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ACTIVITY OF LEAF EXTRACTS OF *COIX LACHRYMA* LINN. AND *ASPARAGUS COCHINCHINENSIS* LINN. AS BREAST ANTICANCER DRUGS

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In the current economic crisis, the use of plant medicine for cancer prevention should be investigated. *Coix lachryma* Linn and *Asparagus cochinchinensis* Linn are among eleven of species of medicinal plants that are noted as plant medicine for cancer in Indonesia, although their mechanism of action are still unknown. The eleven plants were screened using in vitro methods, Sulforhodamin B against breast cancer cells (MCF-7) and skin (KB). The research included a maceration process using ethanol as solvent and an anti-cancer testing process in vitro using Sulforhodamin B indicated by the value of percentage viability. Extracts were classed as being 'active anticancer' if they showed IC_{50} values below 100 ppm.. *Coix lachryma* Linn. and *Asparagus cochinchinensis* Linn. show breast and skin anticancer activity with IC_{50} values 6.51 ppm and 11.3 ppm of MCF-7 cells. The ethanol plant extracts were further extracted using various solvents with increasing polarity: n-hexane, methylene chloride, and ethyl acetate. The methylene chloride extract of *Coix lachryma* Linn. had IC_{50} = 2.75 ppm against MCF-7 cells. Against KB cells, methylene chloride extracts of *Coix lachryma* Linn. gave IC_{50} = 5.16 ppm. For *Asparagus cochinchinensis* Linn., an ethyl acetate extract had IC_{50} = 3.70 ppm against KB cancer cells and IC_{50} = 9.80 ppm against MCF-7 cancer cells. These data indicated that both plants can be used as anticancer drugs on breast and skin cancers.

Keywords: Ethanol extract, breast anticancer, *Coix lachryma* Linn**,** *Asparagus cochinchinensis* Linn, IC_{50}, Sulforhodamin B, MCF-7.

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Introduction

According to data from the Ministry of Health of the Republic of Indonesia, so far around 7500 species of medicinal plants have been discovered. Approximately 10% of these are plant medicines for cancer (Anonymous, 1983), but unfortunately there usefulness in preventing the cancer cell division process has not been studied in depth. Also it is not clear how these natural materials work, whether inhibiting an enzyme or by other means.

Helpful natural medicine is clearly required that can be obtained at a low price, and with side effects mild enough for it to be consumed as an everyday item. Utilization of natural materials, especially plants as medicines or raw materials is one way to overcome the problems of drug raw material supply in Indonesia, which still represents 90% of imported products (Atta-ur-Rahman and Choudary, 1998; Endo, 1987; Hutapea, 1993). Tracing efficacy of traditional medicinal herbs against cancer will provide added value for Indonesia's natural wealth, such as import substitution of raw materials and can be further developed as a dosage phytopharmaca.

In the current economic crisis, for the potential resources of Indonesian medicinal plants, the efficacy of plant medicine in the cancer prevention should be investigated. Medicinal plants used traditionally for the treatment of cancer, researched and known to have pharmacological effects, can be seen in Table 1.

TABLE 1. TRADITIONAL MEDICINAL PLANTS FOR CANCER TREATMENT THAT ARE KNOWN TO HAVE PHARMACOLOGICAL EFFECTS

Local name	Species	Pharmacological effects	References
Arbenan	*Duchesna indica* (Andrz) Focke	Inhibits growth of human esophageal cancer cell line	Dalimarta, 1999 Dalimarta, 2003
Bidara Laut	*Stryhnos nuxvomica* L.	Inhibits growth of human esophageal cancer cell line	Dalimarta, 1999 Dalimarta, 2003
Baru Cina	*Artemisia argyi* Levl. Et vant	Inhibits the growth of HeLa cells	Dalimarta, 2003
Bidara Upas	*Merremia mammosa* Lour. Hall.f.	Based on research of PT. Eisai Indonesia, can inhibit the multiplication of HIV virus	Dalimarta,1999 Dalimarta, 2003
Cakar Ayam	*Selaginella doederleiniin* Hieron	Extract inhibits the growth of L16 cells isolated from human liver cancer	Dalimarta, 2003
Gadung Cina	*Smilax china* L.	Inhibits the growth of brain tumors in rats	Uripi, 2002 Dalimarta, 2003

