Used: Roots

Ethnomedicinal Applications

- Their roots are used for asthma and cough.
- It is diuretic and tonic.

101- Botanical Name: *Dicleptera roxburghiana*
Vernacular Name: Kirich
Family: Acanthaceae
Part Used: Shoots

Ethnomedicinal Applications

- It is used as tonic.

102- Botanical Name: *Dioscorea deltoids*
Vernacular Name: Kanis
Family: Liliaceae
Part Used: Rhizome

Ethnomedicinal Applications

- The decocation is given in fish poison.
- Paste of rhizome is applied to external ulcer.

103- Botanical Name: *Dodonaea viscose* Linn.
Vernacular Name: Sanath
Family: Sapindaceae
Part Used: Leaves and bark

Ethnomedicinal Applications

- Fish poison, topical anti rheumatic

104- Botanical Name: *Ehretia obtusifolia* Hochst exDc.
Vernacular Name: Chamror
Family: Boraginaceae
Part used: Root

Ethnomedicinal Applications

- Used in vernal disease.

105- Botanical Name: *Ficus racemosa*
Vernacular Name: Gularoomul
Family: Moraceae
Part used: Bark, fruit

Ethnomedicinal Applications

- It is edible fruit used as carminative.
- The bark and roots are used as astringent

106- Botanical Name: *Gallium aparine* Linn.
Vernacular Name: Banosha
Family: Rubiaceae
Part used: Sape

Ethnomedicinal Applications

- It is used as diuretic.

107- Botanical Name: *Geranium ocellalum* Canb.
Vernacular Name: Bhanda
Family: Geraniaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- Roots are diuretic and astringent

108- Botanical Name: *Geranium rotunifolium* Linn
Vernacular Name: Bhanda
Family: Gerniaceae
Part Used: Roots

Ethnomedicinal Applications

- Root is diuretic and astringent.

109- Botanical Name: *Hyoscyamus insanus* Stocks.
Vernacular Name: Dewana bhang
Family: Solanaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- The plant used as anti asthmatic.
- It is taken as laxative.
- Used as demulcent.

110- Botanical Name: *Jasminum officinale*
Vernacular Name: Chambely
Family: Oleaceae
Part Used: Young shoots

Ethnomedicinal Applications

- Used for oral candidacies.
- The garland of the flower put in the neck of the child suffered from measles.
- The root is used for curing the ringworm infection.
- Used in heart disease also.

111- Botanical Name: *Lallemantic royleana* Linn.
Vernacular Name: Tukhumbalanga
Family: Lamiaceae
Part used: Seeds

Ethnomedicinal Applications

- Seed are sedative.
- Causes the constipation.

112- Botanical Name: *Litsea monopetala*
Vernacular Name: Maida lakri
Family: Lauraceae
Part Used: Bark

Ethnomedicinal Applications

- Applied to bone fractures, diarrhea and astringent.

113- Botanical Name: *Malva neglecta*
Vernacular Name: Khubasi
Family: Malvaceae
Part Used: Whole Plant

Ethnomedicinal Applications

- Used in Piles.
- Used as expectorant.
- Causes the constipation.

114- Botanical Name: *Malvastrum coromendelianum*
Vernacular Name: Jhar
Family: Malvaceae
Part Used: Leaves and Flower

Ethnomedicinal Applications

- The plant is considered emollient.
- The leaves are applied to inflamed sores and wounds and cooling and healing salve.
- The flowers are given as a pectoral and diaphoretic.

115- Botanical Name: *Martynia annua*
Vernacular Name: Hathjoy
Family: Pedaliaceae
Part Used: Shoot and Fruit

Ethnomedicinal Applications

- Laxative, throat sore and epilepsy

116- Botanical Name: *Cassia fistula* Linn
Vernacular Name: Amaltas
Family: Caesalpiniaceae
Part Used: Root, Leaves, buds, flowers, seeds.

Ethnomedicinal Applications

- The root is useful in skin diseases.
- It is generally given as a tonic.
- It has been found to act as a strong purgative. The leaves lessen inflammation. The flowers are purgative. The seeds are sweetish oily, laxative.
- Carminative, improve the appetite, cure biliousness. Every part of the plant is equally use in the treatment of either snake-bite or scorpion-sting.
- Fruit ash is used to cure whooping cough.

117- Botanical Name: *Cassia occidentalis*
Vernacular Name: Kasunda
Family: Caesalpiniaceae
Part Used: Root, leaves, seeds.

Ethnomedicinal Applications

- The root is useful in ringworm.
- The leaves are tasty; cure cough; asthma; good for sore throat.
- The whole plant is purgative, tonic and febrifuge.
- The seeds and leaves are used in skin diseases.
- A decocation of powdered seed was given in 1:10 with milk as mild purgative.
- The seeds and leaves are used as anti periodic.
- The roasted seeds are used as blood tonic.

118- Botanical Name: *Cassia obtusifolia* Linn
Vernacular Name: Chakunda
Family: Caesalpiniaceae
Part Used: Root and Leaves

Ethnomedicinal Applications

- The root is useful for skin diseases, tuberculosis and ringworm.
- The leaves are bitter with a sharp taste and same flavour; digestible; laxative; cure biliousness; bronchitis; asthma; skin disease; useful in the diseases of heart and in ringworm.
- The flowers are used in urinary discharges and in diabetes
- The fruit is useful in vomiting and thirst.
- The seed is alexipharmic.

119- Botanical Name: *Cassia angustifolia*
Vernacular Name: Caesalpiniaceae
Family: Senna
Part Used: Leaves, Seeds

Ethnomedicinal Applications

- The seeds and leaves are useful in constipation.
- Used in liver complaints and abdominal troubles and dyspepsia.
- The seed are used in anemia and leprosy
- It given in the symptoms of tumors.

120- Botanical Name: *Cassia tora* Linn
Vernacular Name: Pamad
Family: Caesalpiniaceae
Part Used: Leaves, seeds.

Ethnomedicinal Applications

- The leaves are used as a laxative in the form of a decoction.
- Both leaves and seeds constitute are used for the remedy of skin diseases, chiefly for ringworm and itch.

121- Botanical Name: *Tamarindus indica* Linn
Vernacular Name: Imali
Family: Caesalpiniaceae
Part Used: Bark, Leaves, flowers.

Ethnomedicinal Applications

- The bark in used topically for loss of sensation in paralysis.
- The ash of the bark is given for urinary discharge and gonorrhea.
- The leaves are applied to reduce inflammatory swellings and tumors.
- Useful in diseases of the blood, small pone and n eye disease.
- The pulp of fruit is toxic to the heart.

122- Botanical Name: *Bauhinia veriegata* Linn.
Vernacular Name: Kachnar
Family: Caesalpiniaceae
Part Used: Bark, root, buds.

Ethnomedicinal Applications

- The bark is astringent to the bowels tonic to the liver.
- Used in asthma
- The paste of leaves is used on wounds and ulcers externally.
- Used as a gargle in stomatitis.
- The buds are used in piles.
- The infusion of bark taken orally in the eye diseases.
- Used in liver complaints.
- The root is given in dyspepsia and flatulency:

123- Botanical Name: *Prosopis spicigera* Linn.
Vernacular Name: Jundh
Family: Mimosaceae
Part Used: Bark, Leaves, flowers.

Ethnomedicinal Applications

- The bark cures leprosy, dysentery, asthma, leucoderma, tremors of the muscles and wandering of the mind.
- The smoke of the leaves is good for eye troubles.
- The fruit is dry hot, indigestible, destroys the nails and hairs.

124- Botanical Name: *Acacia arabica* Wild
Vernacular Name: Babool
Family: Mimosaceae
Part Used: Bark, Leaves, gum

Ethnomedicinal Applications

- The bark is bitter acrid; cures cough, bronchitis, diarrhea, burning sensation, and leucoderma.
- The leaves cure bronchitis; heal fractures, well for diseases of the eye.
- The gum is astringent to the bowels, anti dysenteric; cures biliousness.

- All parts of the plant are aphrodisiac.

125- Botanical Name: *Acacia rugata*
Vernacular Name: Ritha
Family: Mimosaceae
Part Used: Pod, leaves, seeds.

Ethnomedicinal Applications

- The pod is purgative, improves the appetite.
- It is used as cardio tonic.
- The leaves are cathartic and cure biliousness.
- The seeds are said to facilitate delivery in childbirth.

126- Botanical Name: *Rosa gallica*
Vernacular Name: Chota Gulab
Family: Rosaceae
Part Used: Petals

Ethnomedicinal Applications

- The dried petals are slightly tonic and astringent, and useful in debility.

127- Botanical Name: *Rosa alba* Linn
Vernacular Name: Gulab
Family: Rosaceae
Part Used: Flowers

Ethnomedicinal Applications

- The flower used in stomatitis, purifies the blood, improves the complexion.
- The flowers are used as a cooling medicine in fevers, also in palpitation of heart.

128- Botanical Name: *Rosa indica* Linn
Vernacular Name: Gulab
Family: Rosaceae
Part Used: Fruit

Ethnomedicinal Applications

- The fruits are used as an application to wounds, sprains, injuries and foul ulcers.

129- Botanical Name: *Optuntia monacantha*
Vernacular Name: Danda Thuar
Family: Cactaceae
Part Used: Stems, fruit

Ethnomedicinal Applications

- The infusion of the stems is made into emollient cataplasms.
- The fruit is used as a laxative.

130- Botanical Name: *Opuntia stricta*
Vernacular Name: Thur
Family: Cactaceae
Part Used: Fruit

Ethnomedicinal Applications

- The juice of the fruit is applied to indolent ulcers.

131- Botanical Name: *Opuntia dillenii*
Vernacular Name: Kunda thur
Family: Cactaceae
Part Used: leaves, fruits

Ethnomedicinal Applications

- The leaves are very tasty and stomachic
- The leaves are cure inflammations and pains.
- The flowers cure bronchitis
- The flowers are given in tumors.
- The decoction of the fruit appears to increase the secretion of bile.

132- Botanical Name: *Bupleurum falcatum* Linn
Vernacular Name: Spili
Family: Apiaceae
Part Used: Roots

Ethnomedicinal Applications

- The roots, in combination with other dugs are prescribed in liver troubles and as a diaphoretic.

- The root causes perspiration and is effective in thoracic and abdominal inflammation and fever, and useful in flatulence and indigestion.
- It is used in malaria and various other fevers.

133- Botanical Name: *Bupleuram jucundum*
Vernacular Name: Amurland
Family: Apiaceae
Part Used: Roots

Ethnomedicinal Applications

- The roots are diaphoretic and antipyretic.
- The roots are used in the liver dis order.

134- Botanical Name: *Foeniculum capillacerm*
Vernacular Name: Sonf
Family: Apiaceae
Part Used: Seed, Root, Leaves

Ethnomedicinal Applications

- The seed are purgative, stomachic and anthelmintic.
- The leaves improve the eyesight.
- The decoction and oil of seed is carminative, stimulant; cures intestinal troubles when applied to abdomen of children, useful diseases of the chest, the spleen, in headache, cough, and asthma; lesser inflammations; strengthens the eye.
- The root used as purgative.
- The fruits are used in venereal diseases.

135- Botanical Name: *Angelica glauca*
Vernacular Name: Chora
Family: Apiaceae
Part Used: leaves,

Ethnomedicinal Applications

- Leaves are given to persons suffering from rheumatism
- Seeds are used to expel worm from children.
- Used in flatulence and dyspepsia.

136- Botanical Name: *Vernonia cinerea* Linn.
Vernacular Name: Gandhavaki
Family: Asteraceae
Part Used: Seeds, Flowers

Ethnomedicinal Applications

- The decocation of the plant promote perspiration and in febrile.
- The flowers cure fevers.
- Seeds are used as anthelmintic.
- The whole plant is given as a remedy for spasm of the bladder.
- The root is given for dropsy.
- The expressed juice is given in piles.

137- Botanical Name: *Ageratum conzeoides* Linn
Vernacular Name: Ageera
Family: Asteraceae
Part Used: Stem, leaves, roots

Ethnomedicinal Applications

- The leaves applied to wounds act as aseptic and heal them quickly.
- The leaves are commonly used for wounds and sores.
- Stems are used in skin diseases, more particularly leprosy and eczema.

138- Botanical Name: *Pulchea indica*
Vernacular Name: Mandar
Family: Asteraceae
Part Used: Leaves, root

Ethnomedicinal Applications

- The root and leaves are used as astringents and antipyretics.
- The decoctions of the roots are prescribed in fevers as a diaphoretic, and as infusion of the leaves given internally in lumbago.

139- Botanical Name: *Eclipta alba*
Vernacular Name: Tikka
Family: Asteraceae
Part Used: Leaves and root.

Ethnomedicinal Applications

- Used to prevent abortion.
- The fresh plant is applied with sesamum oil in skin diseases.
- The juice of leaves is given in one teaspoonful doses in jaundice and fevers.
- The root is given to relieve the scalding of urine.

140- Botanical Name: *Helianthus annuus* Linn.
Vernacular Name: Surajmuki
Family: Asteraceae
Part Used: Flower, root, seed, leaves

Ethnomedicinal Applications

- The flower is pungent and hot; anthelmintic; anti periodic, skin diseases, ulcers, hysteria and asthma.
- A decoction of the root strengthens the teeth and cures toothache.
- The leaves are emetic and applied in lumbar pain.
- The seeds are diuretic.
- Used in bronchial, laryngeal and pulmonary infections.

141- Botanical Name: *Achillea millefolium* Linn
Vernacular Name: Biranjassfa
Family: Asteraceae
Part Used: Flower

Ethnomedicinal Applications

- The flower is used as laxative, vulnerary, diuretic, anti pyretic, stimulant and tonic to brain.
- Used in urinary discharges and liver complaints
- The herb is useful in hysteric, flatulence, heart burn, colic and epilepsy.

142- Botanical Name: *Cotula anthemoides* Linn.
Vernacular Name: Babuna
Family: Asteraceae
Part Used: Leaf, root

Ethnomedicinal Applications

- The plant heated with oil is applied externally in rheumatism.
- The infusion is used as an eye wash in most diseases of the eye.
- A decoction used for the remedy for head and chest colds.
- The nostrils are sometimes filled with the crushed leaf for colds.
- The stem, leaf and root used in decoction for colic pain remedy.

143- Botanical Name: *Cotula aurea* Linn
Vernacular Name: Babni
Family: Asteraceae
Part Used: Flowers

Ethnomedicinal Applications

- The flowers are used as a tonic, diaphoretic, anthelmintic, and anti pyretic, anti hysteric and for pain in the bowels.

144- Botanical Name: *Artemisia scoparia*
Vernacular Name: Biur
Family: Asteraceae
Part Used: whole plant

Ethnomedicinal Applications

- The plant is considered as a cure for pain in the ear.

145- Botanical Name: *Artemisia maritima* Linn.
Vernacular Name: Kirmala
Family: Asteraceae
Part Used: Seed, whole plant

Ethnomedicinal Applications

- The seeds are used for abdominal pain and mucous diarrhea.
- The herb is use as laxative, stops expectoration and removes bad humors; cures scorpion sting;

- Useful in toothache and inflammation.

146- Botanical Name: *Artemisia vulgaris* Linn
Vernacular Name: Baniru
Family: Asteraceae
Part Used: Leaves

Ethnomedicinal Applications

- The juice of the leaves is used in asthma and in diseases of children.
- It is applied to head of young children for the prevention of convulsions.
- The leaves and tops are administered in nervous and spasmodic affection connected with debility, in asthma and diseases of the brain.

147- Botanical Name: *Echinops echinatus*
Vernacular Name: Kantalu
Family: Asteraceae
Part Used: Roots

Ethnomedicinal Applications

- The plant is used in liver disorder.
- Useful in diseases of brain.
- Used in ophthalmic, chronic fever, pains in joints and inflammations.
- The root is aphrodisiac.
- The Roots are powdered and mixed with Acacia gum and applied to hair to destroy lice; also the powdered roots are appeared to wounds in cattle to destroy maggots.

148- Botanical Name: *Sonchus oleraceus* Linn
Vernacular Name: Sadi
Family: Asteraceae
Part Used: Root, leaves, stem

Ethnomedicinal Applications

- The gum used in liver disease.
- An infusion of the root and leaves used as tonic.
- The decoction of the stem as a sedative and a tonic.

149- Botanical Name: *Thevetia neriifolia*
Vernacular Name: Pali Kanar
Family: Apocynaceae
Part Used: Seeds

Ethnomedicinal Applications

- The plant is useful in urethral discharges, skin diseases, leucoderma and in piles.
- The oil from the seeds is emetic and purgative.

150- Botanical Name: *Abrus precatorius* Linn
Vernacular Name: Rati
Family: Papilionaceae
Part Used: Root and leaves

Ethnomedicinal Applications

- The root and leaves are sweetish.
- The fruit is aphrodisiac, tonic, remove biliousness; useful in eye diseases; cures leucoderma, itching, skin diseases and wounds, stomatitics, asthma, thirst and caries of the teeth.
- The fruit is tonic to brain.
- The root is emetic.
- The watery extract is useful in relieving obstinate coughs.
- The root is taken for sore throat.
- The juice of green leaves is taken for purifying the blood.
- Seeds are useful in affection of the nervous system.

151- Botanical Name: *Frankenia pulverulenta* Linn
Vernacular Name: Khareeya
Family: Frankeniaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- This plant is valued by native practitioners in the fresh state for its mucilaginous and aromatic properties; exhibited in the froms of decoction in eruptions.

152- Botanical Name: *Indigofera articulate* Linn.
Vernacular Name: Surmaii
Family: Papilionaceae
Part Used: Root and leaves, seeds

Ethnomedicinal Applications

- The roots and leaves are used as tonic.
- The seeds are considered anthelmintic.

153- Botanical Name: *Melilotus alba* Desr.
Vernacular Name: Aspurk
Family: Papilionaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The small fruit is carminative and tonic
- The fruit is useful in leucoderma.
- The herb is used as emollient.

154- Botanical Name: *Portulaca tuberosa*
Vernacular Name: Lunuk
Family: Portulacaceae
Part Used: Leaves

Ethnomedicinal Applications:

- The fresh leaves are used medicinally; an external application is prescribed by native practitioners in erysipelas and infusion in dysuria.

155- Botanical Name: *Dicoma tomentosa*
Vernacular Name: Dayii
Family: Asteraceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The herb is used in the febrile attacks to which women are subject after the childbirth.

156- Botanical Name: *Geisekia pharnacoides* Linn
Vernacular Name: Aluka
Family: Ficoidaceae
Part Used: Whole plant, leaves, stalks

Ethnomedicinal Applications:

- The plant is purgative and anthelmintic.
- It cures scabies.
- Used in heart troubles.
- Used in urinary diseases.

157- Botanical Name: *Rhazya stricta* Dence
Vernacular Name: Ishwarg
Family: Apocynaceae
Part Used: Leaves, fruits

Ethnomedicinal Applications:

- The juice of the leaves is given with milk to children for eruptions and infusion of them is very useful for sore throat.
- The fruit and leaves are considered efficacious in cases of eruptions.
- The plant is used as a cooling medicine.

158- Botanical Name: *Sonchus arvensis* Linn
Vernacular Name: Malii Boti
Family: Asteraceae
Part Used: Root

Ethnomedicinal Applications:

- The plant is diuretic; good in chronic fevers.
- The root is given in jaundice.

159- Botanical Name: *Aerva tomentosa* Forsh
Vernacular Name: Buikallan
Family: Amaranthaceae
Part Used: whole plant

Ethnomedicinal Applications:

- A decoction of the plant is used to remove swellings from the body.

160- Botanical Name: *Amaranthus spinosus* Linn
Vernacular Name: Cholai
Family: Amaranthaceae
Part Used: Roots, leaves

Ethnomedicinal Applications:

- The root is considered a specific in gonorrhea.
- It is used in menorrhagia and eczema'
- The boiled leaves and roots are given to children as laxative.
- The whole plant is used in the treatment of snake-bite.

161- Botanical Name: *Mallotus philippnensis* Muell
Vernacular Name: Kambal
Family: Euphorbiaceae
Part Used: Leaves

Ethnomedicinal Applications:

- The leaves are given as appetitizer, cause flatulence and constipation.
- The glands on the fruit are pungent used in purgative; heal ulcers and wounds, tumors, stone in the bladder,
- The fruit with glands are useful in bronchitis, diseases of abdomen, and enlargement of the spleen.
- The glands and the hairs on the fruit useful in scabies, ringworm and other skin diseases.

162- Botanical Name: *Callicarpa macrophylla*
Vernacular Name: Daya
Family: Verbenaceae
Part Used: Roots, leaves

Ethnomedicinal Applications:

- Aromatic oil from the root is used as a remedy in disorder of stomachic.
- The leaves are heated and applied to rheumatic joints.

163- Botanical Name: *Calligonum polygonoides*
Vernacular Name: Phogalli
Family: Polygonaceae
Part Used: Roots

Ethnomedicinal Applications:

- The roots are boiled in combination with catechu, are used as a gargle for sore gums.

164- Botanical Name: *Convolvulus glomeratus* [Choisy]
Vernacular Name: Loaralli
Family: Convolvulaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The plant is used as a purgative.

165- Botanical Name: *Euphorbia helioscopia* Linn
Vernacular Name: Gandabuti
Family: Euphorbiaceae
Part Used: Plant juice seed

Ethnomedicinal Applications:

- The plant is used as a cathartic, and juice is applied to remove warts.
- The milky juice is applied to eruptions and seeds are given with roasted pepper in cholera.
- The latex is successfully used for the removal of warts.

166- Botanical Name: *Coronopus didymus*.
Vernacular Name: Charini boti
Family: Brassicaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The decocation is used as laxative and diuretic.
- The paste of the plant used on boils.

167- Botanical Name: *Glossonema varians* Benth
Vernacular Name: khurram
Family: Asclepiadaceae
Part Used: Fruit

Ethnomedicinal Applications:

- The juice of fruit used in pain muscles, cough and sore throat.

168- Botanical Name: *Lycium barbarum* Miller.
Vernacular Name: Chirchitta
Family: Solanaceae
Part Used: Leaves, Fruit

Ethnomedicinal Applications:

- The fruit is useful in bleeding piles, scabies and in toothache.
- The juice of the leaves improves the eyesight.

169- Botanical Name: *Petrophurum divierii*
Vernacular Name: Grong
Family: Polygonaceae
Part Used: Whole plant
Ethnomedicinal Applications:

- It is considered a cure for sore throat.

170- Botanical Name: *Tecomella undulata*
Vernacular Name: Luar
Family: Bignoniaceae
Part Used: Bark

Ethnomedicinal Applications:

- The plant is useful in urinary discharges due to enlargement of the spleen and in leucoderma.
- The bark of young branches is used as a remedy for syphilis and for fever cure.

171- Botanical Name: *Coldenia procumbens* Linn
Vernacular Name: Tripunki
Family: Boraginaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- An equal part of the dry plant and seeds rubbed to a fine powder, and applied to warm to boils, quickly brings them to

172- Botanical Name: *Acacia nilotica* (Linn).Del.
Vernacular Name: Kikar/Babul
Family: Mimosaceae
Part Used: Leaves and Pods

Ethnomedicinal Applications:

- Bruised leaves are applied to eye sores in children.
- Pods soaked in cow's milk and dried in shade, grind and mixed with sugar are given in sexual impotency.
- The leaves and pods are astringent in diarrhea.

173- Botanical Name: *Acacia jacquemontii* Benth.
Vernacular Name: Kikari
Family: Mimosaceae
Part Used: Stem

Ethnomedicinal Applications:

- The stem is used as Miswake.

174- Botanical Name: *Calligonum polygonoides* Linn.
Vernacular Name: Phog
Family: Asclepiadaceae
Part Used: Roots

Ethnomedicinal Applications:

- The juice of boiled root with the combination of catechu is used for gargles and sore throat.

175- Botanical Name: *Citrulus colocythis* Schred.
Vernacular Name: Tumba
Family: Cucurbitaceae
Part Used: Fruit and roots

Ethnomedicinal Applications:

- Fruit and seeds are used as purgative.
- Roots are used in jaundice, urinary diseases and rheumatism.

176- Botanical Name: *Haloxylon salicornicum* (Moq.)Bunge.
Vernacular Name: Safed lanra
Family: Chenopodiacae
Part Used: Whole plant

Ethnomedicinal Applications:

- The whole plant burn in a pot to get carbonate of soda that is used as an alternate of soap for washing the cloth.

177- Botanical Name: *Kochia indica*
Vernacular Name: Bui
Family: Chenopodiaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The whole plant used as cardiac stimulant in case of week and irregular heart beat especially following on fever.

178- Botanical Name: *Sophora millis*
Vernacular Name: Lathia
Family: Papillionaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The decocation of the plant used as tonic.

179- Botanical Name: *Eleusine flagellifera*
Vernacular Name: Chhimber
Family: Poaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The infusion used as tonic and astringent.

180- Botanical Name: *Solanum xanthocarpum*
Vernacular Name: Katilla
Family: Solanceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The roots are used as laxative, appetizer, asthma and in bronchitis.
- The decocation of the leaves are given to the children's for the cure of fever.
- The fumigations with the vapours of the burning seeds of this plant are used for burning feet.

181- Botanical Name: *Phoenix dactylifera*
Vernacular Name: Pend
Family: Palmaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- In equal parts of the dry plant and seeds are grinded to a fine powder, and applied to warm to boils, quickly brings them to suppuration.
- The fruit is aphrodisiac in nature, used in bronchitis, fatigue, as expectorant and demulcent.
- It is good for liver.
- Roots are used in leprosy.
- The fruit of the plant used by anemic pregnant women.

182- Botanical Name: *Phoenix sylvestris*
Vernacular Name: Pend
Family: Palmaceae
Part Used: Root, Fruit

Ethnomedicinal Applications:

- The roots are used for spermatorrhoea.
- The fruit is used for cardiac purposes.
- The fruit of the plant is used by anemic pregnant women.

183- Botanical Name: *Phoenix acaulis*
Vernacular Name: Pend
Family: Palmaceae
Part Used: leaves

Ethnomedicinal Applications:

- The decocation is used in genitourinary diseases.

184- Botanical Name: *Salsola kali*
Vernacular Name: Lanan
Family: Chenopodiaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The decocation is used for the remedy for worms.
- The ashes are applied on itching.