Several other plants were used traditionally for the treatment of cancer, especially breast cancer, but not yet investigated pharmacologically, were Bambu tali (*Asparagus cochinchinensis* Lour.), Buah Makasar (Brucea javanica L.), Cakar ayam (*Selaginella doederleinii* Hieron), Jali (*Coix lachrymal* L.), Leunca (*Solanum nigrum* L.), *Iombang* (*Taraxacum mongolicum* Hand-Mazz.), Rumput Mutiara (*Hedyotis corymbosa* L.), Waru Landak (*Hibiscus mutabilis* L.). These plants have been used for the treatment of breast cancer in a single dosage form, and parts of plants used included tubers, fruit, seeds, herbs, leaves, or even the whole plant. Treatment was carried out by boiling the plant parts as a drink for the patient (Hariana, 2006).

Materials and method

Processing section

Plant parts were dried at 40-45 °C in an oven and then pulverized using a "Disk Mill" (Colegate and Molyneux, 1993; Kinghorn, 1985).

Initial extraction of plants for early anticancer screening

Up to 100 g of plant was extracted by maceration using 95% ethanol. Plants in a dry state were soaked overnight in 100 mL ethanol, and the extract collected and evaporated in a vacuum. The process was repeated three times and the resulting extracts combined. After the extract was concentrated, the weight of extract was measured, and then vitro anticancer activity determined (Colegate and Molyneux, 1993; Kinghorn, 1985; Farnsworth, 1966).

Preparation of cancer cells to test anticancer

Cancer cells used in this study were breast cancer (MCF-7), cervical cancer (A431) and oral epidermoid carcinoma (KB). Breast cancer (MCF-7) and cervical cancer (A431) were cultured in Modified Eagle Dulbecco's Medium (DMEM) with 10% FBS, while an oral epidermoid carcinoma (KB) was cultured in Eagle's MEM with 10% FBS. Both of these cells were cultured at 37°C at a humidity of 95% and 5% CO_2 for 3 days until confluent cell culture was 60-70%. After that the old medium was removed, replaced with new medium and incubated again for 24 hours. The cell culture was then washed with PBS 1-2

times and suspended using trypsin-EDTA solution. Cells that were suspended coupled with new media.

In vitro anticancer test

Anticancer testing was by the SRB method (Sulforhodamin B) developed by the National Cancer Institute (Likhitwitayawuid *et al*., 1993). Cells that have been prepared to test as many as 100 μL plus the test sample as many as 10 μL were incubated for 3-4 days at room temperature. After that, cells were fixed with 50% TCA, staining with 0.4% SRB in 1% acetic acid for 30 minutes. SRB color that was not bound was removed with acetic acid 1%, while the bound was extracted with Tris base (pH 10). The intensity of color produced was measured by using ELISA plate reader on the wavelength of 515 nm respectively. Percent viability was calculated as follows:

$$\frac{\text{OD (cell + sample) - OD (negative control)}}{\text{OD (c25ells) - OD (negative control)}} \times 100 = \%\ \text{viability}$$

IC_{50} was calculated by nonlinear regression analysis between percent survival and the concentration (Skehan *et al*., 1990).

Extraction of plant anticancer containing secondary metabolites

Figures 1 and 2 show respectively the flow of extraction from *Coix lachryma* and *Asparagus cochinchinensis*.