185- Botanical Name: *Heliotropium strigosum* wild.
Vernacular Name: Gorakhpamo
Family: Boraginaceae
Part Used: Whole plant

Ethnomedicinal Applications:

- The decocation is used as laxative and diuretic.
- The juice of the plant used as an application to sore eyes gums and boils.
- It is used for cure of sting of nettles and insects.
- It is used to cure the pain of limbs.

186- Botanical Name: *Flueggea leucopyrus* wild
Vernacular Name: karan
Family: Euphorbiaceae
Part Used: Leaves

Ethnomedicinal Applications:

- The past of the leaves with tobacco are used to destroy worms in sores.

3.1.2 Collection of data from local people and Hakims:

The ethno medicinal data of the plants of South Punjab region was collected from the local people and from the local Hakims (**Table 2.2**) by a questionnaire (Appendix-I).

The information was collected from the local peoples or Hakims including local name, local uses drug preparation, part used, occurrence, taste, potency, toxicity, mode of use, chemical composition (on the basis of availability and its ethno medicinal value), personalities involved in the collection and other related information (locally published books and the literature saved in the folk language books).

3.2 Establishment of Prem Madan Herbarium

3.2.1 Collection of Plants

The establishment of Herbarium was an offshoot of the present research. Several visits (2005-2008) were made on seasonal basis to different parts of the Pakistan to collect plants for the herbarium as part of undergraduate studies under supervision of the researcher (Assistant Professor Botany). The name of the herbarium was dedicated to the Prof. Prem Madan: professor of taxonomy, and Head of Department of Botany associated with 35 years of meritorious service. Prem Madan Herbarium was formally inaugurated in Lahore College for Women University, Lahore on 23rd May, 2008 by Prof Dr Zaheer-ud-Din, Chairperson of Botany Department, Government College University, Lahore.

All the voucher specimens were properly dried and after identification, pasted on the herbarium sheets. All the voucher specimens were entered in the register (Printed especially for the entry of voucher specimens) with their proper botanical and local names, date of collection, Name of collector, location, ethno medicinal value and proper photograph if available.

The plants collected from the various locations of Pakistan (2005-2008) are listed in Table **2.3.**

SECTION B

4. BIOLOGICAL ACTIVITY

4. BIOLOGICAL ACTIVITY

4.1 Introduction and Historical Review

4.1.1 Anticancer Activity

Cell division or cell proliferation is a physiological process that occurs in almost all tissues and under many circumstances. Normally the balance between proliferation and programmed cell death is tightly regulated to ensure the integrity of organs and tissues. Mutations in DNA that lead to cancer disrupt these orderly processes.

The uncontrolled and often rapid proliferation of cells can lead to either a benign tumor or a malignant tumor (cancer). Benign tumors do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they extrinsically compress vital structures. Malignant tumors can invade other organs, spread to distant locations (metastasize) [Kleinsmith, L.J. and Pearsons, B.C., 2006] [Mulazim, H.B., *et. al.* 2002] and become life threatening.

4.1.2 Antimicrobial Activity

Current estimates suggest that over 80% of people in developing countries still depend on our traditional medicine for their primary health care needs and about 85% of traditional medicine involves the use of plant extracts. [MPDDRC, 2006] [Goldstein, A. *et.al.* 1974] [Tyler *et.al*.1988] [Kinghom, A.D., 1993] [Lenaz, D., 1993] [Klayman, *et. al*.1984] [Klayman, *et. al.*1985] [Nair, M. SR., 1986] [De Souza, N.J., 1983]

Recently due to increasing development of drug resistance in pathogenic microorganism as well as appearance of undesirable side effects of certain antibiotics or other synthetic drugs antimicrobial properties have been reported in a wide variety of plant extracts with

goal to discover new chemical classes of antibiotics that could resolve these problems. [Piddock, *et. al*.1989]

Some microorganisms and fungi have been approved as new antibacterial and antifungal drugs but there is an urgent need to identify novel substances active towards pathogens with higher resistance. Higher plants can be good source of antibiotics. [Recio, M.C., 1989] [Fridous, *et al.* 1990] [Kumar, *et al.* 2006] [Buwa, *et. al*. 2006] [Zampini*, et. al.* 2005] [Alanís *et. al*. 2005] [Owais, *et al*. 2005]

The potential for developing antibiotics from higher plants appears rewarding, as it will lead to the development of phytomedicine to act against microbes. Plants based on antimicrobial studies have enormous therapeutically potential as they can serve the purpose without any side effect that already is associated with synthetic antibiotics. [Hussain, M.A. and Gorsi, M.S. 2004] [Yasunaka, *et al.* 2005] [Bonjar, G. H., 2004]. Extracts of *Calotropis procera, Chenopodium murale, Pulicaria orientalis, Tribulus terrestris* and *Withania somniferum* displayed a remarkable antimicrobial activity. [Ali *et. al.* 2001] [Ageel, *et. al*.1986]

Behera, K.K in 2006 reported, that the root paste (3mg) of *Heliotropium indicum* L with lime used by rubbing the infected portion of skin as a cure for ring worms and decoction of root (10ml) with honey (2ml) was taken for iron deficiency by a woman against anemia during pregnancy period. [Behera, K.K., 2006] [Muthu, *et. al*. 2006]

Salvadora persica (Arak) and *Azadirachta indica* (Neem) were commonly used as oral hygiene tools in different parts of the world; several studies had demonstrated the anti-plaque, anticarious and antibacterial effect of these sticks. The aim of this study was to compare the effectiveness of antimicrobial activity of Neem and Arak chewing stick's aqueous extracts at various concentrations. Data suggested that both chewing stick extracts are effective at 50% concentration on strept mutans and Strept faecalis. Arak extract was more effective at lower concentrations for *Streptococcus faecalis*. [Almas, K., 1999]

Micro-organisms show resistance to the synthetic drugs and antibiotics. Another discrepancy of the synthetic drugs and antibiotics is that they may also make interactions

with the body system to disturb the metabolic processes. Scientists therefore, are working on the extraction of anti-infect ional compounds including antimicrobial peptides/proteins from natural sources like plants and animals. The anti-inflectional compounds show broad-spectrum bioactivity against infection causing agents such as bacteria, fungi, protocists, protozoans, viruses, yeast etc [Cookson, B.D,2000] [Boyd, R.F., 1995] [Mario, D., 2000][Awadh, A.N.A.,2001]

Petersen, F. C. & Scheie, A. A. (2000) enlists the different strains of bacteria which were resistant to various antibiotics. It is a need of time to find new spectrum of antibiotics.

In 1984, at least 25% of the prescription drugs issued in the USA and Canada were derived from or modeled after plant natural products [Farnsworth, N.R and Soejarto, D.D., 1985].

4.1.3 Synergistic Activity

Klein J.P., and Scholler, M. [1998] has taken view and supported it by strong experimental evidence, that synergism occurs only when the microorganism tend to become drug-fast and is related to inhibition by the second of resistance microorganism surviving the action of first. Secondly it acts in two ways that when drug-fastness does not develop with some facility, synergism commonly does not occur and some degree of antagonism may be observed. The consequence of combining drug is dependent upon the drug, upon the strains of microorganisms and upon the condition under which the organism is subjected to the action of drug.

The synergistic activity of herb and spice extracts can be used as natural antimicrobials for food preservation much better then the single one. [Zempini, et al. 2005]

Brooks, *et al.,* evaluated the antimicrobial activity of plant extracts synergistically with antibiotic against resistant bacteria. The individual extracts and when they were used in lower concentrations with ineffective antibiotics showed high potential against resistant bacteria. [Brooks, *et al*. 1991]

4.2 Selection of Plants for Biological Activity

The plants were selected on the basis of their ethno medicinal importance and multiple medicinal uses in the local community of South Punjab and their abundance. The plants selected were *Coronopus didymus, Withania coagulans, Capparis decidua, Salsola kali, Heliotropium strigosum*, *Salvadora oleoides, Calotropis procera and Tamarix aphylla* (**Table-4.1**).

Table-4.1: Medicinal Plants selected for isolation and biological activity

S. #	Ethno medicinal Plants	Family	Voucher #
1.	*Capparis decidua (Forssk.)Edgew*	Capparidaceae	02
2.	*Coronopus didymus* Linn	Brassicaeae	105
3.	*Heliotropium strigosum*	Boraginaceae	040
4.	*Salsola kali* Linn	Chenopodiaceae	86
5.	*Salvadora oleoides* Linn	Salvadoraceae	28
6.	*Tamarix aphylla*	Tamaricaceae	03
7.	*Withania coagulans* (Stocks) Dunal	Solanaceae	04
8	*Calotropis procera* (Willd.)R.Br	Asclepiadaceae	31

4.3 General Extraction Procedure

The precise mode of extraction depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated.

The best chemical procedure for obtaining organic constituent from dried plant tissue is to continuously extract powder in a soxhlet apparatus with a range of solvents, starting in turn with ether petroleum and dichloromethane and then ethyl acetate and alcohol (for more polar compound) (**Table 7.2**) [Harborne, J. B., and Williams, C. A. 2000] [Tashibangu, J.N. 2002]

5. EXPERIMENTAL

5. EXPERIMENTAL

5.1 Anticancer Activity

5.1.1 Extraction Procedure

The plant material was dried in shade. The plant material was grinded to powder and placed in the thimble of Soxhlet and extracted with methanol at 60°C. The methanol was evaporated under vacuum by rotary evaporator to yield crude methanolic extract (**Table 6.1**). The crude methanolic extract was then fractioned with pet ether (b.p. 40-60°C), and then dichloromethane to evaluate the biological activity of non-polar (Pet ether extract), less polar (dichloromethane extract) and polar compounds (methanol extract).

All solvents used were of analytical grade (Merck).

5.1.2 Preparation of Stock Solution for Biological Activity:

Stock solution of 1000µg/µl was prepared by dissolving 1.0g extracts in 1.0 ml of 1:1(DMSO: Solvent).The pH of the extract was checked. (Since microorganism may not be able to grow in media which are excessively acidic or basic) Then serial dilution was made (250µg/µl, 100µg/µl, 50µg/µl, 25µg/µl, 10µg/µl 0.5µg/µl, and 0.1µg/ µl).

5.1.3 Feeding Routine and Hygiene

The rats were fed at regular intervals with synthetic rat food (National feeds Lahore) and provided tap water by means of glass bottles fitted with nozzles. Food and water were daily refreshed. (**Table 6.1.34**) [Conner, M.J., 1988].

Saw dust was spread on the floor of all rat cages in two centimeters thickness to help absorb the animal feces and moisture. It also helped maintain a steady temperature in the cages. The saw dust was changed after every 24 hours.

5.1.4 Preparation of Animals for Carcinogens

After a week of acclimatization, the dorsal skin of albino rats was shaved with electric clipper after shaving 5 x 5 cm area. It was marked with a permanent marker for the application of carcinogens three days prior to the beginning of the experiment. [Mughal, M.S., *et. al.* 2005] [Heffelfinger, S.C., et. al .2005]

5.1.5 Method (Route) of Dose Administration:

Following carcinogens were applied topically:

*7-12-dimethyl benz (a) antheracene (*DMBA): Carcinogen-7-12-dimelhyl benz (a) antheracene was obtained from Sigma Chemical Company and was dissolved in acetone (10g/100ml) in a concentration of 100µg/mL. The working solution was prepared just before its use and was kept in amber glass at 20°C. The automatic pipette was used for the application of the working solutions. DMBA was applied on the skin of albino rats as a single dose 100µg/ml in groups B-M.

12-O-Tetradecanoylphorbol 13-acetate (TPA): The 12-O-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma Chemical Company. The working solution of TPA was prepared as a10µg/ml. in acetone. A stock of TPA (1g/100ml) and a working solution was stored at 20°C. The 12-O-Tetradecanoylphorbol 13-acetate (TPA) was applied on the skin of rats after two weeks of 7. 12-dimcthyl benz (a) antheracene in groups B-M.

5.1.6 Solvents Used as Dilutors

Acetone was used as a vehicle for all topically applied carcinogens and obtained in sealed amber bottles. The acetone was applied purely on the skin of rats of the control group (C). All the carcinogens prepared in acetone were applied topically through insulin syringes. DMBA and TPA are known carcinogens so high protective measures were taken during the drug application, like using masks, gloves and washing the hands with proper antiseptics. All the syringes were dumped and discarded properly after using the carcinogens. The carcinogens were applied during the resting phase of the hair cycle of the animal and during the dark periods of the day.

Pet ether was used as dilutor in the pet ether extracts of the study plants. The pet ether was applied through insulin syringes on the skin of rats of the control group (D).

Methanol was used as dilutor in the methanol extracts of the study plants. The methanol was applied purely on the skin of rats of the control group (E).

Dichloro methan was used as a dilutor in the Dichloro methan extracts of the plant under study. The Dichloro methan was applied purely on the skin of rats of the control group (F). (**Table 6.1.1**) [Takasaki, M et al 1999]

5.1.7 Record of Animals

The information about each rat's group and its age was recorded at the start of the experiment. Other information that was also recorded at the start and at suitable intervals about the rat, is the duration for which the rats were kept for the experiment, their sex, weight in grams, both at the beginning and at the end of the experiment, the type and quantity of the carcinogen dose and the duration of its application and the period of the placebo treatment. The records were used for analysis of the effect of the experiment on the rats and for the conclusion of the result. (**Table-6.1.4**)

5.1.8 Particulars of Lesions Recorded

Hair loss and other gross morphological tumor appearing features, such as outgrowth and ulcer were weekly observed, counted and measured by Vernire Caliper in the skin of each animal throughout the experiment. The lesions and the surrounding skin of each animal were also examined microscopically after heamatoxyline and eosin staining to determine the histopathological changes, such as atrophy, hyperplasia. parakeratosis, dysplasia, fibrosarcoma, chronic inflammation, squamous cell carcinoma in situ, extensive squamous cell carcinoma and osteoma etc. at the end of the experiment. Then the lesions were diagnosed according to the histopathological changes.

5.1.9 Anesthesia

Ether was used to anesthetize the rats as unlike other gaseous anesthetics like halothane, it can be used, without a vaporizer machine. Cotton gauze plugs were moistened with ether and placed at the bottom of the glass jar. An elevated platform was placed at the bottom to prevent direct contact of the rat with the anesthesia. After the anesthetized, it was removed from the jar and a nosecone (made by using empty syringe case packed with welled ether) was utilized to maintain anesthesia.

5.1.10 Specimen Collection and Preservations

Autopsy (removal of tissues): All the animals were sacrificed after completion of 30 weeks. Rats were placed in the ether jar till death (about 2 minutes).

After trimming the dorsal skin, long section of the tissue surroundings the lesion was removed for re-sectioning. This cancerous and surrounding tissue was washed 2-3 times with saline solution to remove any debris and blood. The dorsal skin was trimmed and specimens were kept in labeled jars for fixation containing 10% formalin.

Fixation of the Specimen: The Jars were labeled according to the groups. In each jar a sufficient amount of 10% formalin was used as a fixative for skin tissue [Hopwood, D.1990]

Gross Examination and Sections Preparation: After 24 hours of fixation each tissue was individually examined grossly in a grossing room. Sections were taken and labeled on the white card.

Processing of the Sections: All the specimens were processed in an enclosed type automated processing machine. The tissues were kept in steel cassettes with their labeled cards and kept in steel baskets. [Gorden, K.C. 1990]

Embedding and Section Cutting: After 24 hours tissues were embedded in paraffin by traditionally prepared Lockhart's 'L' piece rececticle. Blocks were prepared and kept in a refrigerator for further hardening. Each block was trimmed to remove the excessive wax and for the exposure of the tissue on the surface. The paraffin section cutting was performed by a plain wedge sharp blade on rotatory microtome. Flat sections were made with the correct orientation of the ribbons of the sections. Wrinkles were removed by teasing apart, using forceps. These flat sections were picked up correctly on the clean glass slides, by immersing the slide in a thermostatically controlled water bath and lifting it vertically. The sections were flattened on the slides. Albumin was used as an adhesive agent on the slide. [Gorden, K.C. 1990]

5.1.11 Staining Procedure (Haematoxyline and Eosin Stain)

The sections were dewaxed by dipping them in xylene and then they were hydrated through descending grades of alcohol that is 100%, 70% and water. Slides were then kept in Harris Haematoxyline for 10 minutes. Bluing was done in running tap water for 5 minutes, differentiated in acid alcohol for 10 seconds and rinsed in running tap water for 5 minutes. The slides were then stained with 1% eosin for 10 minutes and washed in running tap water for 5 minutes. The stained sections were then dehydrated using

ascending grades of alcohol that is 50%, 70%, 90% and 100% and mounted in canda balsam after xylene rinse. [Stevens, A. 1990]

5.1.12 Experimental Plan

After acclimatization for 7 days all rats were distributed into fourteen groups (A, B, C, D, E, F, G, H, I, J, K, L and M) of ten rats each. Diluters (Acetone, Pet ether, and Methanol and Dichloro methan) were applied for first 15 weeks during the experiment in certain groups (C, D, E, and F). Skin tissues of all animals were taken by line needle biopsy after 15 weeks, while biopsies of all animals of each group were taken at the end of the experiment (i.e. after 30 weeks) all animals were sacarified by decapitation and immediately the required skin tissues were taken out after macroscopic histopathological studies of the skin.(**Table-6.1.1**)

Group A: The group A acted as control and was not given any treatment. After the completion of thirty weeks, biopsies were taken to observe the normal skin.

Group B: In group B DMBA was given as a single dose on the shaved dorsum of the albino rats in a dose of 100µg/ml by topical application. TPA was given, after two weeks of DMBA application, twice a week for the next 15 weeks in a dose of l0µg/ml. At the end of the experiment (30 weeks), the animals were sacrificed and biopsies were taken to see the different lesions developed with chemical carcinogens (DMBA & TPA).

Group C, D, E & F: The animals of these groups were kept for placebo control. The animals of group C, D, E and F however received an application of acetone, pet ether, methanol and Dichloro methan. These are the vehicles used for the dilution of carcinogens and plant extracts. After the completion of the experiment, the albino rats were scarified; biopsies were taken to see the response of the diluter.

Group G-M: The group G-M were further divided into three sub groups each (Ten rats in each sub group).In all groups, DMBA was given as a single dose on the shaved dorsum of the albino rats in a dose of 100µg/ml by topical application. TPA was given, after two weeks of DMBA application, twice a week for the next 15 weeks in a dose of l0µg/ml.

After completion of 15 weeks, carcinogens were stopped but the animals were kept under observation for the next 15 weeks. The animals of group 1 received an application of a dose of l0µg/ml of pet ether extract; 2 received an application of a dose of l0µg/ml methanolic extract, while 3 received an application of a dose of l0µg/ml respectively. **Group-G**: Each sub group of G was administered chemotherapy with three solvent extracts of *Coronopus didymus* twice a week for next 15 weeks. At the end of the experiment (30 weeks), the animals were sacrificed and biopsies were taken to see the effect of methanol, pet ether and dichloro methan extracts of *Coronopus didymus*.

Group H: was given chemotherapy with methanol, pet ether and dichloro methanol extracts of *Salsola kali.*

Group-I: was given treatment with methanol, pet ether and dichloro methan extracts of *Capparis decidua.*

Group J: received an application of a dose of l0µg/ml of methanol, pet ether and dichloro methan extracts of *Salvadora oleoides* and results noted.

Group K: *Withania coagulans* was used as chemotherapeutic agent twice a week for next 15 weeks. At the end of the experiment (30 weeks), the animals were sacrificed and biopsies were taken to see the effect of methanol, pet ether and dichloro methane extracts of *Withania coagulans.*

Group L: All the three sub groups were treated under set experimental procedure with methanol, pet ether and dichloro methane extracts of *Heliotropium strigosum.*

Group M: received an application of a dose of *Tamarix aphylla* twice a week for next 15 weeks. At the end of the experiment (30 weeks), the animals were sacrificed and biopsies were taken to see the effect of methanol, pet ether and dichloro methan extracts of *Tamarix aphylla.*

All animals were sacrificed after giving anesthesia in a glass jar after 30 weeks. The skin biopsies and lesions from other areas were taken to see the gross and microscopic changes after chemical carcinogens to observe the results of the application of the

complex. The Gross and microscopic changes were seen. In group "B" we saw the effects of chemical carcinogens. We saw size and types of tumor in this group.

In groups C, D, E, F, G, H, I, J, K, L and M the same statistical criteria was used to see the response to complex with topical chemotherapy.

5.2 Antibacterial Activity

All chemicals and solvents were of analytical grade purchased from Merck. The experiments were performed in microbial free environment in laminar flow cabinet and all glassware properly sterilized. Solvent free plant extracts were stored at 4°C.

Microbial strains of *Staphylococcus aureus* ATCC 25923 (*S.aureus*). *Escherichia coli* (ATCC 2592) (*E.Coli*), *Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Streptococcus pneumoniae* (ATCC 49619) (*S. pneumoniae*) *Bacillus subtilus* (ATCC 6051) (*B. subtilus*) and *Sarcina lutae* (ATCC 9341) (*S. lutae*) were obtained from Peads Microbiology Laboratory Mayo Hospital, Lahore. These microbial strains are already identified from National Institute of Health, Islamabad and DTL, drug testing Laboratory, Lahore.

5.2.1 Sterilization and Preparation of Culture Media

Antibacterial activity of plants extract was determined by the agar well diffusion method according to NCCCL [NCCCL, 1993] [Norrel, S.A. and Messley, K.E., 1997].

All glassware was washed with water and detergent followed by the rinsing with tap water and then with distilled water. Then they were wrapped with aluminum foil and sterilized in the autoclave at 15 psi and 121°C.

OXOIDCM-1 nutrient broth was used. It was prepared by dissolving 13g/l of broth in sterilized water, mixing well and distributed to test tubes containing 10ml of each and autoclaved at 15psi + 121°C.

Nutrient agar (Merck) for microbiology was used in the experiment. It was prepared by dissolving 20g /liter of sterilized water, heated in a boiling water bath and autoclaved for half an hour at 15psi + 121°C.

The anti bacterial activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones of the growth measured in millimeter, evaluate the MIC and caculate the antibacterial activity in ml/g. The test was carried out in triplicate and their means are recorded.

5.2.2 Minimum Inhibitory Concentration (MIC)

The MIC was evaluated for plant extracts that showed antimicrobial activity. [Hirasawa *et. al.* 1999] Different conc. (0.1-250mg/l) was prepared using sterile distilled water as the diluents. Agar well diffusion method was used and the test was carried out in triplicate and their means were recorded.

5.3 Synergistic Bacterial Activity

As the antibacterial activity of methanol extracts of experimental medicinal plants was better then all other solvent extracts it was selected to study synergistic effect in combination with methanol extracts of eight other plant extracts (**Table 6.3**) in order to enhance the inhibitory effect of crude extracts as drug against different microbes some of which have developed resistant to many available antibiotics. The selection of plants for synergistic activity was done on the basis of their availability and their ethno medicinal importance.

Synergistic activity against bacterial strains was determined by taking equal amount (25µg/ml (1:1)) of different plant extracts by agar well diffusion method as described before. The synergistic activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones of the growth measured in millimeter. The test was carried out in triplicate and their means are recorded.The synergistic antibacterial

activity were recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeter and evaluated the MIC.

5.4 Antifungal Activity

Antifungal activity of plants extract was determined by the agar tube dilution method. [Atta, *et. al.*, 1999]

5.4.1 Fungal Strains:

Fungal strains *Trichoderma viridis* (FCBP# 642) (T.viridis) *Aspergillus flavus* (FCBP# 647) (*A.flavusi*), *Fusarium laterifum* (FCBP# 624) (*F.laterifum*), *Aspergillus fumigatus* (FCBP# 474) (*A.fumigatus*) *Candida albicans* (FCBP# 478) (*C.albicans*) were obtained from the department of Mycology and Plant Patholgy, University of the Punjab, Quaid-e-Azam Campus, Lahore. Two identified fungal strains *Trichophyton mentogrophytes* and *Microsporum canis* were obtained from the Main Microbiology Laboratory of Mayo Hospital, Lahore.

5.4.2 Fungal Bioassay:

For antifungal bioassay of plants extracts, the following steps were taken

- Test samples were dissolved in sterile DMSO to serve as stock solution.
- Different concentrations were prepared from the stock solution (250µg/µl, 100µg/µl, 50µg/µl, 25µg/µl, 10µg/µl ,0.1µg/µl and 0.5µg/µl)
- Sabouraud dextrose agar was prepared by mixing 4% glucose agar and agar in distilled water.
- It was then stirred with a mechanical stirrer to dissolve it and a known amount was dispensed into screw capped test tubes.
- Test tubes containing media were autoclaved at 121°C for 15 minutes.

- Tubes were allowed to cool to 50°C and the test samples of desired concentrations pipette from the stock solution into the non-solidified sabouraud agar media.
- Tubes were then allowed to solidify in a slanting position at room temperature.
- Each tube was inoculated with a 4mm diameter piece of inoculums removed from a seven day old culture of fungi.
- All culture containing tubes were inoculated with 105(CFU)/mL-1 fungal spore suspensions at optimum temperature of 28-30°C for growth for 7-10 days. Humidity (40% - 50%) was controlled by plane an open pan of water in the incubator.
- Pure solvents were used as control as negative control.
- Other wells were supplemented with reference compounds i.e. Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole as positive control.

After the incubation for 7-10 days the test tubes with no visible growth of the microorganism was taken to represent the zone of inhibition of the test sample which was expressed in μg/ml. The test was carried out in triplicate and their means were recorded.

5.4.3 Statistical Analysis

The results of zone of inhibition were analyzed by analysis of variance (ANOVA) with completely randomized block design. The significant difference between extracts and standard discs against seven fungal strains were analyzed by statistically taking the level of significance at 0.05.

6. TABLES

6. TABLES

6.1 Biological Activity (Anticancer)

Table 6.1.1: Distribution of Animals in Different Experimental Groups on the Basis of Administration of Controls and Drug (Plant Extract).

Groups	Drug for Chemotherapy	Animals with Lesions (First week)	Animals without Lesions (After 15 weeks)
A	Normal/without induced cancer	Nil	Nil
B	No chemotherapy	Nil	10
C	Acetone	Nil	10
D	Pet ether	Nil	10
E	Methanol	Nil	10
F	Dichloro methan	Nil	10
G	*Coronopus didymus*	Nil	30
H	*Salsola kali*	Nil	30
I	*Capparis deciduas*	Nil	30
J	*Salvadora oleoides*	Nil	30
K	*Withania coagulans*	Nil	30
L	*Heliotropium strigosum*	Nil	30
M	*Tamarix aphylla*	Nil	30

Table-6.1.2: Solvent (3) extracts of Medicinal Plants (7) Collected from Different Localities of Southern Punjab for Biological Activity

S. #	Plant	Family	Part used	Locality	Voucher # PMH	Fresh Wt (Kg)	Dry Wt (Kg)	Extract Wt		
								Methanolic (gm)	Petether (gm)	Dichloro methane (gm)
1-	*Coronopus didyma*	Brassicaceae	Whole plant	Sahiwal	105	5.0	4.9	33 %	5.25%	6.1%
2-	*Salsola kali*	Chenopodiaceae	Aerial part	Bahawalpur	086	2.7	2.3	38%	14%	2.5%
3-	Withania coagulans	Solanceae	Aerial part and Seeds	Dera Ghazi Khan	004	5.0	4.5	87.9%	4.9%	3.2%
4-	*Cappris decidua*	Capparidaceae	Aerial part	Saki Serwer	002	5.0	4.85	89%	4.92%	3.38%
5-	*Salvadora oleoides*	Salvadoraceae	Aerial part	Cholistan	028	4.8	4.6	36.6%	6.6%	7.88%
6-	*Heliotropium strigosum*	Boraginaceae	Aerial part	Bahawalpur	040	1.50	1.48	13.9%	6.2%	3.86%
7-	*Tamarix aphylla*	Tamaricaceae	Leaves and stem	Rahim-Yar-khan	003	1.4	0.885	19.7%	9.09%	2.02%

Table 6.1.3: Medicinal Plants (8) from Northren areas of Pakistan Used to Enhance the Antibacterial Activity of Experimental Medicinal Plants (7) of Southern Punjab

S. #	Plant	Family	Part used	Locality	Voucher # PMH	Fresh Wt (Kg)	Dry/Wt (Kg)	Methanolic (gm)
1-	*Hypericum perforatum*	Hypericaceae	Whole plant	Muree	0313	1.00	0.795	42%
2-	*Pinus wallichiana (Bark)*	Pinaceae	Bark	Muree	0101	5.0	4.5	12.67%
3-	*Gallium asperuloides*	Rubiaceae	Whole plant	Muree	234	1.0	0.700	37%
4-	*Senecio chrysanthemoides*	Asteraceae	Aerial part	Muree	185	1.0	0.790	42%
5-	*Sarcococca saligna*	Buxiaceae	Aerial part	Muree	0154	1.00	0.780	36%
6-	*Impatients walleriena*	Balsaminaceae	Aerial part	Muree	0103	1.0	0.867	34%
7-	*Anethum sowa*	Apiaceae	Aerial Part	Muree	0384	1.0	0.764	45%
8-	Pinus roxburgii(Bark)	Pinaceae	Bark	Muree	002	1.0	0.820	48%

Table 6.1.4: Division of Animals and the Dose Schedule of Chemical Carcinogens and Plant Extracts

Group (30 weeks)	Carcinogen (For 15 Weeks)				Curative (For 15 Weeks)	
	DMBA In acetone 100µg/ml for 2 weeks		TPA In acetone 10µg/ml After 2 weeks of DMBA for 13 weeks		Plant extracts in dilution solvent 10 µg/ml (After 15 weeks of carcinogenesis start plant extracts)	
	Route	Schedule	Route	Schedule	Route	Schedule
A	Nil	Nil	Nil	Nil	Nil	Nil
B	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
C	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
D	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
E	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
F	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
G1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
G2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
G3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
H1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
H2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
H3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
I-1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
I-2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
I-3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
J1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
J2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
J3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
K1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
K2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
K3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
L1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
L2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
L3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
M1	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
M2	Topical	Single dose	Topical	Twice a week	Topical	Twice a week
M3	Topical	Single dose	Topical	Twice a week	Topical	Twice a week

DMBA 7, 12-dimethylbenz (a) antheracene **TPA** 12-O-Tetradecanoylphorbol 13 acetate

Table 6.1.5: Particulars of Animals of Control (Group A)

Sr. No of rats	Sex	Age (weeks)	WEIGHT (g)		CARCINOGEN PERIOD (WEEK)		Period of treatment (week)	Period of specimen taken after experiment (week)	Diagnosis	Protection
			Start of Research	End of Research	Initiator DMBA	Promoter TPA				
1.	M	5	171	316	Nil	Nil	Nil	30	Normal	Protected
2.	M	6	121	234	Nil	Nil	Nil	30	Normal	Protected
3.	M	6	124	267	Nil	Nil	Nil	30	Normal	Protected
4.	F	6	145	256	Nil	Nil	Nil	30	Normal	Protected
5.	M	5	169	345	Nil	Nil	Nil	30	Normal	Protected
6.	M	5	126	236	Nil	Nil	Nil	30	Normal	Protected
7.	F	5	153	258	Nil	Nil	Nil	30	Normal	Protected
8.	M	6	139	253	Nil	Nil	Nil	30	Normal	Protected
9.	M	5	166	336	Nil	Nil	Nil	30	Normal	Protected
10.	M	6	156	230	Nil	Nil	Nil	30	Normal	Protected

M- Male
F- Female

Table 6.1.6: Lesions Obtained in Animals of Control (Group A)

Sr. No Rats	GROSS EXAMINATION			MICROSCOPIC EXAMINATION			Diagnosis	Protection
	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
2.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
3.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
4.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
5.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
6.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
7.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
8.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
9.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected
10.	Nil	Nil	Nil	Nil	Nil	Nil	Normal	Protected

Table -6.1.7: Experimental Protocol for Animals of GroupB-M

CARCINOGEN PERIOD (WEEK)		Period of treatment (week)	Period of specimen taken after experiment (week)
Initiator DMBA	**Promoter TPA**		
2-2	13-26	15-30	30
2-2	13-26	15-30	30
2-2	13-26	15-30	30
2-2	13-26	15-30	30
2-2	13-26	15-30	30
2-2	13-26	15-30	30

Table-6.1.8: Lesions Observed in Animals (Rats) of Control (Group B) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	F	6	156	123	+ +	Mul 1mm	LH	EHP	ED	Ac.Inf	MUL+HP	Not Protected
2	F	5	146	99	++	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	132	101	+++	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	124	97	+++	4mm	LH	HP+PP+ SQCC	FB	Ac.Inf	MFH +HP	Not Protected
5.	F	5	153	104	++	3mm	LH	HP+PP	OT	Bony Osts	MFH	Not Protected
6	M	6	134	101	Nil	2mm	Nil	EPP+SCI	ED	Bony Osts	HIC	Not Protected
7	F	6	156	113	++	2mm	LH	HP+PP-SQCC	FB	Bony Osts	SQCC	Not Protected
8	F	6	128	96	Nil	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	5	171	121	+++	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	5	168	106	++	Mul 1mm	LH	HP+PP SQCCI	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony osteoma

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.9: Lesions Observed in Animals (Rats) of Control (Group C) in Acetone after Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	167	101	+ +	1mm	SCB	EHP +PP	FB	Ac.Inf	HP+PP	Not Protected
2	M	6	127	95	+ +	2mm	LH	EHP+SQCC	FIB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	124	99	+ +	Mul 5mm	LH+SCB	EHP	MFH	Bony Osts	MUL+HP	Not Protected
4	M	6	145	100	+ -	3mm	LH +SCB	HP+PP	FB	Ac.Inf	DYS+OT	Not Protected
5	F	6	154	102	+ +	3mm	LH	HP+PP	OT	Bony Osts	DYS+OT	Not Protected
6	M	6	172	121	+ +	Mul 4mm	SCB	EPP+SCI	MFH	Bony Osts	HIC+CCIS	Not Protected
7	M	5	137	102	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	M	6	166	115	Nil	4mm	LH+SCB	EHP+DYPS CI	MFH	Ac.Inf	MUL+HP	Not Protected
9	F	5	139	95	+ + -	Mul 3mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	5	156	100	+ + +	Mul 1mm	LH	HP+PPSQC CI	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.10: Lesions Observed in Animals (Rats) of Control (Group D) in Pet ether After Applying Experimental protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	156	103	+ +	3mm	LH	SQCC+PP	ED	Bony Osts	HP	Not Protected
2	M	6	166	111	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	5	139	98	+ + +	5mm	LH	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	M	5	128	96	+ + +	4mm	LH	HP+PP+SQCC	FIB	Ac.Inf	DYS+OT	Not Protected
5	M	6	169	115	+ +	3mm	LH	HP+PP	OT	Bony Osts	HIC	Not Protected
6	M	6	126	95	Nil	2mm	LH	EPP+SCI	MFH	Bony Osts	MFH	Not Protected
7	M	6	153	108	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC +DYP	Not Protected
8	M	6	146	113	Nil	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	HP	Not Protected
9	F	5	126	106	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	HP+SCI	Not Protected
10	F	6	136	94	+ +	3mm	LH	SQCC+PP	ED	Bony Osts	HP	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.11: Lesions Observed in Animals (Rats) of Control (Group E) in Methanol After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	F	5	152	109	+ +	Mul 1mm	LH+SCB	EHP+PP	FB	Ac.Inf	MULSCI	Not Protected
2	M	5	161	105	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	5	124	95	+ + +	3mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	145	114	+ + +	4mm	LH	HP+PAP	FB +OT	Ac.Inf	DYS+OT	Not Protected
5	M	6	162	124	+ +	3mm	LH	HP+PAP	OT	Bony Osts	DYS+PAP	Not Protected
6	M	6	146	106	+ +	Mul 2mm	Nil	EPP+SCI	OT+	Bony Osts	MFH	Not Protected
7	M	6	163	118	+ +	2mm	LH	HP+PAP SCI	FB+OT	Bony Osts	SQCC +HIC	Not Protected
8	F	6	129	95	Nil	3mm	LH+SCB	EHP+PAPS CB	MFH	Ac.Inf	SQCC	Not Protected
9	M	5	166	112	+ + +	Mul4mm	LH	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	6	176	124	+ +	3mm	LH	HP+PAPSQ CC	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.12: Lesions Observed in Animals (Rats) of Control (Group F) in Dichloromethan After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	161	103	Nil	1mm	LH	EHP	ED	Bony Osts	MUL+HP	Not Protected
2	M	6	121	97	+ +	2mm	LH	EHP	FIB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	124	105	+ + +	Mul 1mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MUL+HIC	Not Protected
4	F	6	134	102	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	145	100	+ +	3mm	LH	HP+PP	OT	Bony Osts	DYS+PAP	Not Protected
6	M	5	126	96	+ +	2mm	Nil	EPP+SCI	NOR	Ac.Inf	MFH	Not Protected
7	F	5	125	96	+ +	5mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	M	6	139	100	+ +	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	5	166	106	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	6	167	110	+ +	Mul 2mm	LH	HP+PPSQCCI	ED	Bony Osts	MUL+SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.13: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Coronopus didyma* (Group G1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	158	111	+ +	Mul 1mm	LH	EHP	ED	Ac.Inf	MUL+HP	Not Protected
2	M	6	172	124	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	144	102	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	155	105	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	179	122	+ +	3mm	LH	HP+PP	OT	Bony Osts	HIC+CCIS	Not Protected
6	M	6	143	DEAD	+ + +	5mm	LH	EPP+SCI	FB	Bony Osts	MFH	Not Protected
7	F	5	145	258	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	F	6	164	102	Nil	3mm	LH	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	5	166	DEAD	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MULSQCC	Not Protected
10	F	5	147	DEAD	+ +	Mul 4mm	LH +SCB	HP+PPSQCCI	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.14: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Coronopus didyma* (Group G2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	167	105	+ +	4mm	LH	HP+PP	ED	Bony Osts	MFH	Not Protected
2	M	6	124	96	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	124	100	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	M	6	167	107	+ + +	4mm	LH	HP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	169	107	+ +	3mm	LH	HP+PP	OT	Bony Osts	DYS+PAP	Not Protected
6	M	5	145	112	Nil	2mm	Nil	EPP+SCI	FB+ED	Bony Osts	MUL+HIC	Not Protected
7	M	5	153	106	+ +	Mul2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC +HIC	Not Protected
8	F	6	138	DEAD	+ +	3mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MUL+HP	Not Protected
9	F	5	149	109	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	F	6	171	120	+ +	Mul 1mm	LH	HP+SQCC	ED	Ac.Inf	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT-Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.15: Lesions Observed in Experimental Animals Treated with Dichloromethane Extract of *Coronopus didymus* (Group G3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	172	101	+ +	Mul 3mm	LH	EHP+PPSQCC	MFH	Bony Osts	CCIS	Not Protected
2	F	6	143	96	Nil	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	F	6	125	94	+ + +	Mul 3mm	LH	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	145	111	+ + +	4mm	LH	HP+PP+SQCC	ED	Ac.Inf	DYS+OT	Not Protected
5	M	5	169	105	+ +	3mm	LH+SCB	HP+PP	OT	Ac.Inf	DYS+PAP	Not Protected
6	M	5	126	98	+ +	1mm	LH	EPP+SCI	MFH	Bony Osts	MFH	Not Protected
7	F	5	142	104	+ +	2mm	LH	PAP	FB	Bony Osts	SQCC+HIC	Not Protected
8	M	6	139	103	+ +	3mm	LH	EHP+DYP	MFH	Ac.Inf	HIC	Not Protected
9	M	5	172	121	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MFH	Not Protected
10	F	6	156	100	+ +	3mm	LH	HP+PAPSQCC	ED+FB	Bony Osts	CCIS	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.16: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Salsola kali* (Group H1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	164	112	Nil	2mm	LH	EHP	MFH	Ac.Inf	SQCC	Not Protected
2	M	6	154	109	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
3	F	6	155	DEAD	+ + +	Mul 4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
4	M	5	174	120	+ +	3mm	LH	HP+PAP	OT	Bony Osts	DYS+HIC	Not Protected
5	M	5	127	98	+ +	2mm	LH	EPP+SCI	FB+ED	Bony Osts	MFH	Not Protected
6	F	5	153	118	+ +	4mm	LH	HP+PAP SQCC	FB+ED	Bony Osts	SQCC+PAP	Not Protected
7	M	6	139	253	+ + +	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
8	M	5	166	236	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
9	M	6	156	230	Nil	3mm	LH	HP+PPSQCCI	ED	Bony Osts	SCI	Not Protected
10												

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.17: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Salsola kali* (Group H2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	174	121	+ +	2mm	LH	EHP+SQCC	OT	Ac.Inf	HIC	Not Protected
2	M	6	164	112	Nil	2mm	LH	EHP	MFH	Ac.Inf	SQCC	Not Protected
3	M	6	154	109	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	155	DEAD	+ + +	Mul 4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	174	120	+ +	3mm	LH	HP+PAP	OT	Bony Osts	DYS+HIC	Not Protected
6	M	5	127	98	+ +	2mm	LH	EPP+SCI	FB+ED	Bony Osts	MFH	Not Protected
7	F	5	153	118	+ +	4mm	LH	HP+PAP SQCC	FB+ED	Bony Osts	SQCC+PAP	Not Protected
8	M	6	139	253	+ + +	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	5	166	236	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	6	156	230	Nil	3mm	LH	HP+PPSQCCI	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.18: Lesions Observed in Experimental Animals Treated with Dichloromethane Extract of *Salsola kali* (Group H3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	145	273	+ +	Mul 2mm	LH	EHP	FB	Bony Osts	SCI	Not Protected
2	M	6	167	234	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	F	6	154	267	+ + +	3mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	M	6	145	256	+ + +	4mm	LH	HP+PAP+CCIS	FB+ED	Ac.Inf	DYS+OT	Not Protected
5	M	6	169	245	+ +	3mm	LH	HP+PAP	OT	Bony Osts	DYS+HIC	Not Protected
6	M	5	187	DEAD	+ +	Mul 2mm	LH	EPP+SCI	OT	Bony Osts	MUL+MFH	Not Protected
7	M	5	147	258	+ +	Mul 2mm	LH	HP+PAP SQCC	FB	Bony Osts	MUL+SQCC	Not Protected
8	F	6	176	253	Nil	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	HP	Not Protected
9	M	5	166	236	+ + +	4mm	LH+SCB	EHP+SCB	ED	Bony Osts	HP+HIC	Not Protected
10	M	6	145	230	+ +	2mm	LH	HP+PAPSQCC	PP+CCIS	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.19: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Capparis deciduas* (Group I-1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	175	273	+	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	F	6	145	234	+	Nil	Nil	EHP	NOR	Ac.Inf	SQCC	Less Protected
3	M	6	154	267	Nil	2mm	LH	EHP	NOR	Bony Osts	MFH	Less Protected
4	F	6	178	256	Nil	Nil	Nil	HP+DYS	FB	Nil	DYS+OT	Mild Protected
5	M	6	164	245	+	Nil	LH	PAP	FB	Bony Osts	DYS+PAP	Not Protected
6	F	6	145	236	Nil	Nil	Nil	EPH	NOR	Bony Osts	MFH	Less Protected
7	F	6	156	258	+	2mm	LH	EHP	FB	Bony Osts	SQCC	Not Protected
8	M	6	187	253	Nil	Nil	Nil	EHP	FB	Ac.Inf	MUL+HP	Mild Protected
9	M	5	135	236	Nil	2mm	Nil	EHP	FB	Ac.Inf	MUL+HP	Less Protected
10	M	6	156	230	+	Mul 1mm	LH	EHP	NOR	Bony Osts	SCI	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

Table-6.1.20: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Capparis decidua* (I-2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	161	264	+ +	Nil	Nil	EHP	ED	Ac.Inf	HP	Mild Protected
2	M	6	182	356	+ +	2mm	LH	EHP	ED	Ac.Inf	MILD HP	Mild Protected
3	M	5	173	345	Nil	Nil	Nil	EHP	FB	Ac.Inf	HP	Not Protected
4	F	6	167	365	Nil	Nil	Nil	NOR	FB	Ac.Inf	DYS	Mild Protected
5	M	6	169	214	+ +	1mm	LH	EHP	FB	Ac.Inf	PAP	Less Protected
6	M	6	181	245	Nil	1mm	Nil	SQCC	NOR	Ac.Inf	MILD HP	Mild Protected
7	F	5	183	245	+ +	Nil	LH	SQCC	ED	Ac.Inf	HP	Less Protected
8	M	6	199	213	+ +	Nil	Nil	EHP	FB	Ac.Inf	HP	Less Protected
9	M	5	146	241	+ +	Nil	Nil	EHP	ED	Ac.Inf	HP	Mild Protected
10	M	5	134	230	+ +	2mm	LH	EHP	ED	Ac.Inf	MILD HP	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema
HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema
Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

Table-6.1.21: Lesions Observed in Experimental Animals Treated with Dichloromethane Extract of *Capparis decidua* (Group I-3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	181	123	+ +	2mm	LH	EHP	FB	Ac.Inf	HP	Not Protected
2	M	6	161	101	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	124	98	+ + +	3mm	Nil	EHP+DYP	MFH	Ac.Inf	MFH	Not Protected
4	F	6	165	111	Nil	3mm	LH	HP+PAP	FB	Ac.Inf	OT	Not Protected
5	M	5	149	104	+ +	3mm	LH	HP+PAP	OT	Ac.Inf	HIC	Not Protected
6	M	5	186	112	+ +	2mm	Nil	EPP+SCI	OT	Ac.Inf	MFH	Not Protected
7	F	5	173	122	+ +	2mm	LH	HP+SQCC	FB	Ac.Inf	SQCC	Not Protected
8	M	6	132	123	Nil	2mm	LH	EHP+DYP	MFH	Ac.Inf	HP	Not Protected
9	M	5	167	109	+ + +	4mm	LH	EHP	OT	Ac.Inf	HP	Not Protected
10	M	6	151	103	+ +	1mm	LH	HP+PAP	MFH	Ac.Inf	MFH	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema
HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema
Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

Table-6.1.22: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Salvadora oleoides* (Group J1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	164	122	+ +	2 mm	LH+SCB	EHP+PP	FB	Bony Osts	HP+SQCC	Not Protected
2	F	6	178	104	+ +	Mul1mm	LH	EHP	FB	Ac.Inf	MFH+SQCC	Not Protected
3	M	6	187	123	+ + +	Mul 1mm	LH	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	167	114	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	HIC	Not Protected
5	M	5	187	118	+ +	3mm	LH	HP+PP	OT	Bony Osts	DYS+PAP	Not Protected
6	M	6	165	100	+ + +	Mul2mm	LH+SCB	EPP+SCI	FB	Ac.Inf	Mul+MFH	Not Protected
7	F	5	186	123	+ +	2mm	LH	HP+ SQCC	ED	Bony Osts	SQCC	Not Protected
8	F	6	154	114	+ +	3mm	LH	EHP+PP	MFH	Ac.Inf	SCI	Not Protected
9	F	5	168	118	+ + +	4mm	LH	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
10	F	6	169	115	+ +	3mm	LH	HP+CCIS	MFH	Bony Osts	CCIS	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.23: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Salvadora oleoides* (Group J2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	187	110	+ +	4mm	LH	EHP	ED	Ac.Inf	HP	Not Protected
2	M	6	143	106	+ +	2mm	LH	EHP	FB	Ac.Inf	SQCC	Not Protected
3	M	6	156	112	+ + +	Mul 2mm	LH+SCB	EHP+DYP	MFH	Bony Osts	Mul+DYP	Not Protected
4	F	6	145	108	+ + +	3mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	F	6	154	146	+ +	3mm	LH	HP+PP	OT	Ac.Inf	DYS+PAP	Not Protected
6	F	6	164	120	Nil	2mm	Nil	EPP+SCI	OT	Bony Osts	MFH	Not Protected
7	F	6	153	DEAD	+ + +	DEAD	LH	-----	-----	-----	-----	Not Protected
8	M	6	137	99	Nil	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MFH	Not Protected
9	M	5	167	101	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	HIC	Not Protected
10	M	6	153	111	+ +	Mul 1mm	LH	HP+PPSQCCI	ED	Ac.Inf	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.24: Lesions Observed in Experimental Animals Treated with Dichloromethane Extract of *Salvadora oleoides* (Group J3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	145	105	+ +	Mul 1mm	LH	EHP+SCB	FB	Bony Osts	MUL+HP	Not Protected
2	F	6	158	108	+ +	2mm	LH	EHP	ED	Ac.Inf	SQCC	Not Protected
3	M	6	186	112	+ + +	Mul 5mm	LH+SCB	EHP	MFH	Bony Osts	MFH	Not Protected
4	F	6	178	113	+ + +	4mm	LH	HP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	187	123	+ +	3mm	LH	HP+PP	OT	Bony Osts	HIC	Not Protected
6	F	5	163	102	+ +	2mm	LH +SCB	EHP+SCI	OT	Bony Osts	MFH	Not Protected
7	F	5	187	115	+ +	2mm	LH	HP+PAP SQCC	OT	Bony Osts	SQCC	Not Protected
8	M	6	153	114	+ +	3mm	LH	EHP+DYP	MFH	Ac.Inf	HIC	Not Protected
9	F	5	168	115	+ + +	4mm	LH	EHP	MFH	Bony Osts	CCIS	Not Protected
10	F	6	178	124	+ +	Mul 1mm	LH+SCB	PAP +SQCCI	ED	Bony Osts	Mul +SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema
HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema
Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.25: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Withania coagulans* (Group K1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	189	273	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	F	6	161	234	+	Nil	Nil	EHP	NOR	Ac.Inf	MUL+HP	Mild Protected
3	M	6	164	267	Nil	Nil	Nil	EHP+DYP	NOR	Ac.Inf	DYS	Mild Protected
4	F	6	185	256	Nil	Nil	Nil	HP	NOR	Ac.Inf	DYS	Mild Protected
5	M	5	179	245	Nil	Nil	Nil	HP	NOR	Ac.Inf	DYS	Mild Protected
6	F	6	156	236	Nil	Nil	Nil	EHP	NOR	Ac.Inf	PAP	Mild Protected
7	F	5	153	258	Nil	Nil	LH	EHP	FIB	Ac.Inf	SQCC	Mild Protected
8	M	6	178	253	Nil	Nil	Nil	EHP	FIB	Ac.Inf	MUL+HP	Less Protected
9	M	5	156	236	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Less Protected
10	M	6	176	230	Nil	Nil	LH	HP	ED	Ac.Inf	HP	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema
HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema
Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