FIGURE 1. EXTRACTION OF *COIX LACHRYMA* LEAVES WITTH VARIOUS SOLBENTS (COLEGATE AND MOLYNEUX, 1993; KINGHORN, 1985)

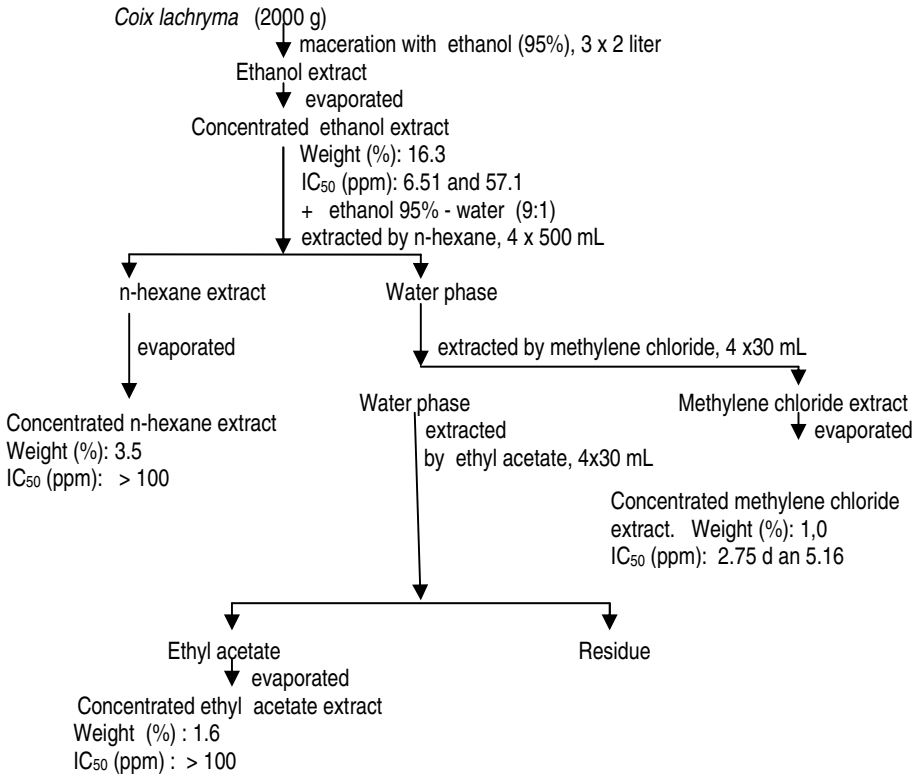


FIGURE 2. EXTRACTION OF *ASPARAGUS COCHINCHINENSIS* LEAVES WITH VARIOUS SOLVENTS (COLEGATE AND MOLYNEUX, 1993; KINGHORN, 1985)

Results and discussion

Early extraction plants for screening anticancer

Eleven Indonesian plants, reported in the literature to have anti cancer activity, were collected (Table 2):

TABLE 2. INDONESIAN PLANTS USED IN THE STUDY

No	Taxonomic name	Local name	Parts of plant used
1	*Asparagus cochinchinensis* L.	Bambu tali	Leaves
2	*Brucea javanica* L.	Buah makasar	Leaves
3	*Selaginella doederleinii* Hieron	Cakar ayam	Leaves
4	*Coix lachryma* L.	Hanjeli	Leaves
5	*Taraxacum mongolicum* Hand. -Mazz.	Jombang	Leaves
6	*Typhonium divaricatum* L.	Keladi tikus	Leaves
7	*Solanum nigrum* L.	Leunca	Leaves
8	*Hedyotis corymbosa* L.	Rumput mutiara	Leaves
9	*Catharantus roseus* L.	Tapak dara putih	Leaves
10	*Curcuma zedoaria* Berg Roscoe	Temu putih	Rhizome
11	*Hibiscus mutabilis* L.	Waru	Leaves

The plants were dried at room temperature and macerated in ethanol solvent for 72 hours. The ethanol was concentrated by evaporation with the yields in Table 3.