Table-6.1.26: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Withania coagulans* (Group K2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	198	373	Nil	Mul 1mm	LH.Sc	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	M	6	156	234	Nil	Mul 1mm	LH	EHP	FB	Ac.Inf	MUL + HP	Mild Protected
3	M	6	176	367	Nil	Nil	Nil	EHP	FB	Ac.Inf	PAP	Mild Protected
4	F	6	145	256	Nil	Nil	Nil	HP+DYS	FB	Ac.Inf	NOR	Mild Protected
5	M	5	156	245	+	Nil	Nil	HP+DYS	ED	Ac.Inf	NOR	Mild Protected
6	M	5	167	236	Nil	Nil	Nil	EHP+DYS	ED	Ac.Inf	MFH	Mild Protected
7	F	5	168	258	Nil	Nil	Nil	EHP	FB	Ac.Inf	MFH	Mild Protected
8	M	6	198	353	+	Nil	Nil	EHP	MFH	Ac.Inf	MUL+HP	Mild Protected
9	M	5	168	236	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Less Protected
10	M	6	165	230	Nil	Nil	Nil	EHP	ED	Ac.Inf	PAP +OT	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.27: Lesions Observed in Experimental Animals Treated with Dichloromethane Extract of *Withania coagulans* (Group K3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	181	345	Nil	Mul 1mm	LH	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	M	6	161	352	Nil	Nil	Nil	EHP	ED	Ac.Inf	PAP	Mild Protected
3	M	6	144	324	Nil	Nil	LH.SC	EHP	MFH	Ac.Inf	MUL + HP	Mild Protected
4	F	6	148	342	Nil	Nil	LH	EHP	FB	Ac.Inf	DYS+OT	Mild Protected
5	M	5	187	383	Nil	Nil	Nil	EPH	FB	Ac.Inf	DYS+OT	Mild Protected
6	M	5	178	356	Nil	Nil	Nil	EHP+SQCC	ED	Ac.Inf	MFH	Mild Protected
7	F	5	187	389	Nil	Nil	LH	EHP	FB + ED	Ac.Inf	PAP+SQCC	Mild Protected
8	M	6	176	362	Nil	Nil	Nil	EHP+DYP	MFH	Ac.Inf	MUL+HP	Mild Protected
9	M	5	187	363	+	Nil	Nil	EHP+	ED	Ac.Inf	MUL+HP	Mild Protected
10	M	6	173	330	Nil	Nil	Nil	EHP	ED	Ac.Inf	SQCC	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.28: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Heliotropium strigosum* (Group L1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	F	6	167	375	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	M	6	156	267	Nil	Nil	Nil	EHP	FB	Ac.Inf	MUL + HP	Mild Protected
3	F	6	167	327	Nil	Nil	LH+ Sc	EHP	MFH	Ac.Inf	MFH	Less Protected
4	F	6	155	345	Nil	Nil	Nil	EHP	FB	Ac.Inf	HP	Mild Protected
5	M	5	179	356	Nil	Nil	Nil	EHP	OT	Ac.Inf	DYS+PAP	Mild Protected
6	M	5	186	345	Nil	Nil	Nil	EHP	NOR	Ac.Inf	MFH	Mild Protected
7	F	5	193	358	Nil	Nil	Nil	EHP	FB	Ac.Inf	MFH	Mild Protected
8	M	6	179	353	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
9	M	6	186	336	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
10	F	6	166	330	Nil	Nil	Nil	EHP	ED	Ac.Inf	HP	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.29: Lesions Observed in Experimental Animals Treated with Methanolic Extract of *Heliotropium strigosum* (Group L2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	5	165	367	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Mild Protected
2	M	6	134	223	Nil	Nil	Nil	EHP	FB+ED	Ac.Inf	PAP	Mild Protected
3	M	6	168	256	Nil	Nil	Nil	DYP	ED	Bony Osts	HP	Mild Protected
4	F	5	123	279	Nil	Nil	Nil	PAP	FB	Ac.Inf	PAP	Mild Protected
5	M	5	168	278	Nil	Mul 1mm	LH.Sc	HP+PP	ED	Bony Osts	MFH	Less Protected
6	M	5	157	289	Nil	Nil	Nil	EHP	NOR	Bony Osts	PAP	Mild Protected
7	M	6	187	289	Nil	Nil	Nil	EHP	FB	Bony Osts	SQCC	Mild Protected
8	M	6	178	353	Nil	Mul 1mm	Nil	EHP	ED	Ac.Inf	MUL+HP	Less Protected
9	M	5	168	236	Nil	Nil	Nil	EHP	NOR	Ac.Inf	MUL+HP	Mild Protected
10	M	6	187	330	Nil	Nil	Nil	EHP	NOR	Bony Osts	HP	Mild Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.30: Lesions Observed in Experimental Animals Treated with Dichloromethane of *Heliotropium strigosum* (Group L3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	167	273	Nil	Nil	Nil	EHP	ED	Ac.Inf	HP	Mild Protected
2	M	6	156	234	Nil	Nil	Nil	EHP	ED	Ac.Inf	HP	Mild Protected
3	M	6	187	338	Nil	Nil	Nil	EHP	ED	Ac.Inf	DYS+PAP	Mild Protected
4	F	6	145	256	Nil	Nil	Nil	PAP	FB	Ac.Inf	DYS+PAP	Mild Protected
5	M	6	169	245	Nil	Nil	Nil	PAP	FB	Ac.Inf	DYS+PAP	Mild Protected
6	M	5	145	236	Nil	Nil	Nil	EHP	FB	Ac.Inf	HP	Mild Protected
7	F	5	153	314	Nil	Nil	Nil	EHP	FB	Ac.Inf	HP	Mild Protected
8	M	6	167	256	Nil	Nil	Nil	EHP	ED	Ac.Inf	MUL+HP	Less Protected
9	M	5	166	234	+	Nil	Nil	DYP	ED	Ac.Inf	MUL+HP	Less Protected
10	M	6	187	356	Nil	Nil	Nil	EHP	ED	Ac.Inf	DYS+PAP	Less Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.31: Lesions Observed in Experimental Animals Treated with Pet Ether Extract of *Tamarix aphylla (*Group M1) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	F	5	167	102	+ +	Mul 1mm	LH	EHP	OT	Ac.Inf	HIC	Not Protected
2	F	6	175	115	+ +	2mm	LH	EHP	FB+ED	Ac.Inf	SQCC	Not Protected
3	M	6	156	101	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	146	100	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	167	114	+ +	3mm	LH	HP+PP	OT	Bony Osts	DYS+PAP	Not Protected
6	M	6	156	121	+ +	Mul 1mm	Nil	EPP+SCI	FB	Bony Osts	HIC	Not Protected
7	F	5	147	113	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	M	6	156	115	+ +	Mul 1mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	6	183	124	+ + +	Mul 1mm	LH+SCB	EHP+SCB	ED	Ac.Inf	MUL+HP	Not Protected
10	M	6	146	100	+ +	Mul 2mm	LH +SCB	HP+PPSQCCI	ED	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.32: Lesions Observed in Experimental Animals Treated with Methanolic of *Tamarix aphylla* (Group M2) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	M	6	145	105	+ +	Mul 1mm	LH	EHP	ED	Bony Osts	MUL+HP	Not Protected
2	M	6	176	111	+ +	2mm	LH	EHP	FB	Bony Osts	SQCC	Not Protected
3	M	6	175	121	+ + +	Mul 5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	187	123	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	HIC	Not Protected
5	M	6	147	115	+ +	3mm	LH	HP+PP	OT	Ac.Inf	DYS+PAP	Not Protected
6	M	5	178	114	Nil	2mm	LH	EPP+SCI	FB	Bony Osts	MFH	Not Protected
7	F	5	153	114	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	M	6	165	102	Nil	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	MUL+HP	Not Protected
9	M	5	176	131	+ + +	4mm	LH+SCB	EHP+SCB	ED	Ac.Inf	HIC	Not Protected
10	M	6	157	121	+ +	Mul 1mm	LH	HP+PPSQCCI	MFH	Ac.Inf	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.33: Lesions Observed in Experimental Animals Treated with Dichloromethane of *Tamarix aphylla* (Group M3) After Applying Experimental Protocol

S. No.	Sex	Mass	Wt. (g)		Gross Examination			Microscpic Examination			Diognosis	Protection
			Before	After	Ulcer	Mass	Other	Epidermis	Dermis	Other		
1	F	6	145	111	+ +	4mm	LH	EHP+SQCC	ED	Bony Osts	MUL+HP	Not Protected
2	M	6	165	123	+ +	2mm	LH	EHP	ED	Bony Osts	SQCC	Not Protected
3	M	6	174	104	+ + +	5mm	LH+SCB	EHP+DYP	MFH	Bony Osts	MFH	Not Protected
4	F	6	145	103	+ + +	4mm	LH	HP+PP+SQCC	FB	Ac.Inf	DYS+OT	Not Protected
5	M	5	163	114	+ +	3mm	LH	HP+PP	OT	Bony Osts	HIC	Not Protected
6	M	5	176	121	+ +	2mm	Nil	EPP+SCI	OT	Bony Osts	MFH	Not Protected
7	F	5	184	132	+ +	2mm	LH	HP+PP SQCC	FB	Bony Osts	SQCC	Not Protected
8	M	6	153	DEAD	+ +	3mm	LH+SCB	EHP+DYP	MFH	Ac.Inf	HIC	Not Protected
9	M	5	171	123	+ + +	4mm	LH+SCB	EHP+DYS	ED	Bony Osts	HIC	Not Protected
10	M	6	152	111	+ +	4mm	LH	HP+PPSCI	FB	Bony Osts	SCI	Not Protected

NOR- Normal
SCI-Severe chronic infection
HP- Hyperplasia
SCB- Scab formation
MUL- Multiple
OT- Ostema

HIC- Histiocytoma
SQCC- Squamous Cell Carcinoma
DYS- Dysplasia
EHP-Epidermal Hyperplasia
FB-Fibrosis
Bony Osts - Bony Ostema

Ac-Inf- Acute infection
CCIS- Severe chronic infection
PAP- Papilloma
LH- Loss of Hairs
MFH- Malignant fibrous histiocytoma
ED- Edema

M- Male
F- Female

Table-6.1.34: Percentage Composition of Synthetic Diet with Normal Amount of Vitamins and Minerals Administered to Experimental Animals (Rats) During the Study

Ingredients	%	Ingredients of Mineral mixture	gm/kg of Mineral mixture	Ingredients of vitamins mixture	gm/kg of Vitamin mixture
Casein	20	Calcium oxalate	550	Thiamin hydrochloride	600mg
Maize Starch	60	Potassium citrate monohydrate	220	Riboflavin	600mg
Cane sugar	10	Sodium chloride	74	Pyridokine hydrochloride	700mg
Corn oil	5	Magnesium chloride	55	Nicotinic acid	3gm
DL methionine	0.5	Potassium sulphate	52	Calcium pentothenate	1.6gm
Mineral mixture	3.5	Magnesium sulphate	4	Cyanocobalamin (B12)	1.0mg
Vitamin mixture	1.0	Ferrous sulphate	3	Vitamin A Retinyl acetate	1.2gm
Total	100	Zinc sulphate	1.6	Vitamin D3 Cholecalciferol	2.5mg
		Copper sulphate	0.4	Vitamin E	35mg
		Potassium iodide	1	Vitamin K	5.0mg
		Sucrose	40	Sucrose	

6.2: Antibacterial Activity

Table 6.2.1: Zone of Inhibition of Different Antibiotics (Controls)

Bacterial Strains	Positive Control							Negative Control		
	Zone of inhibition at 25µg/ml									
	AMP	AMX	LFLX	TCY	VCM	CPR	PNC	Pet ether	MeOH	$CHCl_3$
S. aureus	10	50	100	30	1.0	5.0	10	13	30	11
E. coli	10	80	150	50	5.0	1.0	20	10	17	10
P.aeruginosa	-	50	-	-	-	5.0	-	10	22	10
S.pneumoniae	-	-	20	20	5.0	5.0	-	15	15	12
B. subtilus	250	50	5.0	-	-	-	-	13	10	12
S.lutae	100	-	10	50	-	10	-	15	10	14

AMP = *Ampicillin*
CiPR = *Ciprofloxacin*
LFLX= *Levofloxacin*
TCY = *Tetracycline*
VCM = *Vancomycin*
AMX = *Amoxicillin*
PNC = *Penicillin*

Table 6.2.2: MIC values (µg/ml) of Methanol Extracts of Experimental Medicinal Plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali, Heliotropium strigosum and Coronopus didymus)*

Sr. No.	Bacterial Strains	Withania coagulans	Salvadora oleoides	Capparis decidua	Tamarix aphylla	Salsola kali	Heliotropium strigosum	Coronopus didymus
1-	*Staphylococcus aureus*	-	0.1	0.1	0.1	0.1	0.1	0.1
2-	*Escherichia coli*	-	0.1	0.1	0.1	-	0.5	0.1
3-	*pseudomonas aeruginosa*	0.1	25	0.1	0.1	25	0.5	0.1
4-	*Streptococcus pneumoniae*	0.1	-	-	0.1	0.1	0.1	-
5-	*Bacillus subtilus*	0.1	0.1	0.1	-	0.1	-	0.1
6-	*Sarcina lutae*	0.1	-	0.1	0.1	50	-	0.1
	Extract mg/g	879	366	890	197	380	139	333
ACTIVITY ml/g								
1-	*Staphylococcus aureus*	-	3660	8900	197	3800	1390	3330
2-	*Escherichia coli*	8790	3660	8900	197	-	278	3330
3-	*pseudomonas aeruginosa*	8790	14.64	8900	197	15.2	278	3330
4-	*Streptococcus pneumoniae*	8790	-	-	197	3800	1390	-
5-	*Bacillus subtilus*	8790	3660	8900	-	3800	-	3330
6-	*Sarcina lutae*	8790	-	8900	197	7.6	-	3330

Table 6.2.3: MIC values (µg/ml) of Pet Ether Extracts of Experimental Medicinal Plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali, Heliotropium strigosum and Coronopus didymus*)

Sr. No.	Bacterial Strains	Withania coagulans	Salvadora oleoides	Capparis decidua	Tamarix aphylla	Salsola kali	Heliotropium strigosum	Coronopus didymus
1-	*Staphylococcus aureus*	-	0.1	0.1	0.1	10	0.1	10
2-	*Escherichia coli*	-	0.5	0.1	0.1	-	10	0.5
3-	*pseudomonas aeruginosa*	0.1	10	0.1	0.1	-	10	10
4-	*Streptococcus pneumoniae*	0.1	-	-	0.1	0.1	0.5	-
5-	*Bacillus subtilus*	0.1	0.1	0.1	-	0.1	-	0.1
6-	*Sarcina lutae*	0.1	-	0.1	0.1	-	-	0.1
	Extract mg/g	49.0	66.0	49.2	19.9	14.0	62.2	52.5
ACTIVITY ml/g								
1-	*Staphylococcus aureus*	-	660	492	199	14	622	525
2-	*Escherichia coli*	-	132	492	199	-	6.22	105
3-	*pseudomonas aeruginosa*	490	660	492	199	-	6.22	5.52
4-	*Streptococcus pneumoniae*	490	-	-	199	140	124.4	-
5-	*Bacillus subtilus*	490	660	492	-	140	-	525
6-	*Sarcina lutae*	490	-	492	199	-	-	525

Table 6.2.4: MIC values (µg/ml) of Dichloro Methan Extracts of Experimental Medicinal Plants (*Withania coagulans, Salvadora oleoides, Capparis decidua, Tamarix aphylla, Salsola kali , Heliotropium strigosum and Coronopus didymus*)

Sr. No.	Bacterial Strains	Withania coagulans	Salvadora oleoides	Capparis decidua	Tamarix aphylla	Salsola kali	Heliotropium strigosum	Coronopus didymus
1-	*Staphylococcus aureus*	-	0.1	0.1	1.0	50	0.1	0.1
2-	*Escherichia coli*	-	0.1	0.1	1.0	-	0.5	0.1
3-	*pseudomonas aeruginosa*	0.1	10	0.5	1.0	-	0.1	0.1
4-	*Streptococcus pneumoniae*	0.1	-	-	1.0	10	10	-
5-	*Bacillus subtilus*	0.1	10	0.5	-	0.1	-	0.1
6-	*Sarcina lutae*	0.1	-	50	1.0	-	-	0.1
	Extract mg/g	32.0	78.0	33.8	20.2	59.0	38.6	61.0
ACTIVITY ml/g								
1-	*Staphylococcus aureus*	-	780	338	202	1.18	386	610
2-	*Escherichia coli*	-	780	338	202	-	77.2	610
3-	*pseudomonas aeruginosa*	320	7.80	6.76	202	-	386	610
4-	*Streptococcus pneumoniae*	320	-	-	202	5.90	3.86	-
5-	*Bacillus subtilus*	320	7.80	6.76	-	590	-	610
6-	*Sarcina lutae*	320	-	-	202	-	-	610

Table 6.2.5: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Withania coagulans* with Other Plants

Sr. No.	Bacterial Strains	*Withania coagulans*	*Withania coagulans*+ *Pinus wallichiana*	*Withania coagulans* +*Capparis decidua*	*Withania coagulans*+ *Salvadora oleoides*	*Withania coagulans*+ *Hypericum perforatum*	*Withania coagulans* +*Heliotropium strigosum*	*Withania coagulans*+ *Coronopus didymus*
1-	*Staphylococcus aureus*	-	0.1	0.1	0.1	25	0.1	0.1
2-	*Escherichia coli*	-	0.5	25	0.5	25	-	0.1
3-	*pseudomonas aeruginosa*	0.1	0.5	25	0.5	-	0.1	100
4-	*Streptococcus pneumoniae*	0.1	10	25	10	25	-	100
5-	*Bacillus subtilus*	0.1	-	0.5	0.5	-	0.5	100
6-	*Sarcina lutae*	0.1	-	25	10	-	-	100

Table 6.2.6: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Salsola kali* with Other Plants

Sr. No.	**Bacterial Strains**	***Salsola kali***	***Salsola kali* + *Pinus wallichiana***	***Salsola kali* +*Capparis decidua***	***Salsola kali* + Salvadora oleoides**
1-	*Staphylococcus aureus*	0.1	25	0.1	0.5
2-	*Escherichia coli*	-	0.1	0.1	0.1
3-	*pseudomonas aeruginosa*	25	0.1	0.1	0.5
4-	*Streptococcus pneumoniae*	0.1	0.5	0.1	0.1
5-	*Bacillus subtilus*	0.1	0.1	0.1	0.1
6-	*Sarcina lutae*	100	0.5	-	0.1

Table 6.2.7: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Capparis decidua* with Other Plants

Sr. No.	Bacterial Strains	*Capparis decidua*	*Capparis decidua+Pinus wallichiana*	*Capparis decidua + Coronopus didymus*	*Capparis decidua+ Sarcococca saligna*
1-	*Staphylococcus aureus*	0.1	25	0.1	0.5
2-	*Escherichia coli*	-	0.1	0.1	0.1
3-	*pseudomonas aeruginosa*	25	0.1	0.1	0.5
4-	*Streptococcus pneumoniae*	0.1	0.5	0.1	0.1
5-	*Bacillus subtilus*	0.1	0.1	0.1	0.1
6-	*Sarcina lutae*	100	0.5	-	0.1

Table 6.2.8: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Heliotropium strigosum* with Other Plants

Sr. No.	Bacterial Strains	***Heliotropium strigosum***	***Heliotropium strigosum* + *Hypericum perforatum***	***Heliotropium strigosum*+ *Pinus roxburgii* (Bark)**	***Heliotropium strigosum*+ *Salsola kali***
1-	*Staphylococcus aureus*	0.1	10	0.1	0.1
2-	*Escherichia coli*	0.1	10	10	0.1
3-	*pseudomonas aeruginosa*	0.1	-	0.1	-
4-	*Streptococcus pneumoniae*	-	10	0.5	-
5-	*Bacillus subtilus*	0.1	-	0.5	-
6-	*Sarcina lutae*	0.1	-	10	10

Table 6.2.9: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Salvadora oleoides* with Other Plants

Sr. No.	Bacterial Strains	*Salvadora oleoides*	*Salvadora oleoides* + *Impatients walleriana*	*Salvadora oleoides*+ *Anethum sowa*
1-	*Staphylococcus aureus*	0.1	0.1	0.1
2-	*Escherichia coli*	0.1	0.5	0.5
3-	*pseudomonas aeruginosa*	25	0.5	0.5
4-	*Streptococcus pneumoniae*	-	0.5	100
5-	*Bacillus subtilus*	0.1	0.5	0.5
6-	*Sarcina lutae*	-	0.5	250

Table 6.2.10: MIC values (µg/ml) of Synergistic Activity of Methanolic Extract of *Coronopus didymus* with Other Plants

Sr. No.	Bacterial Strains	*Coronopus didymus*	*Coronopus didymus+ Hypericum perforatum*	*Coronopus didymus+ Pinus wallichiana(Bark)*	*Coronopus didymus+ Sarcococca saligna*
1-	*Staphylococcus aureus*	0.1	10	250	10
2-	*Escherichia coli*	0.1	0.5	0.1	50
3-	*pseudomonas aeruginosa*	0.1	10	0.5	100
4-	*Streptococcus pneumoniae*	-	25	0.1	10
5-	*Bacillus subtilus*	0.1	-	10	-
6-	*Sarcina lutae*	0.1	10	10	-

6.3 Antifungal Activity

Table 6.3.1: In Vitro Antifungal Activity of Pet Ether Extracts of Ethnomedicinal Plants

Fungal strains	Mean of Zone of inhibition in mm						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Aspergillus flavus	18	17	04	20	21	21	15
Fusarium laterifum	20	17	17	17	20	20	18
Aspergillus fumigatus	22	22	18	14	21	22	17
Candida albicans	22	24	17	19	25	16	23
Trichophyton mentogrophytes	19	23	23	13	24	14	22
Microsporum canis	20	18	22	15	24	22	17
Trichoderma viridis	18	14	24	21	21	03	18
Control							
Ketoconazole	12	15	14	20	21	21	15
Econazole	14	18	19	21	20	20	21
Nystatin	22	17	17	22	17	21	24
Amphotericin	16	26	18	23	18	23	21
Clotrimazole	18	19	17	18	19	21	22
Miconazole	18	17	14	20	21	21	15

A- *Coronopus didyma* B- *Salsola kali* C- *Capparis decidua*
D- *Salvadora oleoides* E- *Withania coagulans* F- *Heliotropium strigosum*
G- *Tamarix aphylla*

Table 6.3.2: In Vitro Antifungal Activity of Methanolic Extracts of Ethnomedicinal Plants

Fungal strains	**Zone of inhibition in mm**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Aspergillus flavus	21	17	16	20	21	21	14
Fusarium laterifum	20	17	17	17	21	21	17
Aspergillus fumigatus	20	22	17	14	29	21	18
Candida albicans	22	17	16	19	22	22	17
Trichophyton mentogrophytes	20	23	21	13	22	22	19
Microsporum canis	21	16	22	15	20	21	21
Trichoderma viridis	23	17	21	21	30	17	22
Control							
Ketoconazole	12	15	14	20	21	21	15
Econazole	14	18	19	21	20	20	21
Nystatin	22	17	16	22	17	21	24
Amphotericin	16	25	17	23	18	23	21
Clotrimazole	18	19	17	18	19	21	22
Miconazole	18	17	14	20	21	21	15

A- *Coronopus didyma* B- *Salsola kali* C- *Capparis decidua*
D-*Salvadora oleoides* E- *Withania coagulans* F- *Heliotropium strigosum*
G- *Tamarix aphylla*

Table 6.3.4: In Vitro Antifungal Activity of Dichloro Methane Extracts of Ethnomedicinal Plants

Fungal strains	**Zone of inhibition in mm**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Aspergillus flavus	22	12	17	20	21	22	15
Fusarium laterifum	15	10	12	17	22	14	18
Aspergillus fumigatus	10	13	13	20	22	22	17
Candida albicans	14	11	13	22	23	24	23
Trichophyton mentogrophytes	14	23	15	16	20	23	22
Microsporum canis	10	18	22	24	23	21	17
Trichoderma viridis	11	19	24	14	21	22	18
Control							
Ketoconazole	11	15	14	20	21	21	15
Econazole	14	18	19	21	20	20	21
Nystatin	21	17	16	22	17	21	24
Amphotericin	16	25	17	23	18	23	21
Clotrimazole	18	19	17	18	19	21	22
Miconazole	18	17	14	20	21	21	15

A- *Coronopus didyma* B- *Salsola kali* C- *Capparis decidua*
D-*Salvadora oleoides* E- *Withania coagulans* F- *Heliotropium strigosum*
G- *Tamarix aphylla*

Table 6.3.5: Ethnomedicinal Plant Extracts are Compared with Other Antifungal Drugs Using ANOVA Critical Value $F.05_{(6, 36)} = 2.38$

Plant Extracts		Pet ether	Methanol	Dichloro methan
Coronopus didyma	**F-value**	1.96	2.34	1.56
	Conclusion	Insignificant	Insignificant	Significant
Salvadora oleoides	**F-value**	2.12	1.98	2.25
	Conclusion	Insignificant	Insignificant	Insignificant
Tamarix aphylla	**F-value**	1.97	2.67	1.67
	Conclusion	Insignificant	Significant	Insignificant
Salsola kali	**F-value**	2.12	2.78	1.89
	Conclusion	Insignificant	Significant	Insignificant
Withania coagulans	**F-value**	2.67	3.98	2.99
	Conclusion	Sgnificant	Significant	Significant
Capparis decidua	**F-value**	2.39	2.98	2.54
	Conclusion	Sgnificant	Significant	Significant
Heliotropium strigosum	**F-value**	2.89	2.42	2.76
	Conclusion	Sgnificant	Significant	Significant

7. DISCUSSION

7. DISCUSSION

7.1 Anticancer Activity

For biological activity seven popular Ethnomedicinal plants collected from Southern Punjab namely *Capparis decidua* (Capparidaceae), *Coronopus* didymus (Brassicaeae), *Heliotropium strigosum* (Boraginaceae), *Salsola kali* (Chenopodiaceae), *Salvadora oleoides* (Salvadoraceae), *Tamarix aphylla* (Tamaricaceae) and *Withania coagulans* (Solanaceae) were collected, dried away from the sunlightand extracted in methanol for microbial and anticancer activity.(**Table 6.1.2**)

Topical anti tumor activity of these seven ethno medicinal plants have been investigated in detail for the first time. No tumor was observed in the control group (A) (**Table 6.1.5**). All animals received topical application of DMBA and TPA except group A. All other groups' animals developed benign lesions which were epidermal hyperplasia, dysplasia, papilloma and osteoma. The malignant lesions which were squamous cell carcinoma in situ, squamous cell carcinoma, malignant fibrous histiocytoma were also produce in all groups except the group A.

Most of the rats except group A had chronic inflammation and precancerous changes in early weeks. Hair loss was observed on specific area in the third week where DMBA and TPA were applied locally and post application of DMB and TPA, slight bleeding and ulceration which was not too deep was observed at the 14th week. Small size out growths (pinkish white colour) were also observed (Papilloma) at 15th week in the treated area.

The group-A (control) animals did not receive any application of DMBA and TPA or any other treatment. No tumor was produced in these animals. (**Table 6.1.6**)

All the groups B-M animals received the application of DMBA and TPA only. In all the animals' benign lesions which were epidermal hyperplasia, papilloma and dysplasia and

malignant lesions squamous cell carcinoma, squamous cell carcinoma in situ and malignant fibrous histiocytoma after 15 weeks of carcinogenesis were developed. (**Table 6.1.7**)

Chemotherapy of any type was not given to group B animals so all the benign lesion and malignant lesions remained the same as after the 30th week. All the animals were in very bad condition, their ulcer were bleeding and they lost their weight and hairs. These animals were used only to observe the different lesions developed with chemical carcinogens. (DMBA & TPA) (**Table 6.1.8**)

The animals of group C, D, E and F received chemotherapy of solvents only (Acetone, Methanol, Pet ether and Dichloromethane), their entire benign lesion and malignant lesions remained the same as they were before chemotherapy. These results showed that these solvents did not cure the malignant or benign lesions. (**Table 6.1.9 – 6.1.12)**

The animals of group G-M were divided into three subgroups (G-1, G-2, G-3-M-1, M-2, M-3) according to pet ether, methanol and dichloromethane extracts of tested medicinal plants.

The animals of group G-1 received chemotherapy of a dose of l0µg/ml of pet ether extract of *Coronopus didymus*. All the animals of group G1 were not cured; their malignant or benign lesions remained the same. Three rats were dead. There were seven animals who developed malignant lesions, squamous cell carcinoma, squamous cell carcinoma insuit, histiocytoma and malignant fibrous and all the animals suffered from bony ostema. (**Table 6.1.13**)

The animals of group G-2 received chemotherapy with methanolic extract of *Coronopus didymus*. After the chemotherapy all the animals of group G2 were not cured and their malignant or benign lesions remained the same. One rat was dead. (**Table 6.1.14**) Similarly group of G-3 receiving chemotherapy of a dose of dichloromethane extract of *Coronopus didymus* were not. (**Table 6.1.15**)

The animals of group H (H_1, H_2, and H_3) received extracts of *Salsola kali* were not cured. (**Table 6.1.16- 6.1.18**)

The animals of group I (I-1, I-2, I-3) received chemotherapy with pet ether extract of *Capparis decidua*. After chemotherapy all the animals of group I-1 were cured which were suffering from benign lesions while squamous cell carcinoma in situ, squamous cell carcinoma and malignant fibrous hyperplasia were not cured, however, there were no death of any animal in this group and no further progression of malignancy was seen in this group. All animals in this sequence of tests that had been cured appeared to be healthy, with sleek fur weight gained. (**Table 6.1.19**)

The animals of group I-2 received chemotherapy of a dose of l0µg/ml of methanolic extract of *Capparis decidua* for next 15 weeks. When chemotherapy was given 08 animals were cured which were suffering from benign lesions while squamous cell carcinoma in situ, squamous cell carcinoma and malignant fibrous hyperplasia were not cured, however, there were no death of any animal in this group and no further progression of malignancy was seen in this group. (**Table 6.1.20**)

The animals of group I-3 received chemotherapy of dichloromethane extract of Capparis decidua were not cured. (**Table 6.1.21**)

The animals of group J-1, J-2 and J-3 were not cured, and their malignant or benign lesions remained the same. One rate died during experimental period in group J-3, (**Table 6.1.22 - 24**).

The animals of group K (K-1, K-2, and K-3) after 15 weeks of carcinogenesis received chemotherapy of a dose of l0µg/ml of *Withania coagulans* extracts for next 15 weeks.

When chemotherapy was given to the animals of group K-1 with pet ether extract of *Withania coagulans* all the animals of group K-1 were cured which were suffering from benign lesions while squamous cell carcinoma in situ, squamous cell carcinoma and malignant fibrous hyperplasia were mildly cured and no further progression of malignancy was seen in this group. All animals in this sequence of tests that have been cured and appeared to be healthy, with sleek fur weight gained. This study was very encouraging as it suggested that the pet ether extract of *Withania coagulans* given in pre malignant phases of tumor development, cured benign lesions and decreased the risk of

malignant transformation. There was an immense scope for further research on this plant extract as an anti tumor agent. (**Table 6.1.25**)

The animals of group K-2 also received chemotherapy of methanolic extract of *Withania coagulans*. Animals which were suffering from benign lesions and squamous cell carcinoma in situ were cured. While mild recovery from malignant fibrous hyperplasia was observed. The methanolic extract of *Withania coagulans* gave best results. (**Table 6.1.26**)

The animals of group K-3 received chemotherapy of dichloromethane extract of *Withania coagulans* for next 15 weeks. All the animals were cured. (**Table 6.1.27**)

The animals of group L (L-1, L-2, and L-3) received chemotherapy with pet ether, methanol and dichloromethane extracts of *Heliotropium strigosum* respectively.

When chemotherapy was given to all the animals of group L-1 were cured which were suffering from benign lesions while squamous cell carcinoma in situ ,squamous cell carcinoma and malignant fibrous hyperplasia were mildly cured and no further progression of malignancy was seen in this group. There is an immense scope for further research on this plant extract as an anti tumor agent. (**Table 6.1.28**)

The animals of group L-2 received chemotherapy with methanolic extract of *Heliotropium strigosum* and all the animals were cured from benign lesions while squamous cell carcinoma in situ, squamous cell carcinoma and mildly cured from malignant fibrous hyperplasia. However there were no death of any animal in this group and no further progression of malignancy was seen in this group. The methanolic extract of *Heliotropium strigosum* had potential for anti cancer activity. (**Table 6.1.29**)

The animals of group L-3 received chemotherapy with Dichloromethane extract of *Heliotropium strigosum* and all animals were cured (**Table 6.30**).These resuts were in agreement with the findings of Barclay, A.S, (2006) that whole plant (root, stem, leaves and flower) of *Heliotropium curassavicum* (collection No.1618) showed antitumor activity. [TWBAWP, 2006]

The animals of group M (M1, M2, and M3) received chemotherapy of a dose of pet ether methanol and dichloromethane extracts of *Tamarix aphylla* respectively. After the chemotherapy all the animals of group M-1, M-2 and M-3 were not cured, and their malignant or benign lesions remained the same. (**Table 6.1.32- 6.1.33**)

This study was very encouraging. The plant extracts of *Heliotropium strigosum* and *Withania coagulans* showed best result for anti tumor activity .The methanolic extract of *Withania coagulans* and *Capparis decidua* were shown to have good potential as anticancer agents for the first time.

7.2 Antibacterial Activity

Antibacterial activity of different extracts of ethnomedicinal plants were checked by using agar well diffusion method [Carron, *et. al.*, 1987] against six different species of bacterial strains i.e. *Staphylococcus aureus, Escherichia coli, Bacillus subtilus, streptococcus pneumoniae, Sarcine lutae and Pseudomonas aeruginosa* The zones of inhibitions were measured and evaluate the MIC. Methanolic, pet ether and Dichloromethane extract of all plants (*Coronopus didymus, Salsola kali, Capparis decidua, Salvadora oleoides, Withania coagulans*, *Heliotropium* strigosum and *Tamarix aphylla*) with concentration (250, 100, 50, 10, 0.5 and 0.1µg/ml) were used against each of the six bacterial strains. (**Table 6.2**)

From the results obtained it is observed that methanol was the best solvent for extracting antimicrobial substances for tested medicinal Plant based on the number of organisms inhibited and the diameter of inhibitory zones produced. It could also be seen that different extracts were different in their antimicrobial effectiveness depending on the extractive solvent used. This result agrees favorably with the suggestion of Oloke and Kolawole (1988) that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. (**Table 6.2.1**)

The methanolic extract of *Coronopus didymus* showed highest activity against *Sarcine lutae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and B. subtilis*

(3310 ml/g) except *Streptococcus pneumoniae*. The pet ether extract of *Coronopus didyma* showed poor activity against the *Staphylococcus aureus and B. subtilis* (525ml/g), *Escherichia coli* (105ml/g) and *Pseudomonas aeruginosa* (5.25 ml/g). The dichloromethane extract of Coronopus didyma showed very poor antibacterial activity against the *Escherichia coli, Bacillus subtilus, Streptococcus pneumoniae and Staphylococcus aureus* (610 ml/g) while it totally susceptible to *Pseudomonas aeruginosa.* (**Table 6.2.2-4**)

The pet ether extract of Salsola kali showed non significant result against all the strains. The dichloromethane extract of *Salsola kali* showed the same result. The methanolic extract of *Salsola kali* showed highest antibacterial activity against the *Staphylococcus aureus, Bacillus subtilus* and *Streptococcus pneumoniae* (3800ml/gm). This extract inhibited the growth of *Escherichia coli*. (**Table 6.2.2-4**)

An excellent activity showed by crude methanolic extract of *Capparis decidua* against all the tested bacterial strains (8900 ml/gm) except the *Streptococcus pneumoniae*. The pet ether extract of *Capparis decidua* showed the insignificant against the *Sarcine lutae, B. subtilis* , *Escherichia coli , Pseudomonas aeruginosa* and *Staphylococcus aureus* (492 ml/g).The dichloromethane extract of *Capparis decidua* showed the same result of pet ether extract. (**Table 6.2.2-4**)

The pet ether extract of *Salvadora oleoides* showed poor activity against *all the strains* (660 ml/g). The crude methanolic extract of *Salvadora oleoides* showed good activity against Staphylococcus *aureus, Escherichia coli and Bacillus subtilus* (3660 ml/g) only. The dichloromethane extract of *Salvadora oleoides* showed worst antibacterial activity against all the bacterial strains. Their activity value was 7.80 ml/g only. (**Table 6.2.2-4**)

The methanolic extract of *Withania coagulans* showed best activity against *Pseudomonas aeruginosa, Streptococcus pneumoniae, Bacillus subtilus, Escherichia coli and Sarcine lutae* (8790 ml/g) while it inhibited the growth of bacteria against *Staphylococcus aureus*. The crude pet ether extract of *Withania coagulans* showed insignificant activity against the *Bacillus subtilus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Sarcine lutae* (490 ml/gm). The dichloromethane extract of *Withania coagulans* totally inactive

against *Bacillus subtilus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Sarcine lutae* (320 ml/g). This extract inhibits the growth of *Staphylococcus aureus* and *Escherichia coli.* (**Table 6.2.2-4**)

All the extracts of *Heliotropium strigosum* showed very poor activity against the all tested strains. The value of bacterial activity of pet ether extract of *Heliotropium strigosum* is 622 ml/g. The crude methanolic and dichloromethane extracts result were 278 ml/g, 77.2 ml/g.

Tamarix *aphylla* methanolic, dichloromethane and pet ether extracts showed por activity against the all the tested bacterial strains. (197 ml/g, 202 ml/g, 199 ml/g)

The antibacterial activity of the extracts of the tested medicinal plants showed better activity as compared with the different commercially available antibiotics like Ampicillin, Amoxicillin, Levofloxacin, Tetracycline, Vancomycin, Ciprofloxacin and Penicillin. (**Table 6.2.1**)

7.3 Synergistic Bacterial Activity

The synergistically screening of the methanolic extract of the selected medicinal plants with each other and with other eight popular plants collected from north Punjab namely *Hypericum perforatum* (Hypericaceae), *Pinus wallichiana* (Bark) (Pinaceae), *Gallium asperuloides* (Rubiaceae),Senecio chrysanthemoides (Asteracea), *Sarcococca saligna* (Buxiaceae), *Impatients walleriena* (Balsaminaceae), *Anethum sowa*(Apiaceae) and *Pinus roxburgii* (Bark) (Pinaceae) were done against six bacterial strains namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae, Bacillus subtilus* and *Sarcina lutae*. (**Table 6.3**)

The crude methanolic extract of *Withania coagulans* synergistically responded good with the methanolic extracts of *Pinus wallichiana (Bark), Heliotropium strigosum* and *Salvadora oleoides*. Synergistic activity (inhibition is doubled compared to one of the constituent) was observed in as low in amounts as 1µg/ml (1000µg/ml) methanolic extract of *Withania coagulans mixed with Heliotropium strigosum.* The growth of

Streptococcus pneumoniae and E.coli inhibited completely. *Withania coagulans* showed insignificant result with other methanolic plant extracts. (**Table 6.2.5**)

Methanolic extract of *Salsola Kali* with Heliotropium *strigosum* and *Senecio chrysanthemoides and Gallium asperuloides* gave good response against the S. *aureus* and *S. pneumoniae*. (**Table 6.2.6**)

The methanolic extract of *Capparis decidua* with *Pinus wallichiana (Bark) and Sarcocooa saligna* showed highest activity against all the tested bacterial strains. Synergistically Capparis decidua and Coronopus didymus showed insignificant result against all the strains. (**Table 6.2.7**)

Salvadora oleoides methanolic extract showed best result with *Impatiens walleriana and* moderate activity with *Anethum sowa.* (**Table 6.2.9**)

Heliotropium strigosum showed best synergistic activity with *Pinus roxburgii* (bark) only. The growth of all tested bacterial growth was asserted by mixing the methanolic extracts of *Heliotropium strigosum and Hypericum perforatum.* (**Table 6.2.8**)

The extracts of *Coronopus didymus* and *Salsola kali* showed poor activity against *P. aeruginosa S. pneumoniae Sarcina lutae, Streptococcus mutans .B.sutilus and S. aureus* while *Coronopus didymus* methanolic extract with the extracts of *Pinus wallichiana* (Bark) and *Sarcocooa saligna* showed good activity against some tested strains. (**Table 6.2.10**)

7.4 Antifungal Activity

Antifungal activity of different extracts of ethno medicinal plants were checked by using serial dilution method [Atta *et. al.* (2001)] against seven different strains of fungal strains i.e. *Trichoderma viridis, Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Microsporum canis and Candida albicans.* The zones of inhibitions were measured and statistical analysis was applied on the results of antifungal assay. Methanolic, pet ether and Dichloromethane extract of all plants (*Coronopus didyma, Salsola kali, Capparis decidua, Salvadora oleoides, Withania coagulans, Heliotropium strigosum and Tamarix aphylla)* at the concentration of 25µg/ml were used against each of the seven fungal strains. The fungal strains were checked against the following standards Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole as positive control. (**Table 6.4**)

The pet ether, methanolic and Dichloromethane extract of *Coronopus didymus* showed best activity against all the tested fungal strains *Microsporum canis, Trichophyton mentogrophytes. Aspergillus fumigatus, Fusarium laterifum, Aspergillus flavus*, *Trichoderma viridis* and *Candida albicans*. (**Table 6.3.2**) All the extracts of *Coronopus didymus* showed better result as compared to the antifungal standards. Statistically analysis showed the results were not significant against any fungal strain.

Salsola kali methanolic extract showed highest activity against *Trichophyton mentogrophytes, Trichoderma viridis, Aspergillus fumigatus*, *Aspergillus flavus, Fusarium laterifum, Microsporum canis* and *Candida albican.* (**Table 6.3.3**) The F-value conclusion showed significant result (F-Value 2.78). The Dichloromethane and pet ether extract of *Salsola kali* showed in significant result against *Trichoderma viridis, Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Microsporum canis and Candida albicans*. The methanolic extract showed significant result against the standards. (Antifungal) (**Table 6.3.4**)

All the extracts of *Capparis decidua* showed significant activity against all the tested fungal strains. The F- value of pet ether extract was 2.39, methanolic extract value 2.98 and dichloromethane extract was 2.54. (**Table 6.3.5**)

The extracts of *Salvadora oleoides* showed zone of inhibition against the all fungal strains but their zone of inhibition was not more significant as compared to the antifungal standards (Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole). Their extracts showed zone of inhibition against all the fungal strains but their zone of inhibition was not more significant as compared to the antifungal standards (Ketoconazole, Econazole, Nystatin, Amphotericin, Clotrimazole and Miconazole). Statistically all the results were insignificant.

The pet ether, methanolic and dichloromethane extract of *Withania coagulans* showed highest activity against all the tested fungal strains *Trichoderma viridis, Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Microsporum canis* and *Candida albicans.*Their caculated F-vaules were greater then the F-table value. (2.67, 3.98 and 2.99) (**Table 6.3.5**)

The dichloromethane, Methanolic and pet ether extracts of *Heliotropium strigosum* showed significant activity against *Aspergillus flavus, Fusarium laterifum, Aspergillus fumigatus, Trichophyton mentogrophytes, Trichoderma viridis*, Microsporum *canis,* and *Candida albicans.* (**Table 6.3.5**)

The methanolic extract of *Tamarix aphylla* showed only the best activity against the fungal strains (F-vaue-2.67) while rest of the other extracts showed insignificant results. (**Table 6.3.5**)

SECTION C

8. ISOLATION OF BIOLOGICALLY ACTIVE COMPOUNDS

8. ISOLATION OF BIOLOGICALLY ACTIVE COMPOUNDS

8.1 Introduction and Historical Review

The search for medicinal chemicals from plants is an enormous task. According to a rough estimate, of the 250,000 plant species on earth only 2% have been thoroughly screened for chemicals with potential medicinal use. Though there is a large potential of exploration of plants for new drug discoveries it is feared that a number of plant species would be lost due to extensive use of herbs and plants if some sort of discipline is not applied in this field.

Discovery of plant medicines is a trial and error method. Most of the decoctions used in the folklore indeed yielded some valuable medicines like salicylic acid, the active ingredient in aspirin.

According to one estimate about 80,000 species of plants are utilized by the different system of Indian medicine [Prajapati *et.al.* 2006]. The codified traditions have about 25,000 plant drugs formulations that have emerged from indigenous knowledge. In addition to this over 50,000 formulations are believed to exist in the folk and tribal traditions as is described in The Vedas, epic. The Ayurveda system of medicine in subcontinent dates back to 1500 B.C. which grew into a respected and widely used system of healing in India. People from numerous countries came to Indian Ayurvedic schools to learn about this world medicine in its completeness. Chinese, Tibetans, Greeks, Romans, Egyptians, Afghanistan's, Persians, and more traveled to learn the complete wisdom and bring it back to their own countries.

According to the World Health Organization (WHO) more than 1 billion people rely on herbal medicines to some extent. The WHO has listed 21,000 plants have reported medicinal uses around the world. India has a rich medicinal plant flora of some 2500

species, of these, 2000 to 3000 at least 150 species are used commercially on a fairly large scale [http://www.botanical.com/ site/ column_ poudhia/open_university.html].

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by micro-organisms, insects, and herbivores.

8.2 Secondary Metabolites

Secondary metabolites are compounds which are not essential for growth and reproduction, but enhance plant prospects of survival. These are chemicals for which allow plants to ward off microorganisms, insects, and other animals harmful to them. There are about twenty thousand known secondary plant metabolites used for medicinal purposes to fight infections and diseases in human beings.

Plants have the ability to produce a large variety of secondary metabolites, such as terpenoids, phenylpropanoids, flavonoids, and alkaloids, which together account for over 200,000 compounds [Dixon, RA, *et. al*. 2003]. The National Cancer Institute has identified a host of compounds found in foods and plants that possess cancer preventing properties. Among these are antioxidants, phytosterols, carotenoids, triterpenes, saponins, tannins, and flavonoids. These phytochemicals may augment immune function, inhibit the formation of cancer-causing nitrosamines, hinder hormonal activity, as well as induce Phase I or Phase 2 detoxification enzymes, thus protecting the body against chronic diseases, such as cancer. Even so, a substantial amount of additional research is needed in order to obtain a better understanding of the role these agents play in cancer chemoprevention.

Flavonoids, including the anthocyanins, flavonols and flavones, are among the most intensely studied secondary products with over 6,000 known compounds [Harborne, 2000]. Many of them play important roles as flower and fruit pigments, UV protectants,

signaling molecules between plants and microbes, and regulators of auxin transport. [Dooner, 1991, Dixon, 1991] The flavonoids are also thought to have antioxidant, anti-allergenic, and anti-inflammatory effects, thus contributing to human health. [Scalbert, 2005, Ross, 2002]

Secondary metabolites are organic chemical compounds such as alkaloids, glycosides, terpenes, steroids, having physiological activity in humans and warding of a number of deceases. (**Table 8.1**)

The search for new pharmaceuticals from plants is possible using a number of distinct strategies. Random collecting of plants by field gathering is the simplest but least efficient way.

The chances are much greater that new compounds of medicinal value will be discovered if there is some degree of selectivity employed by collecting those plants that a botanist knows are related to others already having useful or abundant classes of secondary metabolites. Even more relevant is to collect plants already targeted for specific medicinal purposes, possibly among indigenous or ethnic peoples who use traditional, plant-derived medicines often with great success to provide for their well-being. Such data are part of ethno-botany, when researchers often obtain detailed information on the plants people use to treat illnesses, such as the species, specific disease being treated, plant part preferred, and how that part is prepared and used for treatment.

Taking the ethno-botanical approach, a specific part of the targeted Ethnomedicinal plant is extracted, usually in a solvent like ethanol, and then studied in bio directed assays and then isolated and characterized by spectroscopic techniques.

Table 8.1: Some Medicinal Plants and their Uses

Scientific Name	Common Name	Family	Compound Class	Compounds	Uses
Atropa belladonna, Duboisia myoporoides	Belladonna	*Solanaceae*	Alkaloid	Atropine, scopolamine	Anticholinergic, motion sickness, mydriatic
Catharanthus roseus	Madagascar periwinkle	*Apocynaceae*	Alkaloid	Vincristine, vinblastine	Anticancer (antileukemia)
Chondrodendron tomentosa, Curarea toxicofera	Curare	*Menispermaceae*	Alkaloid	(+)-Tubocurarine	Reversible muscle relaxant
Cinchona calisaya, Cinchona officinalis	Jesuits' bark	*Rubiaceae*	Alkaloid	Quinine, quinidine	Antimalaria (quinine), antiarrhythmia (quinidine)
Digitalis lanata, Digitalis purpurea	Foxglove	*Scrophulariaceae*	Cardiac glycoside (steroidal)	Digoxin, digitoxin, lanatosides	Heart failure and irregularity
Dioscorea species	Yam	*Dioscoreaceae*	Saponin glycoside (steroidal)	Diosgenin, precursor of human hormones and cortisone	Female oral contraceptives, topical creams
Ephedra sinica	Ephedra, Ma huang	*Ephedraceae*	Alkaloid	Ephedrine	Bronchodilator, stimulant
Pilocarpus species	Jaborandi	*Rutaceae*	Alkaloid	Pilocarpine	Glaucoma
Podophyllum peltatum	May-apple	*Berberidaceae*	Resin	Podophyllotoxin, etoposide	Anticancer
Rauwolfia serpentina		*Apocynaceae*	Alkaloid	Reserpine	Antihypertensive, tranquilizer
Taxus brevifolia	Pacific yew	*Taxaceae*	Diterpene	Taxol	Anticancer (ovarian, breast)

8.3 Bio Flavonoids

Flavonoid is any member of a class of widely distributed biological natural products containing aromatic heterocyclic skeleton of flavan (2-Phenyl benzopyran) but no nitrogen in plants. Generally, flavonoids are biological pigments providing colours from red to blue in flowers, fruit and leaves. Besides their coloring in plants, flavonoids have important roles in the growth and development of plants; protection against UV-B radiation; forming antifungal barriers; antimicrobial, insecticidal and oestrogenic activities; plant reproduction. Flavonoids also exhibit a wide range of biological properties including anti-microbial, insecticidal and oestrogenic activities. Flavonoids are usually classified into main 6 subgroups as below plus flavans, neoflavonoids, flavonols, aurons, catechins according to the structural patterns.

- Flavonols (Hydroxy derivatives of flavone): Fisetin, Galangin, Kaempferide, Kaempferol, Morin, Myricetin, Myricitrin, Quercetin, Quercetrin, Rhamnetin, Robinin, Rutin, Spirenoside
- Flavones (skeleton: 2-phenylchromen-4-one): Apigenin, Baicalein, Chrysin, Diosmetin, Diosmin, Flavone, Luteolin, Rpoifolin, Tangeretin, Techtochrysin, Rhamnazin, Nobiletin, Natsudaidain.
- Isoflavones (skeleton: 3-phenylchromen-4-one): Daidzin, Genistein, Irilone, Luteone, Prunetin, Pratensein,
- Flavonones (derivation by reduction of the 2(3) C═C bond): Eriodictyol, Hesperidin, Hesperetin, Likvirtin, Naringin; Naringenin; Pinocembrin
- Flavanols (derivation by reduction of the keto group):(+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-Epicatechin 3-gallate (ECG), (-)-Epigallocatechin 3-gallate (EGCG), Theaflavin, Theaflavin 3-gallate, Theaflavin 3-gallate, Theaflavin 3,3' digallate, Thearubigins
- Anthocyanidins (aglycones of the glycoside anthocyanins): Apigeninidin, Cyanidin, Delphinidin, Diosmetinidin, Guibourtinidin, Fisetinidin, Luteolinidin, Malvidin, Pelargonidin, Peonidin, Robinetinidin, Tricetinidin, Capensinidin, Petunidin, Europinidin, Aurentinidin, Columnidin, 5-Desoxy-malvidin, 5-Desoxy-peonidin, Hirsutinidin, Rosinidin

8.4 Selection of Plant

8.4.1 *Heliotropium strigosum* (Boraginaceae)

The heliotropes (*Heliotropium*) are genus of plants in the family Boraginaceae with 250 to 300 species. The name "heliotrope" derives from the fact that these plants turn their leaves to the sun. *Helios* is Greek for "sun", *tropein* means "to turn". The old English name "turnsole" has the same etymology [Selvi, F. and Bigazzi, M.2001]

João Sammy N *et.al.*(2005) has isolation two alkaloids having moderate antioxidant activity from *Heliotropium indicum* .Reports on the occurrence of flavonoid aglycones in Boraginaceae are scarce so far. Family Boraginaceae has been found to be rich in flavonoids (Table 8.2) there is practically no information on the type of falvonoids isolated from *Heliotropium strigosum.* The willd plant *Heliotropium strigosum* (Gorakhpamo) is used locally as laxative, diuretic and for cure the pain of limbs. It is also found to be effective as an application to sore eyes, gums, boils and also to cure sting of nettles and insects. In the present research *Heliotropium strigosum* was selected for identification and isolation of flavonoids.