TABLE 3. PERCENTAGE YIELDS IN PLANT EXTRACTS

No	Name	Dried Weight (gram)	Weight of Concentrated extracts (gram)	Yield (Acquisition) (%)
1	*Asparagus cochinchinensis* (L.)	26	2.7108	10.4
2	*Brucea javanica* (L.)	10	0.9725	9.7
3	*Selaginella doederleinii* Hieron.	4	0.3074	7.7
4	*Coix lachryma* L.	6	0.0526	0.87
5	*Taraxacum mongolicum* Hand.-Mazz.	10	0.3271	3.3
6	*Typhonium divaricatum* L.	12	0.7317	6.1
7	*Solanum nigrum* L.	10	0.6078	6.1
8	*Hedyotis corymbosa* L.	15	1.1423	7.6
9	*Catharantus roseus* (L)	10	1.3598	13.6
10	*Curcuma zedoaria* (Berg) Roscoe	26	0.6061	2.3
11	*Hibiscus mutabilis* .L	8	0.6442	8.05

The concentrated ethanol extracts were then used to test anticancer bioactivity against breast cancer cells (MCF-7) with the results shown in Tables 4, 5 and 6.

TABLE 4. IC_{50} VALUES OF ETHANOL PLANT EXTRACTS USING MCF-7 CELL LINE

No	Plants	Regression equation	IC_{50} (ppm)
1	Cisplatin (standard)	Y = -53.05 log X + 65.54	1.96
2	*Asparagus cochinchinensis* L.	Y = -60.69 log X + 113.92	11.3
3	*Brucea javanica* L.	Y = -29.17 log X + 125.75	> 100
4	*Selaginella doederleinii* Hieron.	Y = -38.14 log X + 113.64	> 100
5	*Coix lachryma* L.	Y= -34.14 log X + 58.72	6.51
6	*Taraxacum mongolicum* Hand.-Mazz.	Y = -8.25 log X + 69.64	> 100
7	*Typhonium divaricatum* L.	Y = -24.57 log X + 122.60	> 100
8	*Solanum nigrum* L.	Y = -33.66 log X + 140.25	> 100
9	*Hedyotis corymbosa* L.	Y = -34.66 log X + 120.71	> 100
10	*Catharantus roseus* L.	Y = -43.48 log X + 107.65	> 100
11	*Curcuma zedoaria* (Berg) Roscoe	Y = -42.41 log X + 92.37	> 100
12	*Hibiscus mutabilis* L	Y = -32.64 log X + 115.95	> 100

Notes: extracts were considered to be anticancer active with IC50 values <100 ppm

TABLE 5. IC_{50} VALUES OF ETHANOL PLANT EXTRACTS USING KB CELL LINE

No	Plant Names	Regression equation	IC_{50} (ppm)
1	Cisplatin (standard)	Y = -40.80 log X + 53.89	1.44
2	*Asparagus cochinchinensis* L.	Y = -91.81 log X + 163.78	17.4
3	*Brucea javanica* L.	Y = -99.69 lg X + 247.14	> 100
4	*Selaginella doederleinii* Hieron.	Y = -30.96 log X + 88.76	> 100
5	*Coix lachryma* L.	Y = -88.36 log X + 205.22	57.1
6	*Taraxacum mongolicum* Hand.-Mazz.	Y = -97.24 log X + 241.60	> 100
7	*Typhonium* divaricatum L.	Y = -50.44 log X + 127.74	> 100
8	*Solanum nigrum* L.	Y= -113.55 log X + 283.37	> 100
9	Hedyotis *corymbosa* L.	Y = -58.89 log X + 179.06	> 100
10	*Catharantus roseus* L.	Y= -103.19 log X + 190.31	> 100
11	*Curcuma zedoaria* (Berg) Roscoe	Y= -108.26 log X + 224.38	> 100
12	*Hibiscus mutabilis* L	Y = -61.22 log X + 137.88	> 100

Notes: extracts were considered to be anticancer active with IC50 values <100 ppm