Table- 8.2: Reported Flavonoids of Boraginaceae (1992-2003)

No	Name of Flavonoids	Trivial Name	Formula	MW	Plant Name	References
Anthocyanins						
1	Cy3,5-di-glc, Cys3-{6-(rha)glc, Dp3,5-d-glc, dp3-[6-(rha)glc], Dp3-glc	-			*Lobostemon* flowers	Van-Wyk, B.E. Winter, P.J.D. and Buys, M.H., 1997
Flavones						
2	5,7-dihydroxyflavone	Chrysin	$C_{15}H_{10}O_4$	254	*H.pycnophyllum*	Wollenweber, E. et al.,2002
3	5,7,3'-trihydroxy4-methoxyflavone	Diosmetin	$C_{16}H12O6$	300	*Nonea rosea*	Wollenweber, E. et al.,2002
4	5-hydroxy7,3',4'-trimethoxyflavone	-	$C_{18}H_{16}O_6$	328	*Nonea pulla*	Wollenweber, E. et al.,2002
5	5,7,3',5'-tetrahydroxy4'-methoxyflavone	-	$C_{16}H_{12}O_7$	316	*Nona lutea Nona pulla*	Wollenweber, E. et al.,2002
6	5,7,5'-trihydroxy3',4'-dimethoxyflavone	Apometzgerin	$C_{17}H_{14}O_7$	330	*Nona pulla*	Wollenweber, E. et al.,2002
7	5,7-dihydroxy3',4',5'-trimethoxyflavone		$C_{18}H_{16}O_7$	344	*Nona pulla*	Wollenweber, E. et al.,2002
Flavonols						
8	5,7-dihydroxy3-methoxyflavonol	-	$C_{16}H_{12}O_5$	284	*H. filifolium* *H. huascuense* *H. megalanthum* *H. pycnophyllum* *H. sinuatum* *H. stenophyllum*	Urzua,A. et al.,2000 Villarroe, l. et al.,2001 Urzua, A. et al., 2000 Wollenweber, E. et al.,2002 Torres, r. et al.,1996 Wollenweber, E. et al.,2002
9	5-hydroxy3,7-dimethoxyflavonol	-	$C_{17}H_{14}O_5$	298	*H. huascuense* *H. megalanthum* *H. pycnophyllum*	Villarroe, l. et al.,2001 Urzua, A. et al., 2000 Wollenweber, E. et al.,2002
10	3,5,4'-trihydroxy7-methoxyflavonol	Rhamnocitrin	$C_{16}H_{14}O_5$	300	*H. stenophyllum*	Wollenweber, E. et al.,2002

11	3,4'-dihydroxy3,7-dimethoxyflavonol	Kumatakenin	$C_{17}H_{14}O_6$	314	*H. chenopodiaceum* *var. ericoideum* *H. pycnophyllum* *Alkanna orientalis*	Urzua,A. et al.,2000 Wollenweber, E. et al.,2002 El-Sohly, H. N. et al.,1197
12	3,5-dihydroxy7,4'-dimethoxyflavonol	-	$C_{17}H_{14}O_6$	314	*H. stenophyllum*	Wollenweber, E. et al.,2002
13	5-hydroxy3,7,4'-trimethoxyflavonol	-	$C_{18}H_{16}O_6$	328	*H. stenophyllum*	Wollenweber, E. et al.,2002
14	5,7,4'-trihydroxy3,6-dimethoxyflavonol	-	$C_{17}H_{14}O_7$	330	*Alkanna orientalis*	El-Sohly, H. N. et al.,1997
15	5,3',4'-trihydroxy3,7-dimethoxyflavonol	-	$C_{17}H_{14}O_7$	330	*H. pycnophyllum* *H .stenophyllum*	Wollenweber, E. et al.,2002
16	5,7,4'-trihydroxy3,3'-dimethoxyflavonol		$C_{17}H_{14}O_7$	330	*H. sinuatum* *H. stenophyllum*	Torres, r. et al.,1996 Wollenweber, E. et al.,2002
17	3,7,4'-trihydroxy5,3'-dimethoxyflavonol		$C_{17}H_{14}O_7$	330	*H. stenophyllum*	Urzua, A. et al., 2000
18	3,5,4'-trihydroxy7,3'-dimethoxyflavonol	Rhamnazin	$C_{17}H_{14}O_7$	330	*H .stenophyllum*	Wollenweber, E. et al.,2002
19	5,4'-dihydroxy3,7,3'-trimethoxyflavonol	Pachypodol	$C_{18}H_{16}O_7$	344	*H. sinuatum*	Torres, r. et al.,1996
20	5,3'-dihydroxy3,7,4'-trimethoxyflavonol	Ayanin	$C_{18}H_{16}O_8$	344	*H. chenopodiaceum* var. *ericoideum* *H .pycnophyllum*	Urzua,A. et al.,2000 Wollenweber, E. et al.,2002
21	5,7,4'-trihydroxy3,6,3'-trimethoxyflavonol	Jaceidin	$C_{18}H_{16}O_7$	360	*Alkana orientalis*	El-Sohly, H. N. et al.,1997
22	3,5-dihydroxy7,3',4',5'-tetramethoxyflavonol		$C_{19}H_{18}O_8$	374	*H. magalanthum*	Urzua, A. et al., 2000
Flavanone						
23	5,2'-dihydroxy7,4',5'-trimethoxyflavanone		$C_{18}H_{18}O_7$	346	*Onosma hispida*	Bohm, H. et al.,1998

H= Heliotropium

8.4.2 *Withania coagulans* (Solonaceae)

Withania coagulans (Stocks) Dunal, commonly known as Indian rennet, panir or vegetable rennet belongs to family Solanaceae and subfamily Solanoideae [USDA, 2006] .It is commonly found in Afghanistan, India and southern Pakistan. The seeds contain a powerful coagulating agent which is used in preparing vegetable rennet ferment for making cheese. The seed extract possessed strong coagulating powers even when used in small amounts [Harden and Macallum, 1914]. Budhiraja, *et.al*, studied the pharmacological properties of *Withania coagulans* [Budhiraja *et.al,* 1977] and also isolated a new withanolide with a unique chemical structure similar to the aglycones of the cardiac glycosides [Budhiraja, *et. al,* 1983]. A number of new withanolides (steroidal lactones) were isolated from the whole plant of *Withania coagulans* by Atta-ur-Rahman *et.al,* and their structures were deduced by spectral analysis [Atta-ur-Rahman, *et.al,* 1998] [Atta-ur-Rahman *et.al*, 2003]. Administration of aqueous extract of fruits of Withania coagulans Dunal significantly lowered the blood sugar, serum cholesterol, serum LPO, and hepatic LPO in streptozotocin induced diabetic rats and it also exhibited free radical scavenging activity in an in vitro system using DPPH [Hemalatha, *et.al,* 2004]. The aqueous extract of *Withania coagulans* also showed hypolipidemic activity when tested on albino rats [Hemalatha, *et.al,* 2006]. It was selected to identify its volatile constituents by GC MS for the first time.

8.4.3. *Calotropis procera* (Asclepiadaceae)

Calotropis procera is widely used in folk medicine as a rich source of biologically active compounds capable of promoting diverse benefits such as control of dermal fungal infections, antimicrobial activities and pain relief among other useful properties. The identification and cultivation of plants rich in hydrocarbons as renewable sources of chemicals for use as fuel and chemical feedstock has generated considerable interest (Nielsen *et al*., 1977; Buchanan *et al*., 1978a*,* b; Calvin, 1978; Saxon, 1980; Wang & Human, 1981; Adams & Machesney, 1982; Campbell, 1983; Jenkins and Ebeling, 1985; Abbott *et al*., 1990; Seiler *et al*., 1991). Ismat Naeem *et. Al.,* discovered that the roots of *Calotropis procera* removed arsenic from drinking water very efficiently (Naeem, I.

et.al., 2008) and the arsenic removing property was better then *Pteris vittata* and *Ichohornia cressipes (*Abida,T. *et al*., 2009). Due to its medicinal value, abundance and wild nature and use as heavy metal ion remover it was selected for identification of its constituents having functional groups responsible of removing Cr (III) by spectroscopic techniques.

9. EXPERIMENTAL

9. EXPERIMENTAL

9.1 Material and Methods

All reagents were of analytical grade. Quercetin, Myricetin, Kampherol, were purchased from Sigma Aldrich. Silica gel 60 (70-230 mesh, Merck) and Sephadex LH-20 (Pharmacie) was used for column chromatography. TLC was performed on silica gel sheets over polyethylene. [Kieselgel 60 F254, 0.20 mm, Merck]

IR spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrometer. Mass spectra data (EI-MS and HR-EIMS) were acquired on a Shimadzu QP5050A and a JEOL CGMate II instrument, through direct probe and operating at 70 eV.

NMR experiments were performed on a Bruker DRX-500 [1H (500 MHz) and 13^{o}C (125 MHz)] and Varian UM-400 [1H (400 MHz) and 13C (100 MHz)] spectrometers.

The HPLC system (Waters 1500 series), was used with a UV detector (2487). Column was a C18, (250 × 4.6 mm, 5 mm particle sizes). Acetonitrile was purchased from Merck. Water deionized and double distilled and acidified with 1 % acetic acid. Qualitative analysis was made with samples, in isocratic mode, with acetonotrile/water 1:1 maintaining a flow-rate of 1 ml/min^{-1}. The injection volume was 10 ul and elute was monitored at 254 nm.

9.1.1. Extraction and Separation

The plant material *Heliotropium Strigosum* (1.00 kg) was dried away from the sunlight, powdered and exhaustively extracted with methanol using Soxhlet extraction method to give solvent free crude methanolic extract (6.120 %). These methanolic extracts were then acid hydrolyzed and column chromatographed on silica gel (250-400 mesh) by

gradient elution using *n*-hexane, dichloromethane, *n*-butanol and methanol to get 100 fractions. Fraction obtained with *n*-butanol and methanol (50:50) was acid hydrolysed and flavonoid aglycone purified by TLC and identified by standards.

9.1.2 Extraction of Essential Oils

Twelve hundred grams of powdered *Withania coagulans* fruits were taken in five litre reaction vessel and attached to a steam generator. A water cool condenser was also attached with reaction vessel. Steam generator produced the steam which passed through the sample condensed and collected with essential oils. The oil was separated by separating funnel. Then added the anhydrous magnesium sulphate and stored at 4°C before use. The yield based on fresh weight of the sample was calculated (0.175 %). The obtained essential oil was taken in very small amount about 0.2 micro liter dissolved in dichloromethane and used for GC-MS analysis

9.1.3 Acid Hydrolysis

Controlled acid hydrolysis was carried out with 10% acetic acid under reflux for 3.5 hours. These fractionated samples were then analyzed by HPLC without any further separation [Imperato F. 1984]

9.1.4 HPLC Analysis:

The HPLC system (Waters) consisted of a pump 1500 series), and a UV detector (2487). Column was a C18, (250 × 4.6 mm, 5 mm particle sizes). Acetonitrile was purchased from Merck. Water was HPLC grade and acidified with 1 % acetic acid. Qualitative analysis was made with samples, in isocratic mode, with acetonotrile/water 1:1 at a flow-rate of 1 ml min^{-1}. The injection volume was 10 ul and elute was monitored at 254 nm. The filtered methanol extract (0.5 microns) of areal parts of *Heliotropium strigosum* were injected under these conditions and compared with authentic standard of quercetin injected under similar conditions.

9.1.5 GC-MS Analysis

The volatile constituent's analysis was achieved on a Shimadzu GC-MS-QP 2010 with data system Lab Solution and Company Shimadzu. The DB-5 Column of Version 2.2 was indirectly coupled to the mass spectrometer. The DB-5 column was 3 cm in length, 0.25 mm id and 0.5μm in thickness (Agilent Technologies, J and W Scientific Products, Folsom, CA, USA). Carrier gas was Helium (BOC) with a flow rate of 1 ml/min and Pressure of 122KPa. Scanning rate was 2S/decade. Split was 1/10. Injector was Split/Split less. GC oven temperature programming was 50°C hold for 1min, raised at 5°C/min 40°C, and hold for 5min. Injection Temperature was 250°C. Detector Temperature was 280°C. Mass Spectra was 1.5-1024. Mass range was from 40-300 amu at 1scan/s. Ion Source Temperature was 200°C. Ion Source was EI. The mass spectrometer was operating in the EI-mode at 70eV.

9.1.6 Phytochemical Absorption of Cr (III) on *Calotropis procera*

Calotropis procera roots were washed with distilled water to remove any soil or debris.The washed samples were oven dried at a temperature of 333 K for two days. Dried roots were ground and sieved to 100 mesh sizes. This biomass was stored in air tight glass bottles to protect it from humidity.

9.1.7 Fourier Transform Infrared Analysis:

FTIR spectroscopy was used to identify the chemical groups present in roots.The samples were examined using Midac FTIR 2000 spectrometer within range 406-7800 cm^{-1} KBr was used as background material in all the analysis. 0.0035 g roots powder was mixed with 0.5g KBr and pressed to form a pellet. An FTIR spectrum of roots was compared before and after adsorbtion.

9.2 Isolation of Compounds of *Heliotropium strigosum*

9.2.1 Taxifolin (1)

It was isolated as a white solid (6.2* 10-4%) by repeated column chromatography of *n*-butanol fraction with gradient elution and finally by prep TLC using methanol:*n*-butanol:water (40:50:10).

Yellow powder

m.p. 250 with decomposition

[(z] 23 +22.0 ~ (c 1.68, MeOH)

FAB-MS

*m/*z 305 [M+H]+

IR (KBr): 3366, 2370, 1640, 1468, 1360,

Spectral Data

^{1}H **NMR** (600 MHz, CD3OD) δ: 4.51 (dd, J = 11.2, 6.1 Hz, **1H**, 3-H), 5.00 (d, J = 11.2 Hz, **1H**, 2-H), 5.76 (d, J = 6.1 Hz, **1H**, 3-OH), 5.88, 5.93 (each d, J 2 Hz), 6.95 (**1H**, d, J = 2.0 Hz, H-2'), 6.84 (**1H**, dd, J = 2.0, 8.0 Hz, H-6'),

9.2.2 Quercetin (2)

It was identified by HPLC using standard quercetin (purchased from Merck) from methanol extract of *Heliotropium strigosum* (Boraginaceae) using procedure given in section 9.1.1.7.

Yellow powder

MF: $C_{15}H_{10}O_7$

m.p. 310-316°

MS: ***m/z*** 302.24

IR (KBr): 3412, 1652, 1606, 1506, 1300, 1211, 1170, 1114 cm^{-1}

Spectral data:

^{1}H (δ) 12. 87 (OH-5), 6.96,(2H,*s*, H-2 and H-6), 6.61 (1H, *s*, H-3),6.42 (1H, *d*, J=1.8 Hz, H-8), 6.20(1H, *d*, J = 1.8 Hz, H-6), and 3 B ring protons resonated at 7.61 (1H, *s*, H-2′), 6.89 (1H, *s*, H-5′) and 7.52 (1H, *s*, δH-6. [Fathiazad et al. 2006]

9.3 Indentification of Compounds of *Withania coagulans* (Essential oil) by GC MS

Twelve hundred grams of powdered *Withania coagulans* fruits (60% of fresh fruits) yielded essential oil (0.175 %) by steam distillation of which about 0.2 micro liters were dissolved in dichloromethane and used for GC-MS analysis

Table 9.1: Compounds (%age) found in *Withania coagulans* by GC MS

Compound No	Name of Compound	R:T	Mol. Formula	Mol.Wt.	Percentage
(5)	Cyclohexane	2:300	C_6H_{12}	84	0.21%
(6)	Borane carbonyl	2:310	CH_3BO	42	0.22%
(7)	3-methyl ,hexane	2:350	C_7H_{16}	100	3.20%
(8)	Heptane	2:550	C_7H_{16}	100	1.20%
(9)	Hexanoic acid	2:700	$C_6H_{12}O_2$	116	2.00%
(10)	Nonanoic acid	2:710	$C_9H_{18}O_2$	158	3.50%

9.4. Identification of Compounds of *Calotropis procera* (roots) by GC-MS.

Roots of *Calotropis procera* (1 Kg) yielded (13.1 %) *n*-haxane extract which on column chromatography yielded an oily fraction (1.2 %). It was subjected to GC MS analysis. The following compounds were identified (**Table-9.2**) by comparison with NIST data library and on the pattern of fragmentation.

Table 9.2: Compounds (%age) found in *Calotropis procera* by GC MS

Compound No.	Name of Compound	R:T	Mol. Formula	Mol.Wt.	Percentage
(9)	*n*-Heptanoic acid methyl estere	2:210	$C_8H_{16}O_2$	144	2,4 0 %
(10)	*n*-Decanoic acid	2:310	$C_{10}H_{20}O_2$	172	0.46 %
(11)	*n*-Nonoic acid methyl ester	2:350	$C_9H_{18}O_2$	158	7.20 %
(12)	*n*-Decenoic acid methyl estere	2:550	$C_{11}H_{20}O_2$	184	1.60 %

9.4.1 GC-MS Spectral Data

9.4.1.1 *n*-Heptanoic acid methyl estere (9)

m/z: 144 (M^+,$C_8H_{16}O_2$, 6%) 113 (M^+ -31, 4%) 101 (.M^+ -43, 2%) 87 (7%) 73 (100%).

9.4.1.2 *n*-Decenoic acid (10)

***m/z*:** 170 (M^+, $C_{10}H_{18}O_2$, 25%), 127(M^+-43, 7.5%), 113(M^+-14,8%), 99(M^+-14, 11%), 85(M^+-14, 49%), 71(M^+-14,69%), 57(M^+-14, 100%), 43($^+$-14, 28%).

9.4.1.3 *n*-Nonanoic acid methyl ester (11)

***m/z*:** 172(M^+, $C_{10}H_{20}O_2$,11%), 157(M^+-15, 37%), 129 (M^+-43, 8%),111(27%) 97(11%), 83, (37%) 69(28%), .55(100%).

9.4.1.4 *n*-Decenoic acid methyl ester (12)

***m/z*:** 184(M^+, $C_{11}H_{20}O_2$, 21%), 152(M^+32, 2%), 127(M^+57, 11%), 113(12%), 99(15%), 85(75%), 74(100%).

9.3 Identification of Functional Groups for Cr(III) by *Calotropis procera* Roots (Methanol Extract)

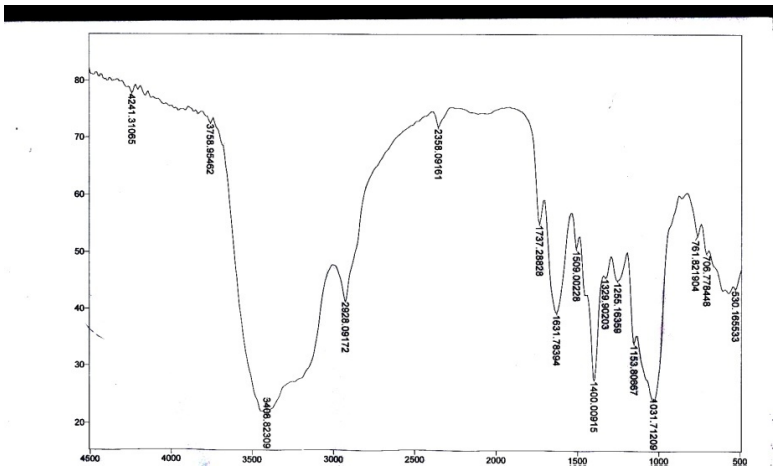


Fig-9.1(a): Infrared spectrum of *Calotropis procera* roots before adsorbtion of Cr(III) on it

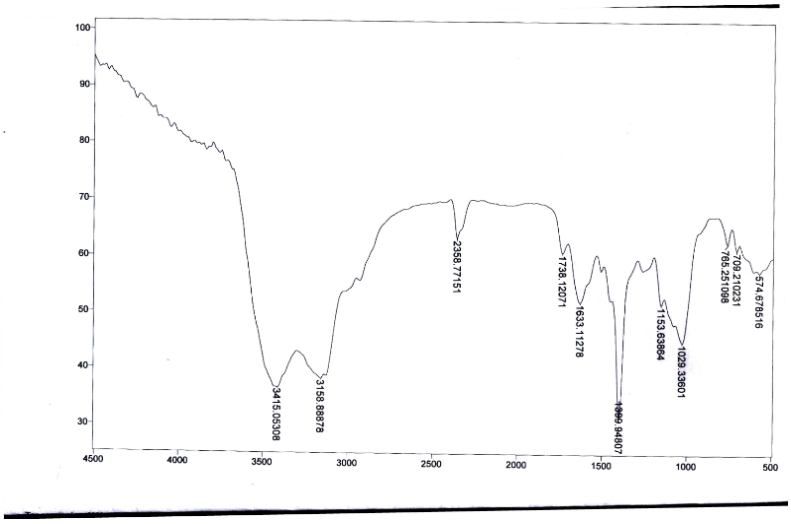


Fig-9.1(b): Infrared spectrum of *Calotropis procera* roots after adsorbtion of Cr(III) on it.

Table-9.3: Differnce Between Adsorbtion Bands (cm^{-1}) of Calotropis procera Roots Before and After Adsorbtion of Cr (III) on it.

IR Peak	Absorption bands (cm^{-1})			Assignment
	Before adsorption	After adsorption	Difference	
1	3408	3415	+7	Bonded —OH groups, —NH stretching
2	2928	3158	+230	Carboxilic acids —OH stretching —CH STRECH
4	1737	1738	+1	C═O streching
5	1631	1633	+2	C═C strech
7	1153	1153	0	C—O strechs
8	1031	1029	-2	R—O strech

10. DISCUSSION

10. DISCUSSION

10.1 Compounds Isolated from *Heliotropium Stirgosum*

10.1.1 Taxifolin (Dihydroquercetine) (1)

The HREI MS of (1) exhibited the M+ peak at m/z 304.0541 analyzing for $C_{15}H_{12}O_7$ (calcd. 304.0583). Hence it possessed ten degrees of instauration. Three of these were accounted for tri-cyclic skeleton of flavone; six were due to endocyclic double bonds of benzene rings and one due to carbonyl function. The IR spectrum (CHCIs) showed intense absorption at 1720 cm^{-1} characteristic of ketonic function. The IR spectrum showed the characteristic absorption of carbonyl function at 1720 cm^{-1} and a broad band at 3600 cm^{-1} indicating the presence of hydroxyl groups. The 1H-NMR spectrum of compound (1) exhibited a one proton C-2 doublet at δ 4.9 (J = 4.8 Hz) showing COSY interaction with C-3 proton resonating at δ 4.51 (J = 4.8 Hz) characteristic of dihydroflavanols.

A one proton singlet at δ 6.02 and one proton singlet at δ 5.07 were assigned to C-6 and C-8 respectively in the ring A of compound (1) on the basis of HMQC correlations. The one proton signals at δ 6.96 (C-2′), δ 6.85(C-5′) and δ 6.78 (C-6′) indicated the compound to be an aglycone. On comparison with literature the compound was identified to be Taxifolene aglycone [Fossen, T et al. 1998].

Taxifolin **(1)**

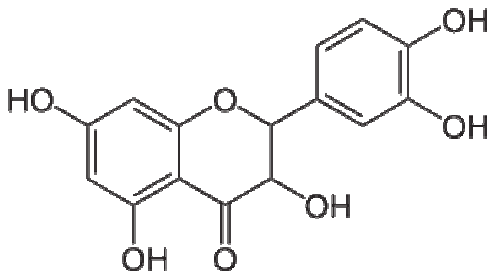


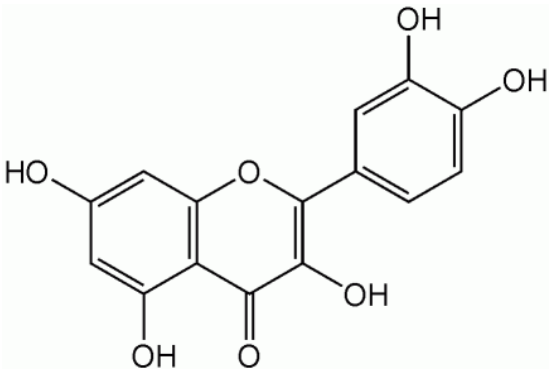
Table 10.1: ^{13}C-NMR Spectral Data of Taxifolin

Carbon ppm Multiplicity δ ppm Carbon Multiplicity	Multiplicity	δ ppm	Carbon	Multiplicity	Δ
C-2	CH	83.8	C-10	C	164.0
C-3	CH	72.3	C-1	CH	128.6
C-4	C	197.1	C-2	CH	114.6
C-5	C	167.5	C-3	C	145.0
C-6	CH	96.1	C-4	C	145.8
C-7	C	163.0	C-5	CH	114.8
C-8	CH	95.0	C-6	CH	119.6
C-9	C	100.5			

10.1.2 Quercetin (2)

It was isolated as a yellow powder m.p. 310-316° Mass spectrum analysis showed a molecular ion peak at m/z 302.24 corresponding to a molecular formula $C_{15}H_{10}O_7$.

The 1H spectrum showed three phenolic OH signals (δH 12. 87 (OH-5), 10.92 and 9.57), two equivalent B-ring protons (δH 6.96, s, 2H, H-2 and H-6), a singlet at δ H 6.61 typical of a flavone H-3, two meta-coupled A-ring protons (H 6.42 and 6.20, J = 1.8 Hz, H-8 and H-6), and 3 B ring protons resonated at (δH-2′ 7.61, δH-5′ 6.89 and δH-6′ 7.52) respectively. On comparison of NMR with literature it was deduced that compound 1 was a 5, 7, 3, 4′, 5′-pentahydroxyflavonol. [Fathiazad et al. 2006]



Quercetin (2)

10.2 Identification of Compounds from *Withania coagulans*

10.2.1. Cyclohexane (3)

Compound (**5**) was identified by GC MS analysis of pet ether extract of *Withania coagulans*.The molecular ion peak at *m/z* 85 corresponded to molecular formula C_6H_{12}. An ion peak at *m/z* 56 was due to the elimination of one C_2H_2 radical. The compound was identified by comparison of its mass spectrum with given Library spectrum [Heller, S.P and Mike, G.W. NIST 27].

R.T : 2:300

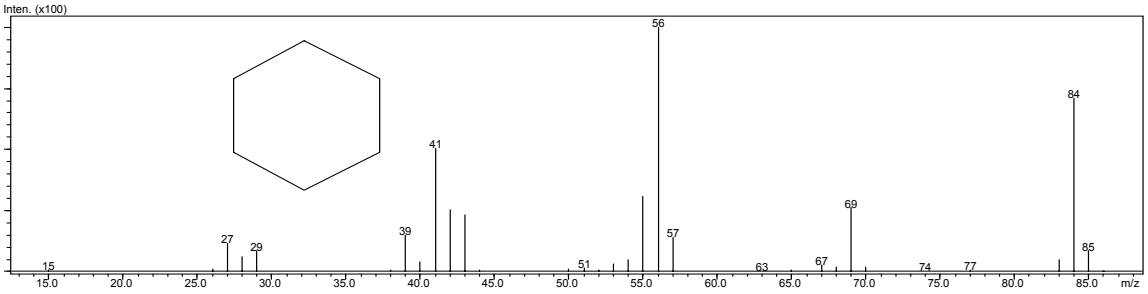


Fig-10.1: Mass Chromatogram of Cyclohexane (3)

10.2.2 Borane carbonyl (4)

The molecular ion peak at *m/z* 42 corresponded to molecular formula CH_3BO. An ion peak at *m/z* 40 was due to the elimination of two hydrogen atoms. The compound was identified by comparison of its mass spectrum with given Library spectrum. [Heller, S.P and Mike, G.W. NIST 27]

R.T : 2:310

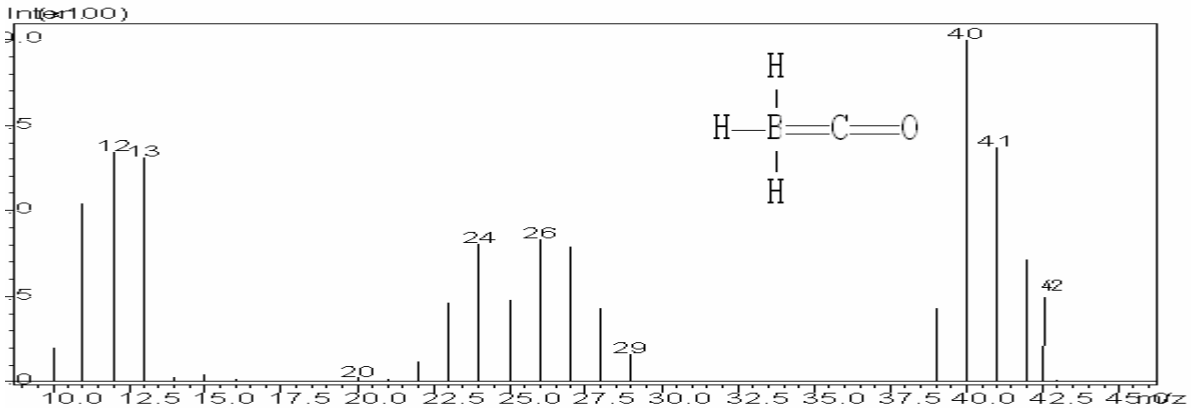


Fig-10.2: Mass Chromatogram of Borane carbonyl (4)

10.2.3. Hexane 3-methyl (5)

The molecular ion peak at *m/z* 100 corresponded to molecular formula C_7H_{16}. An ion peak at *m/z* 85 was due to the elimination of one methyl radical. The ion peak at *m/z* 71 was due to the elimination of one CH_2 radical. The compound was identified by comparison of its mass spectrum with given Library spectrum. [Heller, S.P and Mike, G.W. NIST 27]

R.T: 2:350

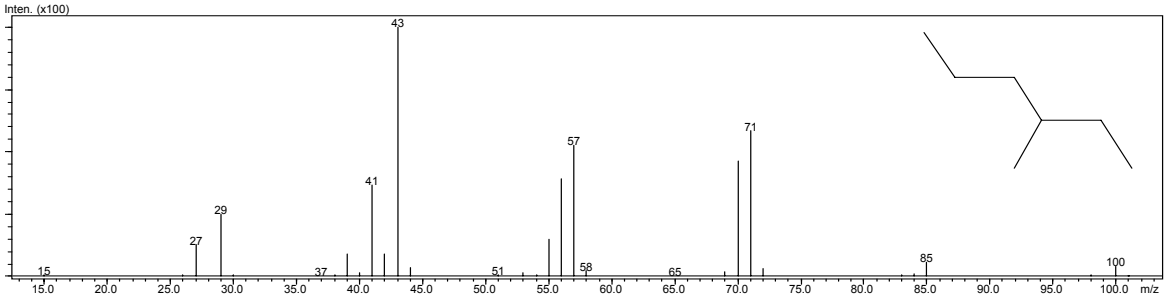


Fig-10.3: Mass Chromatogram of Hexane 3-methyl (5)

10.2.4. Heptane (6)

The molecular ion peak at *m/z*100 corresponded to molecular formula C_7H_{16}. An ion peak at *m/z* 85 was due to the elimination of one methyl radical. The ion peak at *m/z* 71 was due to the elimination of one CH_2 radical. The compound was identified by comparison of its mass spectrum with given Library spectrum [Heller, S.P and Mike, G.W. NIST 27].

R.T : 2:55

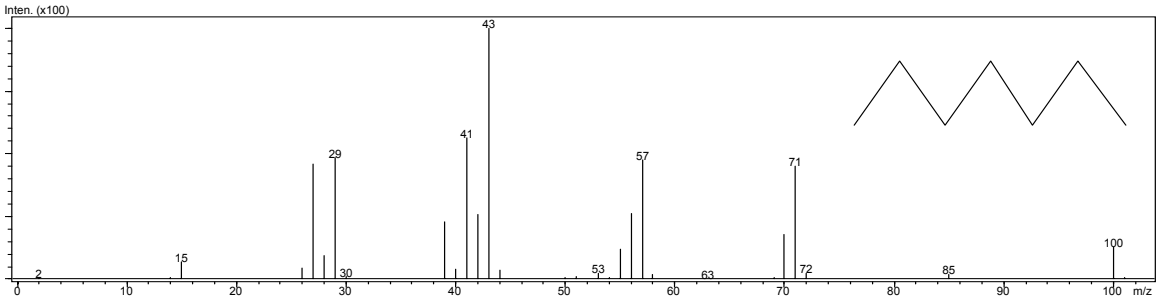


Fig-10.4: Mass Chromatogram of Heptane (6)

10.2.5. Hexanoic acid (7)

The molecular ion peak at *m/z* 116 corresponded to the molecular formula $C_6H_{12}O_2$. An ion peak at *m/z* 99 was due to the elimination of one CHO radical. This compound was identified by comparison with given Library spectrum. [Heller, S.P and Mike, G.W. NIST 27].

R.T : 2:700

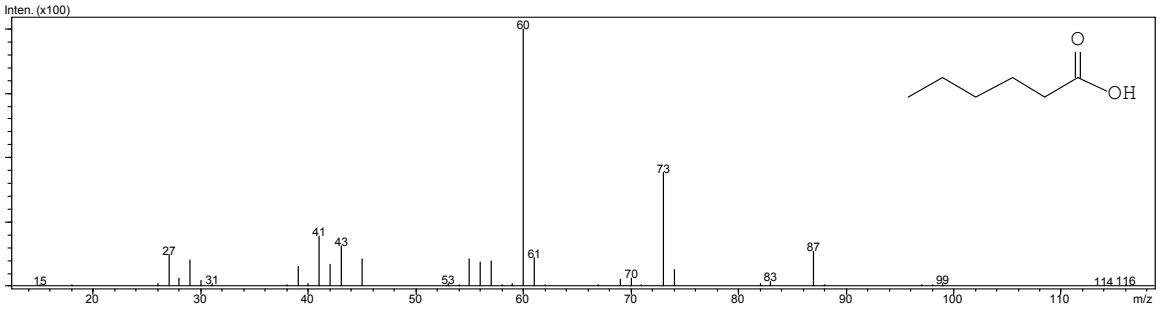


Fig-10.5: Mass Chromatogram of Hexanoic acid (7)

10.2.6 Nonanoic acid (8)

The molecular ion at *m/z*158 corresponded to the molecular formula $C_9H_{18}O_2$. An ion peak at *m/z* 129 was due to the elimination of one CHO radical. The ion peak at *m/z* 115 was due to the elimination of one CH_2 radical. The ion peak at *m/z* 98 was due to fragment ion C_7H_{12}. This compound was identified by comparison with given Library spectrum. [Heller, S.P and Mike, G.W. NIST 27]

R.T : 2:710

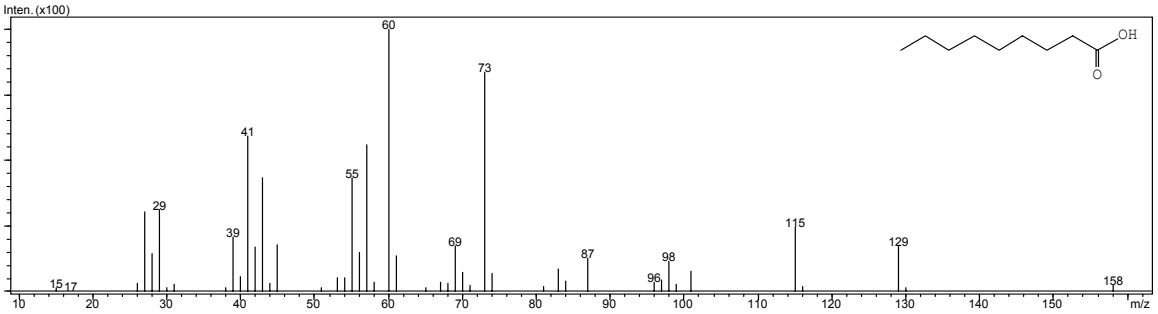


Fig-6: Mass Chromatogram of Nonanoic acid (8)

10.3 Compounds Identified from *Calotropis procera*

The GC-MS showed the presence of 3 saturated 1 unsaturated fatty acids methyl esters which were identified in *Calotropis procera* (roots) by comparison of their spectral data (NIST) and fragmentation pattern.

10.3.1 *n*-Heptanoic acid methyl ester (9)

A molecular ion peak at *m/z* 144 corresponded to molecular formula $C_8H_{16}O_2$ indicating one degree of unsaturation accounted for the presence of one carbonyl group of carboxylic acid function. An ion peak at *m/z* 129 was due to the elimination of a methyl radical suggesting that iit was a methyl ester. Another *m/z* peak at 113 was due to the elimination of a methoxy radical from the molecular ion.hence compound **(9)** was a methyl ester of *n*-heptanoic acid.

$CH_3\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2COOCH_3$
n-Heptanoic acid methyl ester **(9)**

10.3.2. *n*-Decanoic acid (10)

A molecular ion peak at *m/z* 170 corresponded to molecular formula $C_{10}H_{18}O_2$ indicating two degrees of unsaturation accounted for the presence of one carbonyl group of carboxylic acid function and one double bond. An ion peak at *m/z* 127 was due to the elimination of a propyl radical suggesting there was no peak at *m/z* 155 sugesting the absence of a methyl ester. Hence compound **(10)** was *n*-decanoic acid.

$CH_3\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2COOH$
***n*-**Decanoic acid **(10)**

10.3.3 *n*-Nonanoic acid methyl ester (11)

A molecular ion peak at *m/z* 172 corresponded to molecular formula $C_{10}H_{20}O_2$ indicating one degree of unsaturation accounted for the presence of one carbonyl group of carboxylic acid function. An ion peak at *m/z* 157 was due to the elimination of a methyl radical suggesting that iit was a methyl ester. Another *m/z* peak at 129 was due to the elimination of a propyl radical from the molecular ion.hence compound **(9)** was a methyl ester of *n*- nonanoic acid.

$CH_3\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2\,CH_2COOH$
n-Nonanoic acid methyl ester **(11)**

10.3.4. *n*-Decanoic acid methyl ester (12)

A molecular ion peak at *m/z* 184 corresponded to molecular formula $C_{11}H_{20}O_2$ indicating two degrees of unsaturation one accounted for the presence of one carbonyl group of carboxylic acid function and second for a double bond. Most abundant ion peak at *m/z* 74 was due to Mclafferty rearrangement characteristic of methyl esters of carboxylic acids.. The position of the double bond could not be ascertained through MS only.

$CH_3\,CH_2\,CH_2\,CH_2\,CH_2{=}CH_2\,CH_2\,CH_2\,CH_2COOH$
n-Decanoic acid methyl ester **(12)**

10.4 Organic Functional Groups Responsible for Phytosorption of Lead (CrIII) by *Calotropis procera*

The FT-IR spectra before and after adsorption of *Calotropis procera* roots were shown in **Fig-9a** and **b**. The functional groups before and after adsorption on Calotropis procera roots and the corresponding infrared absorption bands (**Table-9.3**) displayed a number of absorption peaks, indicating the complex nature of *Calotropis procera* roots. These band shifts indicated that bonded —OH groups and/or —NH and carboxyl groups especially played a major role in chromium (III) biosorption on *Calotropis procera* roots.

GC MS analysis of *Calotropis procera* **(Section**: **10.3)** showed the presence of carboxylic acids in roots of *Calotropis Procera* which may be playing a dominant part in biosorption of Cr (III).

11. REFERENCES

11. REFERENCES

- Abbott, T.P., R.E., Petterson, L.W. Tjark, D.M. Palmer and M.O. Bagby. (1990). Major extractable components in *Asclepias linaria* (Asclepiadaceae) and *Ilex verticillata* (Aquifoliaceae) two potential hydrocarbon crops. *Econ. Bot*., 44: 278-284.

- Adams, R.P. and J.D. Machesney. (1982). Phytochemicals for liquid fuel and petrochemical substitutions: extraction procedures and screening results. *Econ. Bot*., 37: 207-215.

- Ageel A.M, Parmar N.S, Mossa J.S, Al-Yahya M.A, Al-Said MS, Tariq M,(1986) Anti-inflammatory activity of some Saudi Arabian medicinal plants. 17(3-4):383-4.

- Alanís A.D., Calzada F., Cervantes J.A., J. Torres and G.M. Ceballos. (2005) Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. *Journal of Ethno pharmacology*, 100,(1-2)153-157

- Ali, S.I., Ome, S. and Qaisar, M., (2001). Flora of Pakistan: in Mohammad Afzal and Shehzad A. Mufti Natural History research in Pakistan. PASTIC, Islamabad.

- Ali, N.A, Julich, W.D, Kusnick, C. and Lindequist, U.(2001) Screening of Yameni medicinal plants for antibacterial and cytotoxic activities, J, Ethnopharmacology..Vol,74 (2): 173-9

- Almas K, (1999). The antimicrobial effects of extracts of Mezadirachta indica (Neem) and Salvadora persica (Ark). Indian. Dent. Res. 10(1), 3-26.

- Ajab, M. and Ilyas, I (1999). Ethno botanical information of Malam Jaba, Distt; Swat, Pakistan. M, Phil- Thesis submitted to Department of Biology, Quaid-i-Azam University, Islamabad, pp: 37-40

- Amin, M. (1961). A note on the plants and pharmaceutical industry in Pakistan..J. Sc. Ind. Res.: 4: 217-218.

- Arshad, M and Akram,S.(1999) Medicinal Plants of Arid Agriculture University, Rawalpindi. Hamdard Medicus. 42(3); 46-50.

- Atta- ur-Rahman, Dur- e-Shahwar, Aniqa Naz and M. Iqbal Choudhary (2003) Withanolides from Withania coagulans Photochemistry, 63,(4) 387-390

- Atta-ur-Rehman, Choudhary, M.I and William, J.T. (1999), Manual of Bioassay Techniques for Natural Product Research. Harward Academic Press, Amsterdam.82-84

- Atta-ur-Rahman, Muhammad Shabbir, Muhammad Yousaf, Samina Qureshi, Dur e-Shahwar, Aniqa Naz, M. Iqbal Choudhary (1999),Three withanolides from Withania coagulans Phytochemistry, Volume 52, Pages 1361-136
- Awadh Ali N. A., Jülich W. -D., C. Kusnick and U. Lindequist (2001), Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities Journal of Ethnopharmacology, 74(2) 173-179
- Badshah, L, Hussain, F. and Muhammad, Z, (1996). Floristic and Ethno botanical study on some plants of Pirgarharh Hills, South Waziristan Agency, Pakistan. Pak. J. Bot. 2(2). 167-177.
- Balick, M.J, (1996) Transrforming Ethno botany for the new millennium .Annuals of the Missourib Botanical Garden .83(1):58-66
- Baquar, S .R. (1989). Medicinal and Poisonous plants of Pakistan, Prints, Karachi, Pakistan.
- Barclay, A.S. (2006) The world Botanical Associates Web Page. (TWBAWP) brkesfield (A 93380-1145)
- Behera, K.K, *et. al.*, (2006) Ethno medicinal plants used by the tribals of similipal Biooreserve, Orissa, India. A pilot stud.
- Bohm, H. et al., (1998), Flavonols, flavone and anthocyanins as natural antioxidants of food and their possible role in the prevention of chronic diseases: 37, 147.
- Bonjar, G.H. Shahidi, (2004) Evaluation of Antibacterial Properties of Iranian Medicinal-Plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumoniae and Bordetella bronchoseptica Asian Journal of Plant Sciences 3 (1): 82-86ISSN 1682-3974
- Boyd, R.F., (1995). Basic Medical Microbiology Dis., 24: 211-215. (5th Edn.). Little, Brown and Company: Boston.
- Brooks, G.F., J.S. Butel, L.N. Ornston, E. Jawetz, J.L. Melnick and E.A. Adelberg, (1991) Medical Microbiology . Appleton and Lange: New York: 245-246
- Buchanan, R.A., I.M. Cull, F.H. Otey and C.R. Russell. (1978). Hydrocarbon and rubber producingcrops: evaluation of U.S.plant species. *Econ. Bot*., 32: 131-135.
- Buchanan, R.A., I.M. Cull, F.H. Otey and C.R. Russell. (1978). Hydrocarbon and rubber producingcrops: evaluation of U.S.plant species. *Econ. Bot*., 32: 146-153.

- Buwa L.V. and Staden J. van, (2006) Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa ,Journal of Ethno pharmacology 103 (1)139-142
- Calvin, M. (1978) Petroleum plantations for fuel and materials, *Bioscience*, 29: 533-538.
- Campbell, T.A. (1983). Chemical and agronomic evaluation of common milkweed *Asclepias syriaca. Econ. Bot*., 37: 174-180.
- Chaudari, I.I (1958) Medicinal plants resources of Pakistan and their development. Pak. J. Sci. 10:34-56.
- Chaudari, I.I. (1961) Distribution of some important medicinal plants of Pakistan. Pak.J.Sci.4:207-211.
- Chaudari, I.I, M.N. and R.A, Qureshi. (1991). Pakistan endangered flora II. A checklist of rare and seriously threatened taxa of Pakistan. Pakistan Systematics 5 (12): 1-84.
- Coelho.G., Haas,G. L., SchapovalS.S.(2004) vol(90), 135-143
- Colombo, M.L. and Bosisio, E. (1996) Pharmacological activities of Chelidonium majus L (Papaveraceae). Pharmacology, Res. 33, 127-134.
- Cookson, B.D., 2000. Methicillin-resistant Staphylococcus aureus in the community. New battlefronts, or are the battles lost? Control of Hospital Epidemiol. 21: 398-403.
- Corron R.A, Maran J.M. Montero, F, and Dominguez, A.A, (1987) Journal of Ethnopharmacology, 77(2-3)247 -252.
- Conner, M.J.(1988) Oxidation of retinol to retinoic acid as a requirement for biological activity in mouse epidermis cancer. Res 48: 7038-40
- Davis.L, and Kuttan, G., (2001) Effect of Withania somnifera on DMBA induced carcinogenesis. J, Ethnopharmacolog; 75 (2-3):165-8
- Dastur, H. (1952). Medicinal plants of India and Pakistan Taraporevela, Bombay.
- DeSouza NJ, Dohadwalla AN, Reden J. (1983) Forskolin: a labdane diterpenoid with antihypertensive positive inotropic, platelet aggregation inhibitory, and adenylate cyclase activating properties. 3(2), 201–219.
- Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A.(1999) An ethno botanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology. ; 37:80–91.
- Dixon, R. A., and Paiva, N. L. (1991) Stress-induced phenylpropanoid metabolism Plant Cell 7, 1085-1097.

- Dixon, R. A., and Strack, D. (2003) Phytochemistry meets genome analysis and beyond. Phytochemistry. 62 (6), 815-816.
- Dooner, H. K., Robbins, T. P., and Jorgensen, R. A. 1991. Genetic and developmental control of anthocyanin biosynthesis. Annual. Rev. Genet. 25, 173-199.
- Eisenberg, D.M, Davis R.B, and Ettner ,S.L, (1998)Trends in alternative medicine use in the United States, 280:1569-75
- El-Sohly, H. N. et al., (1997), Antiviral flavonoids from Alkana orientalis, Planta Med., 63, 384.
- Fakim, A.G.(1999) Int .J. crude Drug .28,297-308
- Farnsworth, N.R. (1993) Ethno pharmacology and future drug development: the North American. 38: 145-152.
- Farnsworth, N. R., O. Akerele, A. S. Bingel, D. D. Soejarto, and Z.-G. Guo. (1985). Medicinal plants in therapy, 63:965-981.
- Farnsworth, N. R., and D. D. Soejarto, (1985) Potential consequences of plant extinction in the United States on the current and future availability of prescription drugs. Econ. Bot. 39(3):231-240.
- Fathiazad, F., Delazar, A. Roya.A.and sarker,S.D., (2006) Iranian Journal of Pharmaceutical Research : 3 : 222-227
- Fossen, T. Pedersen, A. T. and Andersen, M. (1998) Flavonoids from red onion (Allium cepa), Phytochemistry, Volume 47, Issue 2, 281-285
- Fridous, A.J., Islam, S.N.L.M, Faruque, A.B.M, (1990) Antimicrobial activity of the leaves of Adhatoda vasica, Calotropis gigantean, Nerium odorum and Ocimum sanctum, Bangladesh Journal. Bot.227.
- Edeoga HO, Okwu DE, Mbaebie BO. (2005)Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. ; 4:685–688.
- Goldstein A, Aronow L. and Kalman S. M., (1974) Principles of drug action: The basis of Pharmacology, 2nd Ed. John Wiley and Sons, New York, 741.
- Goodman, S.M. and A. Ghafoor. (1992) The ethno botany of Southern Baluochistan, Pakistan, with particular reference to Medicinal plants. (31):1-84.
- Gordon, K.C. (1990): Edinburgh: Churchill living stone: 43-45.
- Gupta MP, Solis PN, Calderon AI, Guionneau-Sinclair F, Correa M, Galdames C, Guerra C, Espinosa A, Alvenda GI, Robles G, Ocampo R.

(2005) Medical ethno botany of the Teribes of Bocas del Toro, Panama. Journal of Ethnopharmacology. ; 96:389–401.

- Harborne, J. B., and Williams, C. A. (2000) Advancese in flavonoid research since 1992. Phytochemistry. 55, 481-504.
- Heller, S.P and Mike, G.W. NIST 27 Library
- Haq, I. and Shah, M. (1986) Medicinal plants of District Peshawar NWFP, Hamdard Medicus. 3(40); 76-83.
- Haq,I. and Hussain, M. (1987) Medicinal plants of Mansehra, NWFP, Pakistan. Hamdard Medicus 36(3): 63-100.
- Haq, l. and Hussain, Z, (1995) Medicinal plants of Palandri, Disirict Poonch (Azad Kashmir). Pak.J. PI. Sci., 1 (1): 115-126.
- Haq.I, (1993) Medicinal plants of Mansehra District, N.W.F.P., Pakistan, Hamdard Medicus. 34 (3); 63-99.
- Hirasawa, M., Shoujii, N., Neta, T., Fukushima, K. and Takada, K. (1999). Three kinds of Antibacterial substances from Lentinus edobes (Berk) Sing, Shitake, an edible mushroom Int. J. of Ant. Agents 11, 151 – 157.
- Hocking, G.M. (1962) Pakistan Medicinal Plants IV. Quality Plantarium Material Vegetable 9: 103-119.
- Hamid, A and Sitepus, D.(1990). An understanding of native herbal medicines in Indonesia.Industerial Crops research ,J.3(1): 11-17
- Heffelfinger, S. C, Gear. R.B., Taylor. K., Miller M.A., Shneider. J., LaDow, K., et. al (2005). DMB induce mammary pathologies are angiogenic in vivo and vitro .Lab invest ; 80: 485-92
- Hussain M.A and Gorsi M.S. (2004) Antimicrobial Activity of Nerium oleander Asian Journal of Plant Sciences 3 (2): 177-180, ISSN 1682-3974 Department of Botany, University of Azad Jammu and Kashmir Muzaffarabad-13100, Pakistan.
- Hussain, F. and A, Khaliq, (1996), Ethnobotanical studies of some plants of Dabragii Hill Swat, Proceeding of first Training workshop on Ethnobotany and its application to conservation .NARC, Islamabad. pp: 207-215
- Hopwood, D. (1990) Edinburgh: Churchill living stone: 21-42.
- Ikram, M. and S.F. Hussain. (1978) Compendium of medicinal plants. Pak. Council Sci, Peshawar.
- Imperato F. (1984) Two New Phenolic Glycosides in Asplenium septentrionale. *American Fern Journal*, 74(1), 14-18.

- Jain A, Katewa S.S, Chaudhary BL, Galav P. (2004) Folk herbal medicines used in birth control and sexual diseases by tribals of southern Rajasthan, India. J Ethnopharmacol. 90 (1):171-7.
- Jenkins, B.M. and J.M. Ebeling. 1985. Thermo chemical properties of biomass fuels. *Calif. Agric*., 39: 14-16.
- Jeric, S., Popovic, Z., Macukanovic, J.M., Djuredjevic.L., Miljatovic.M, Karadiz, B. and Pavlovic.P., (2007) An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Moutain (central Serbia) .J.Ethnopharmacol.Vol.111 (1):160-75
- João Sammy N. SouzaI; Luciana L. MachadoI; Otília D. L. PessoaI; Raimundo Braz-FilhoII; Cassia R. OverkIII; Ping YaoIII; Geoffrey A. CordellIII; Telma L. G. Lemos (2005). Pyrrolizidine alkaloids from Heliotropium indicum, J. Braz. Chem. Soc. vol.16 no.6b, published on web.
- Kelin, J.P and Scholler M. (1998) Recent advances in Development of a Streptococcus mutans vaccine. 6:121
- Khan, A.U, (1994) History of decline and present status of natural tropical Thorn forest in Punjab, Pakistan. Biol.conser 63 (3): 205-210.
- Khan, A.K. (1962).Studies on growth and cultivation characteristics of medicinal and other economic plants under semi-temperate condition. Pak. J. of Botany 236-273.
- Klayman, D.L., Lin A. J., Acton N., Scovill J. P., Hoch J.M., Milhouse W.K. (1984) Antimalarial Activity of Some Kenyan Medicinal Plants. J.Nat. Prod., 47, 715.
- Klayman, D.L (1985) Medicinal Plants. J. Science, 288, pp 1049.
- Kleinsmith, L.J. and Pearson, B.C. (2006) Principles of cancer Biology, 12-34
- Kumar V. Prashanth, Neelam S. Chauhan, Harish Padh and M. Rajani. Mathabe, R.V. Nikolova, N. Lall and N.Z. Nyazema M.C (2006) Antibacterial and antifungal agents from selected Indian medicinal plants *Journal of Ethnopharmacology* .6:234-256.
- Lenaz L., and Defuria M.D., (1993) Taxol, a novel natural product with significant anticancer activity, Fitotrapia, LXIV, Suppl. N.I.
- Malik, A. R. (1958) Arid Zone Research 11, Pattern exhibited by some desert species from distt: Khairpur, Sind, Pakistan. Sind: Uni. Res. Jour. 17(2) : 73-85.
- Manandhar, N.P., (1987) Fitoterapia,64:266

- Mario. D. and Irene. D, *J. Agric. Food Chem.*, 2000, 48 (7), pp 2659–2662
- Marwat, Q. and Shinwari, Z- K. (1996) Ethnobotanical studies in Upper Siran Mansehra, Pakistan.Proceding of Ethnobotany workshop, NARC, Islamabad, Pakistan. pp: 73-82.
- Matsuda, H., Murakami, T., Kishi,A., and Yoshikawa.A., (2001), Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian Withania somnifera Dunal and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum.; 9 (6):1499-507
- MPDDRC (2006) Medicinal Plants and Drug Discovery Research Center medpladdrc@asmara.uoa.edu.er
- Mughal, M.S.; Ahmed, T. and Azizullah:(2005) Biologia: 51(2):201 -206
- Mulazim, H.B., et al (2002) Role of chemical carcinogens in epithelial and mesechymal neoplasms with tumor imitiation promotion protocol and the effect of 13-Cis retinoic acid in chemo prevention. JCPSP, Vol.12 Pp 302-306
- Muthu,C, Ayyanar,M. Raja,N and Savari, M. (2006) Medicinal Plants used by traditional healers in Kancheepuram District of Tamal nadu,India Pp :141
- Naeem.I., Taskeen, A., Moeen, T. and Mateen, B., 2008, A new Biomaterial for Removal of Arsenic from Drinking Water, JERAD, India, Vol 2 (3), 295-302
- Nasir, E. & S. I. Ali, (1970). Flora of [West] Pakistan. (F Pak)
- Nair, M.S.R., Acton, N., Klayman, D.L., Kendrick, K., Basile, D.V., and S. Mante., (1986) Production of artemisinin in tissue cultures of Artemisia annual. J. Nat. Prod. 49: 504-507.
- National Committee for Clinical Laboratory Standards (1993), ed.4 M2-T4.NCCLS, Villanous.
- Norrel S. A and Messley, K.E., (1997) Microbiology Laboratory Mannual, Principles and Applications. Prenticen Hall Upper Saddle River New Jersey
- Owais M., Sharad K.S., Shehbaz A. and Saleemuddin M., (2005) Antibacterial efficacy of Withania somnifera (Ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine, 12(3)229-235
- Paris, R. and G. Dillemann. (1960) Medicinal plants of the Arid zone II Today and tomorrow's Printers.