TABLE 6. IC_{50} VALUES OF ETHANOL PLANT EXTRACTS USING A432 CELL LINE

No	Plant Names	Cell Types	Regression equation	IC_{50} (ppm)
1	Cisplatin (standar)	A431	Y = -25.91 log X + 64.63	3.7
2	*Asparagus cochinchinensis* L.	A431	Y = -31.69 log X + 120.21	> 100
3	*Brucea javanica* L.	A431	Y = -21.09 log X + 96.70	> 100
4	*Selaginella doederleinii* Hieron	A431	Y = -39.58 log X + 134.23	> 100
5	Co*ix lachryma* L.	A431	Y = -33.99 log X + 123.75	> 100
6	*Taraxacum mongolicum* Hand.-Mazz	A431	Y = -13.40 log X + 82.62	> 100
7	*Typhonium divaricatum* (L.)	A431	Y = -34.83 log X + 132.14	> 100
8	*Solanum* nigrum L.	A431	Y = -30.12 log X + 117.93	> 100
9	*Hedyotis corymbosa* L.	A431	Y = -16.95 log X + 118.84	> 100
10	*Catharantus roseus* L.	A431	Y = -18.89 log X + 93.40	> 100
11	*Curcuma zedoaria* (Berg) Roscoe	A431	Y = -14.40 log X + 89.39	> 100
12	Hibisc*us mutabilis* L	A431	Y = -26.21 log X + 139.30	> 100

Notes: extracts were considered to be anticancer active with IC50 values <100 ppm

The bioactivity test used anticancer compound cis-Diammineplatinum (II) dichloride (cisplatin) as the standard. From the observation of anticancer bioactivity tests, only two plants were potent in inhibiting the growth of breast cancer cells (MCF-7), namely *Coix lachrymal* L. and *Asparagus cochinchinensis* L., because both these plant extracts had IC_{50} close to standard anticancer compounds (cisplatin).

Table 4 shows that *C. lachryma* had the lowest IC_{50} value followed by *A. cochinchinensis*. This situation indicates that the ethanol extract of both species provide a good antiproliferative activity against breast cancer cell line MCF-7.

Anticancer bioactivity test observations using KB cells (Table 5) for the ethanol extracts of *C. lachryma* and *A. cochinchinensis* gave IC_{50} of 51.7 ppm and 17.4 ppm respectively; it indicates that the ethanol extract of antiproliferasi *A. cochinchinensis* provides better activity than the one of antiproliferasi *C. lachryma* against skin cancer cells.

Table 6 shows that none of the tested plants provides anti-cancer activity against A431 cells. This means that the plant *Coix lachryma* L. and *Asparagus cochinchinensis* L. have a fairly selective cytotoxic activity.

Extraction and fractionation of* Asparagus cochinchinensis *L. and* Coix lachryma *L.

After selection of plants with breast cell anticancer activity (MCF-7) was done, the next step was to multiply the amount of ethanol from plant extracts selected. Two kilograms each of *Coix lachryma* L. and *Asparagus cochinchinensis* L. were macerated with ethanol for 72 hours, and the extract obtained by ethanol evaporated and condensed to for 325.1 g and 194 g respectively of extract. Subsequently further extraction was carried out using organic solvents with increasing in polarity. The results of further extraction of the extract of *C. lachryma* and *A. cochinchinensis* are shown in Tables 7 and 10.

TABLE 7. EXTRACTION OF THE LEAVES *OF COIX LACHRYMA* L. WITH VARIOUS SOLVENTS

Extract / Fractions	Weight (g)	(%)*
Ethanol	325,1	16,3
n-Hexane	70,2	3,5
Methylene chloride	20,7	1,0
Ethyl acetate	31,8	1,6

Notes: * Calculated on a dry weight of plants

Extraction and fractionation *Coix lachryma* L. consecutively with the n-hexane, methylene chloride and ethyl acetate aimed to increase polarity, with a percentage (w/w) of successive extracts being 3.5, 1.0, and 1.6 as can be seen in Table 7.

Anticancer activity test extracts or fractions of *C. lachryma* showed that the fraction of methylene chloride gave the highest anticancer activity against breast cancer cell line MCF-7 (IC_{50} = 2.75 ppm) (Table 8). This means that antiproliferative activity against breast cancer cells is caused by the presence of secondary metabolites that are semi polar. Anticancer activity of this fraction showed greater activity than the anticancer activity of ethanol extract of *C. lachryma* (IC_{50} = 6.51 ppm) (Table 4), which may imply that further chromatographic separation column can enhance the anticancer activity. But this separation was not yet completed enough for secondary metabolite; because TLC analysis showed the methylene chloride fraction *C. lachryma* still contained more than one component.