- Pei, S.,(1995) .Ethno botany and sustainable use of plant resources in the HKH Mountain region. Planning workshop on ethno botany and its application to conservation and community development in Hindu Khush Himalayan (HKH) region, Nepal.
- Petersen, F. C. & Scheie, A. A. (2000) Oral Microbiol. Immunol. 15, 329–334.
- Piddock, K.J.V. and R. Wise, (1989). Mechanism of resistance to quinolones and clinical perspective. J. Antimicrob. Chemotherap, 23:475-483.
- Prajapati, N.D., Prajapati, T. & Jajapura, S. (2006) Advances in Medicinal plants, Vol. I, Asian medicinal plants and health care trust, Jodhpur
- Qureshi, R. (2002) Ethnobotany of Rohri Hill, Sindh, Pakistan. Hamdard Medicus .XLV. No. 1:86-94.
- Recio, M.C., (1989) A review of some antimicrobial isolated from medicinal plants reported in the literature 1978-1988. Phytotherap. Res., 3: 117-125.
- Rojas J.J, Ochoa VJ, Ocampo SA, Muñoz J.F.(2006) Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. *BMC Complementary and Alternative Medicine.***6**:11 :86-92
- Ross, J. A., and Kasum, C. M. (2002) Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu. Rev. Nutr. 22, 19-34.
- Sadaqat, M. 1995. Medicinal plants of family Cucurbitaceae (Part-11). Hamdard Medicus. 34 (3): 91-101.
- Sandhu DS, Heinrich M. (2005) The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Research.; 19:633–42.
- Saxon, E.C. 1980. Tuberous legumes: preliminary evaluation of tropical Australian and introduced species as fuel crops. *Econ. Bot*., 35: 163-173.
- Scalbert, A., Johnson, I. T., and Saltmarsh, M. (2005) Polyphenols: antioxidants and beyond Am. J. Clin. Nutr. 81, 215S-217S
- Seiler, G.J., M.E. Carr and M.O. Bagby. 1991. Renewable resources from wild sunflowers (*Helianthus* spp., Asteraceae). *Econ. Bot*., 45: 4-15.
- Sher, H., Ahmad, M. and Iqbal, C.M. (2000) Market Survey of Medicinal Plants in Major cities of Pakistan, their uses and future prospects. Technical Report .submitted to Swiss –Inter-Cooperation. Swiss Rural Development Cooperation, Berne, Switzerland. Pp: 33-47.

- Sher, H. (2001) Medicinal and economic plants of alpine and sub alpine regions of district Swat and Chitral, Pakistan, technical report submitted to IUCN-P. 23-56.
- Shinwari, Z.K. and Shah .M. (1996) The Ethnobotany of Kharna District, Balochistan Proceedings of the first Workshop on Ethno botany and its application to Conservation, NARC, Islamabad. Pp:124-132.
- Shinwari, M.I., Z.K. Shinwari and Khan, B.A.I (1995) Ethnobotany of Kaghan Valley (Mansehra), Proceeding of Ethnobotany workshop. NARC, Islamabad, Pakistan. Pp: 94-103.
- Shinwari, Z K. (1996) The Ethnobotany in Pakistan: Sustainable and Participatory approach. Proceeding of first Training workshop on Ethnobotany and its application to conservation. NARC, Islamabad, pp: 14-25.
- Shinwari, Z.K. and M.A. Khan. (1999) Ethnobotanical conservation status of Margalla Hills National Park, Islamabad. Journal of plant resources and Environment. 8(2): 53-60.
- Shinwari, Z.K. and M. Shah. (1996) The Ethnobotany of Kharna District, Baluhchistan Proccedings of the first Workshop on Ethnobotany and its application to conservation, NARC, Islamabad.Pp:124-132.
- Shinwari,Z.K, and S.S. Gilani, 2003, Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). Journal of Ethno-pharmacology. 84: 289-298.
- Stevens, A. (1990) The haematoxylins Edinburgh: Churchill living stone: 107-18.
- Sultana, K, Z., Shinwari and F, Iftikhar. (1996) Diversity of edible Mushrooms in Pakistan, Proceedings of The Training first Workshop on Ethnobotany and its application to Conservation. National Herbarium, PARC,. Islamabad, pp. 46-50.
- Selvi,F. and Bigazzi,M.(2001) Leaf surface and anatomy in Boraginaceae tribe Boragineae with respect to ecology and taxonomy .Flora 196.: 269-285
- Takasaki, M.,Kanoshima .J.,Tokuda.H.,Masuda.K.,Aria.Y.,Shiojima ,K. Anti-carcinogenic activity of Taraxacum Plant (1999).J.Biot Pharm. Bull; 22:602-5
- Taskeen,A., Naeem, I*, Mobeen, H. and Moeen, T., 2009, Comparison of Biomass of Different Plants for Phytoremediation of Arsenic, Asian Journal of Chemistry, Vol.21(4), 2857-2860.

- Tshibangu, J.N., K. Chifundera, R. Kaminsky, A.D. Wright and G.M. Konig, 2002, Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. J. Ethnopharmacol., 80: 25-35
- Torres, R. et al., (1996) Flavonoids det exododo resinoso de Heliotropium sinuatum, Bol. Soc. Quim., 41, 95.
- Tyler, V. E., Brady, L.R. and Robbers, J. E., (1988) Pharmacognosy. 9th ed. Philadelphia: Lea and Febiger. von Oettingen, WF 19-58.
- Urzua, A. et al., (1993) Flavonoids in the resinous exudates of Chilean Heliotropium species from Cocharanea section, Biochem.syst. Ecol. 21, 744.
- Urzua, A. et al., (2000) External flavonoids from Heliotropium megalanthum and H. huascoense. Chemotaxonomic considerations, Soc. Chil. Quim, 45, 23.
- Urzua, A. et al., (2000) Comparative leaf surface chemistry from Senecio cerberoanus and Senecio viscosissimus, Biochem. Syst. Ecol., 28, 399
- Van-Wyk, B.E. Winter, P.J.D. and Buys, M.H., (1997) The major flower anthocyanins of Lobostemon (Boraginaceae), Biochem. Syst. Ecol., 25, 39.
- Veilleux, C, and King, S.R, (2002) An introduction to ethnobotany,htt://www.accessexcellence.org/RC/Ethnobotany/Pp.2html
- Villarroe, l. et al., (2001) Heliotropium huascoense resin exudates chemical constituents and defensive properties, J. Nat. Prod., 64, 1123
- Wang, S.C. and J.B. Human. 1981. Botanochemicals: supplements to petrochemicals. *Econ. Bot*.,35: 369-382.
- Yasunaka K. F, Ariaki, N. Hikaru, O. Lucio.L, Edith, L. Elizabeth, E. Muñiz, A.A. and Ricardo.R, (2005) Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones ,Journal of Ethnopharmacology, 97(2)293-299
- Zaman, M.B., A.A. Khan and A, Ahmad. (1972) Contribution to the knowledge of medicinal plants. PFI, Peshawar.
- Zampini Iris C., Vattuone Marta A and Isla Mari. I.(2005) Antibacterial activity of Zuccagnia punctata Cav. ethanolic extracts. Journal of Ethnopharmacology, 102 (3) 4.

LIST OF PUBLICATIONS

Publications

1- Ismat Naeem1, Tahira Mughal2, Uzma Maslahuddin1, Bushra Mateen1 and Hafeez Ikram3, 'Synergistic Activity of *Withania coagulans* with some other Medicinal Plants of Pakistan, International Conference of Chemistry, Lahore College for Women University, Nov.2-3, 2007. Abstract: page 110.

2- Tahira Mughal, Ammara Abass and Ismat Naeem, ' Antimicrobial and Synergistic studies of *Heliotropium strigosum'*, Proceedings of International Seminar on Medicinal Plants, Lahore College for Women University, 21-23 May, 2008, 182-188.

3- Hafsa Mobeen , Ismat Naeem*,Abida taskeen, and Tahira Mughal 2008 'Equilibrium studies of Cr removal from water and waste water using *Calotropis procera* roots', manuscript # 8353/2008 accepted 24 October 2008, Asian Journal of Chemistry, vol. 21, 2009

Publications (Proposed)

1. Plant extracts chemical carcinogens in epithelial and mesechymal neoplasms with tumor imitiation promotion protocol and the effect of extracts of *Heliotropium strigosum* in chemoprevention.

2. Ehnobotanical studies of medicinal plants used by the local people in South Punjab,Pakistan

3. Antibacterial activity from indigenous ethnomedicinal plants of South Punjab,Pakistan

4. Evaluation of antifungal activity of some ethnomedicinal plants of South Punjab,Pakistan

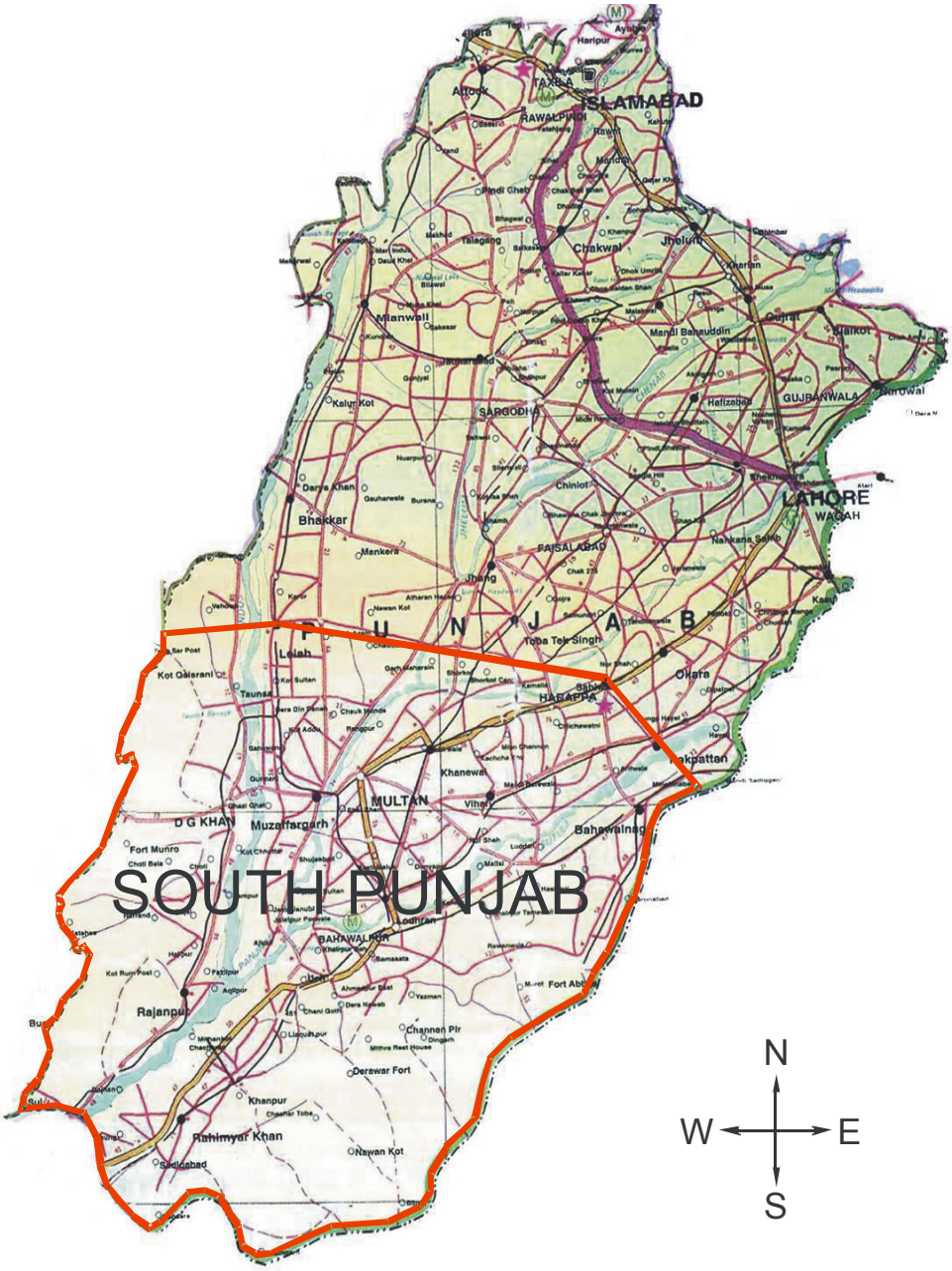
5. Effect of extracts of *Withania coagulans* and *Capparis decidua* on DMBA induced cancer.

6. Synergistic antibacterial activity of medicinal plants of Southern and Northern region of Pakistan.

7. Two new flavonoides isolated from *Heliotropium strigosum*

APPENDIX - I

Map of South Punjab



— **Area of study**

Questionnaire

Name of the Consultant .....

Botanicla name of the plant .....

Local Name of Plant .....

Locality .....

Ethnomedicinal uses .....

Parts of plant .....

(i) Root .....

(ii) Stem .....

(iii) Leaf .....

(iv) Flower .....

(v) Bud .....

(vi) Seed .....

(vii) Fruit .....

Side effects .....

.....

.....

.....

Dosage administration .....

.....

Date: _____ **Signature**

APPENDIX - II

Fig. 1: A view of Suleman Ranges (The Fort Munro) Punjab Southern Regions

Fig. 2: A view of Tribal Area, D.G. Khan

Fig. 3: A view of Cholistan Desert near Bahawalpur

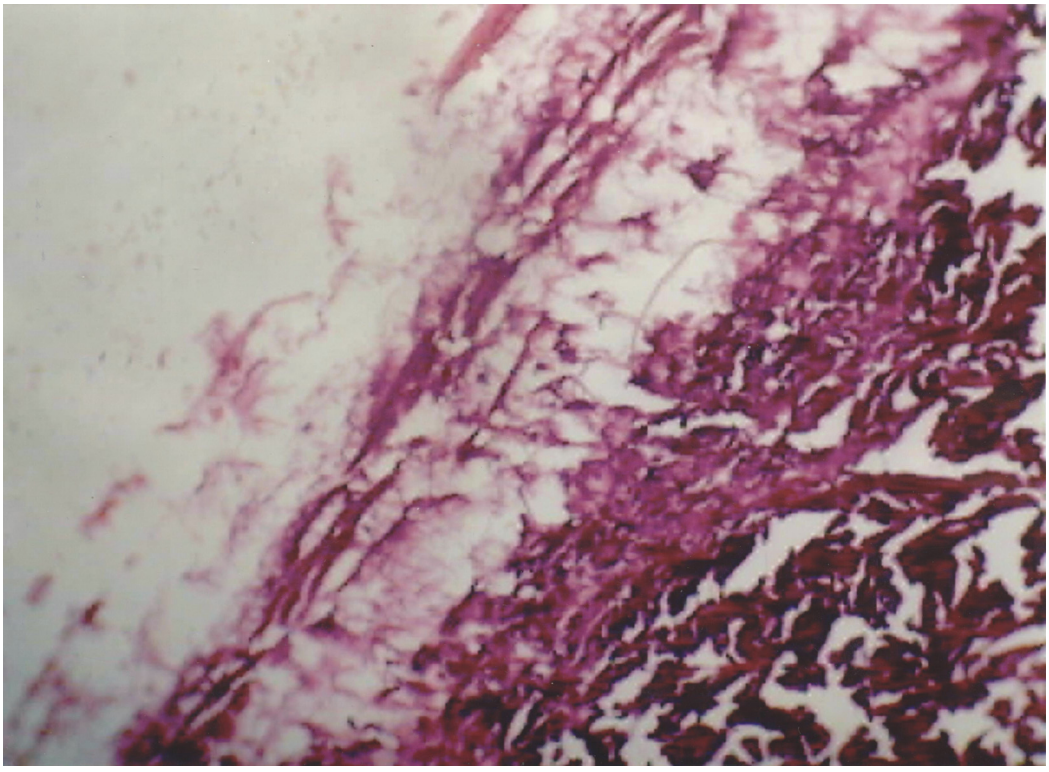
Fig. 4: Roadside view along Multan to Muzaffargarh

Fig. 5: A view of *Desmotachya bipinnata* near River Chenab

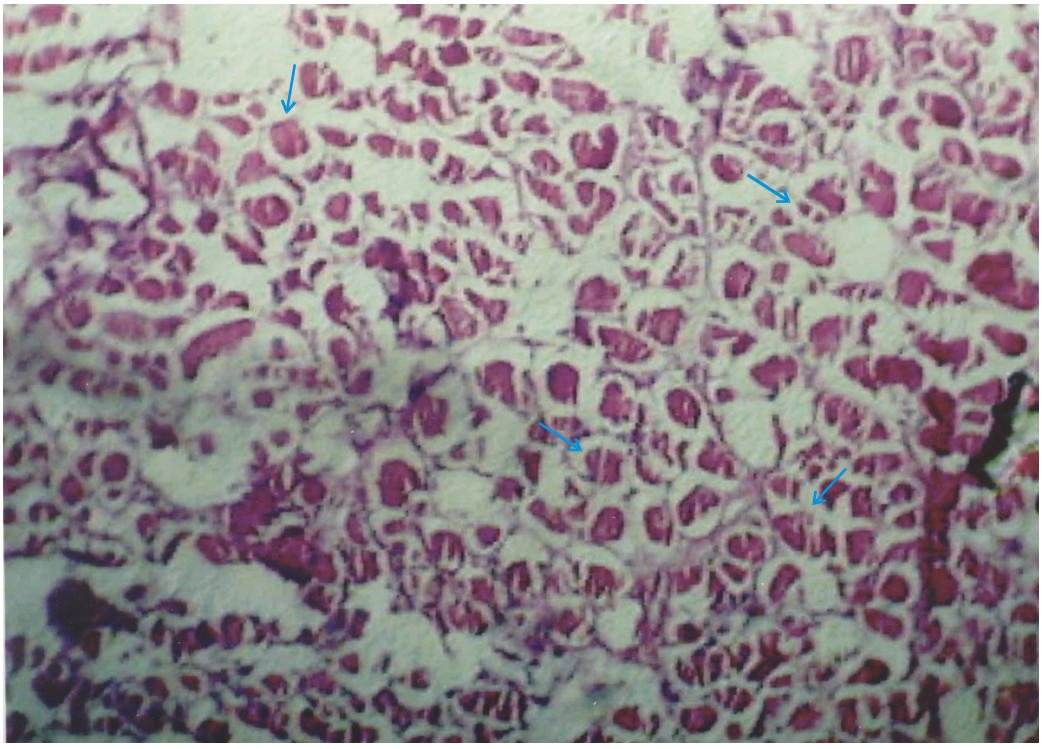
Fig. 6: Author collecting the *Tamarix aphylla* near Saki Sarwar

Fig. 7: Author interviewing the local people

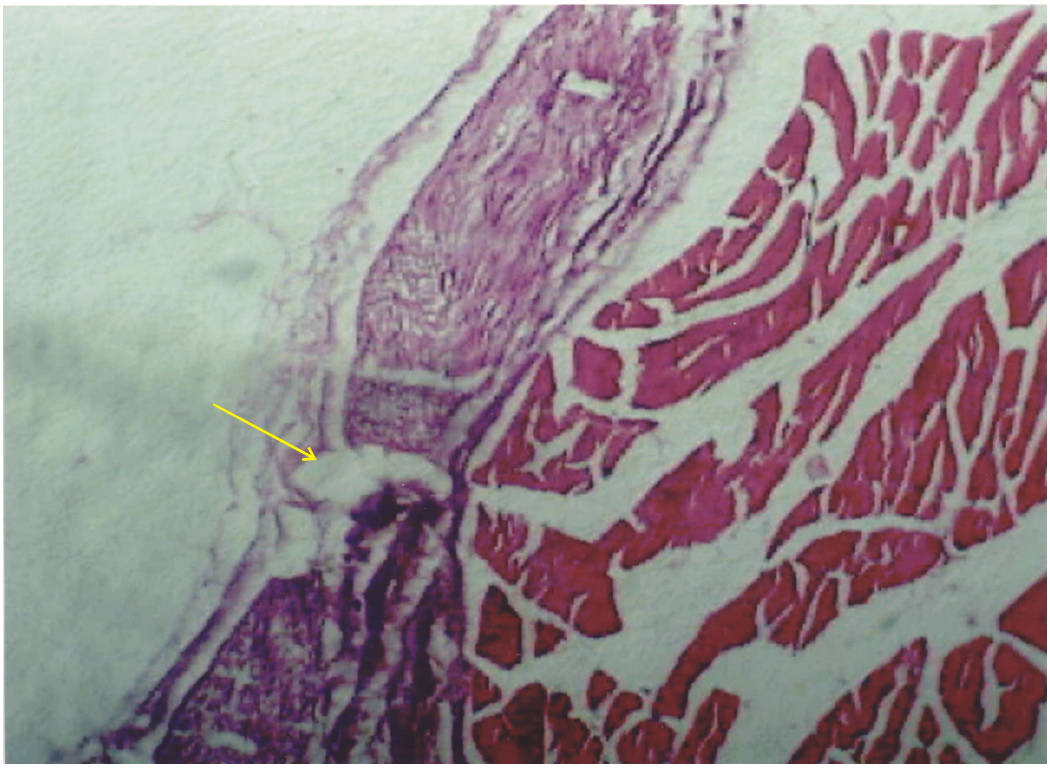
Fig. 8: Ulcered Albino rat



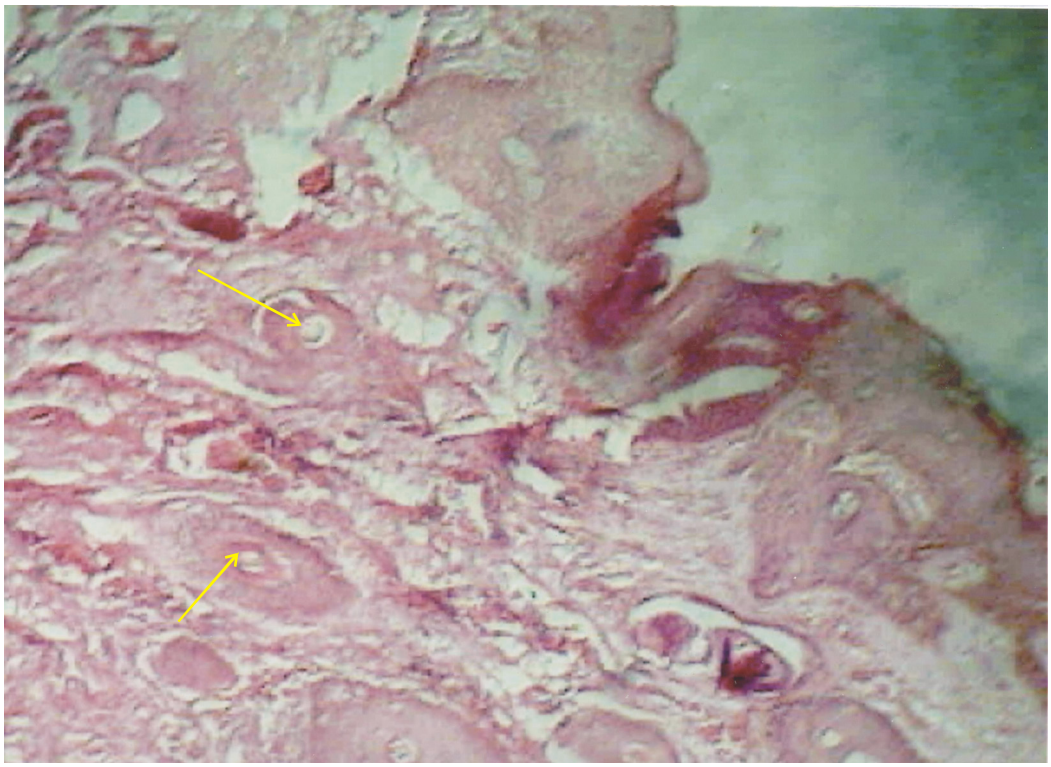
Pic. 9 Normal skin of Albino rat



Pic. 10 Abnormal mitotic stages of cancer in Albino rat



Pic. 11 Carcinoma insituin Albino rat



Pic. 12 The vacuolated skin of albino rat