TABLE 8. IC_{50} VALUES OF PLANT EXTRACTS OF *C. LACHRYMA* USING MCF-7

No	Extract / Standard	Regression equation	IC_{50} (ppm)
1	Cisplatin	Y= -23.16 log X + 51.29	1.53
2	n-Heksan extract	Y = -18.89 log X + 93.40	> 100
3	Methylene chloride extract	Y = -39.91 log X + 67.55	2.75
4	Ethyl acetate extract	Y = -16.95 log X + 118.84	> 100

Notes: extracts were considered to be anticancer active with IC50 values <100 ppm

The same thing happened to test anticancer activity of extract or fraction *C. lachryma* using KB cell skin cancer. The methylene chloride fraction gave the highest anticancer activity with IC_{50}=5.16 ppm (Table 9). This means that antiproliferative activity against skin cancer cells was also caused by the presence of secondary metabolites that are semipolar. Anticancer activity of this fraction showed much greater activity than the anticancer activity of ethanol extract of *C. lachryma* (IC_{50} = 57.1 ppm) in Table 5, which may imply that further chromatographic separation column can enhance the anticancer activity.

TABLE 9. IC_{50} VALUES OF PLANT EXTRACTS OF *C. LACHRYMA* KB CELL

No	Extract / Standard	Regression equation	IC_{50} (ppm)
1	Cisplatin	Y = -16.46 log X + 31.69	1,08
2	n-Heksan extract	Y = -15.07 log X + 79.89	> 100
3	Methylene chloride extract	Y = -22.96 log X + 66.35	5,16
4	Ethyl acetate extract	Y = -26.21 log X + 139.30	> 100

Notes: extracts were considered to be anticancer active with IC50 values <100 ppm

Fractionation of the methylene chloride fraction of *C. lachryma* was performed according to Figure 3. Results of fractionation gave 10 fractions with the last fraction (10) having the greatest weight is 60.1% and fraction 4 having the smallest weight of 0.1% (Table 10). But this separation was not yet pure enough for secondary metabolites.

Subsequently, fraction 10 was separated by column chromatography with further receipts of hexane, hexane-ethyl acetate and ethyl acetate. These results of further fractionation produced several fractions and one of them as single fraction. Single fraction was kept for further analyzed using NMR to determine its structure later.

Extraction and fractionation *A. cochinchinensis* with the solvent n-hexane, methylene chloride and ethyl acetate aimed to obtain extracts with different polarity, i.e n-hexane extract, extract of methylene chloride and ethyl acetate extracts with a percent (w/w) the weight of successive extracts of 3.9, 0.5 and 1.5 as can be seen in Table 11.

FIGURE 3. FURTHER SEPARATION OF METHYLENE CHLORIDE FRACTION OF *C. LACHRYMA*

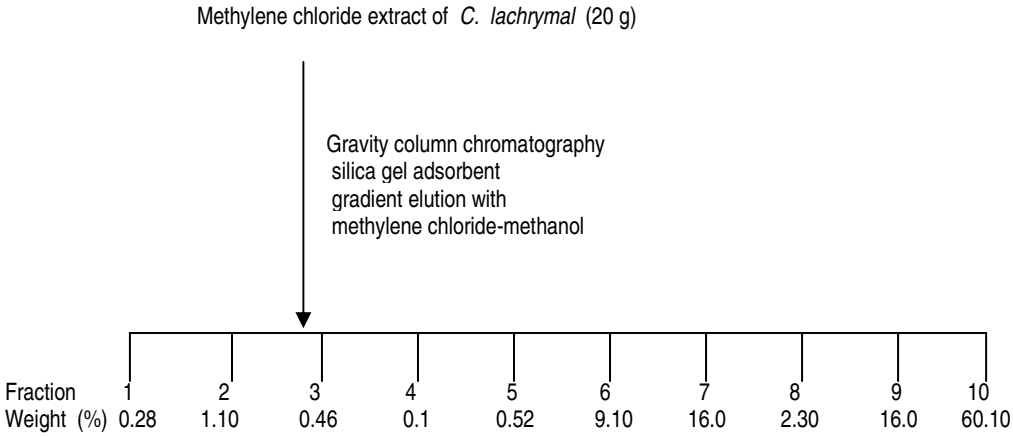


TABLE 10. WEIGHT FRACTIONS OF METHYLENE CHLORIDE EXTRACT OF *C. LACHRYMA*

Fractions	Weight (gram)	Percentage (%)*
1	5.46	0.28
2	22.18	1.10
3	9.10	0.46
4	1.82	0.10
5	10.54	0.52
6	181.80	9.10
7	320.0	16.0
8	46.0	2.30
9	320.0	16.0
10	1213.2	60.10

Notes: * Calculated on a dry weight of plants

Anticancer activity test extracts or fractions *A. cochinchinensis* using KB cancer cells are shown in Table 12. It can be seen that the ethyl acetate fraction gave the smallest IC_{50} value (IC_{50} = 3.7 ppm), followed by the hexane fraction (IC_{50} = 5.5 ppm) and the methylene chloride fraction (IC_{50} = 8.1 ppm). These data indicated that the activity against cancer cells antiproliferative against Oral epidermoid carcinoma (KB / skin cancer) was caused by the presence of polar secondary metabolites. Anticancer activity of this fraction showed greater activity than the anticancer activity of ethanol extract of *A. cochinchinensis* (IC_{50} = 17.4 ppm) in Table 5, which may imply that further chromatographic separation column can enhance the anticancer activity.

TABLE 11. *ASPARAGUS COCHINCHINENSIS* L. EXTRACTION WITH VARIOUS SOLVENTS

Extract / Fractions	Weight (g)	(%)*
Ethanol	194.0	9.7
n-Hexane	77.6	3.9
Methylene chloride	10.4	0.5
Ethyl acetate	9.4	0.5

Notes: * Calculated on a dry weight of plants

TABLE 12. IC_{50} PLANT EXTRACTS OF *A. COCHINCHINENSIS* USING KB CELL

No	Extract / Standard	Regression equation	IC_{50} (ppm)
1	Cisplatin	Y = -14.06 log X + 41.41	0.25
2	n-Hexane extract	Y = -30.95 log X + 72.87	5.50
3	Methylene chloride extract	Y = -42.05 log X + 88.06	8.10
4	Ethyl acetate extract	Y = -25.91 log X + 64.63	3.70

Notes: Extracts were considered to be anticancer active with IC50 values <100 ppm

The test of anticancer activity of extracts or fractions *A. cochinchinensis* using cancer cell line MCF-7 (breast cancer) showed that n-hexane, methylene chloride and ethyl acetate gave IC50 values respectively of 84.5 ppm; >100 ppm and 9.80 ppm (Table 13). These data indicated that antiproliferative activity against breast cancer cells was also caused by the presence of polar secondary metabolites. Anticancer activity of this fraction showed greater activity than the anticancer activity of ethanol extract of *A. cochinchinensis* (IC_{50} = 11.3 ppm) in Table 4, which may imply that further chromatographic separation column can enhance the anticancer activity.

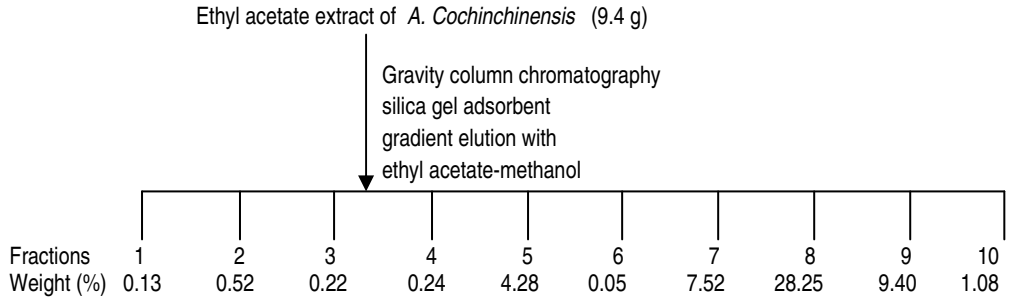
TABLE 13. IC_{50} PLANT EXTRACTS OF *A. COCHINCHINENSIS* USING MCF-7

No	Extracts / Standard	Regression equation	IC_{50} (ppm)
1	Cisplatin	Y = -16.46 log X + 31.69	1.08
2	n-hexane extracts	Y = -12.97 log X + 74.99	84.5
3	Methylene chloride extracts	Y = -10.93 log X + 88.50	>100
4	Ethyl acetate extracts	Y = -10.43 log X + 60.36	9.80

Notes: Extracts were considered to be anticancer active with IC50 values <100 ppm

Fractionation of ethyl acetate fraction of *A. cochinchinensis* was performed as in Figure 4, giving 10 fractions with fraction 8 having the greatest weight of 28.25% and fraction 6 having the smallest weight of 0.05% (Table 14). But this separation was not yet pure enough for secondary metabolites.

FIGURE 4. FURTHER SEPARATION OF METHYLENE CHLORIDE FRACTION *A. COCHINCHINENSIS*



Results of further fractionation of No.8 produced several fractions and one of them as a single fraction. Single fraction was kept for further analyzed using NMR to determine its structure later.

TABLE 14. WEIGHT FRACTIONS OF METHYLENE CHLORIDE EXTRACT OF *A. COCHINCHINENSIS*

Fractions	Weight (gram)	Percentage (%)*
1	2.57	0.13
2	10.43	0.52
3	4.23	0.22
4	4.96	0.24
5	85.45	4.28
6	0.86	0.05
7	150.40	7.52
8	570.20	28.25
9	187.55	9.40
10	21.62	1.08

Notes: * Calculated on a dry weight of plants

Phytochemical analysis of extracts / fractions Plants

Chemical analysis was conducted to predict the qualitative class of compounds contained in the extracts or fractions of isolated plants. Qualitative examination of groups of chemical compounds of plant extracts and fractions of *C. lachryma* showed that the ethanol extract contained terpenoids, steroids, and phenolics; the methylene chloride fraction contained steroids and phenolics; the n-hexane fraction contains terpenoids, steroids, and phenolics; and the ethyl acetate fraction contains steroids and phenolics (Table 15). Anticancer active fraction was indicated by the fraction of methylene chloride (Table 8 and Table 9); it gives the sense that the anticancer activity generated by the extract / fraction of plants of *C. lachryma* was alleged to be caused by a group of compounds contained in the fraction.

Phytochemical screening conducted on plant extracts and fractions of *A. cochinchinensis* (Table 16) showed that ethanol extract, methylene chloride fraction and n-hexane fraction contained terpenoids, steroids and phenolics; and the phenolic fraction contained ethyl acetate. Anticancer active fraction of *A. cochinchinensis* was shown by ethyl acetate (Table 12 and Table 13); it gives the sense that the anticancer activity generated by the extract / fractions of this plant is alleged to be caused by one group of compounds contained in this fraction.

TABLE 15. PHYTOCHEMICAL TEST OF *C. LACHRYMA* EXTRACT

Class of chemical compounds	Ethanol extract	n-hexane extract	Methylene chloride extract	Ethyl acetate extract
Alkaloid	-	-	-	-
Phenolic	+	+	+	+
Flavonoid	-	-	-	-
Tannin	-	-	-	-
Triterpenoid	-	-	-	-
Terpenoid	+	+++	-	-
Steroid	+	+	+++	+
Saponin	-	-	-	-

Notes: + there is a positive reaction to the chemical test; - No positive reaction to the chemical test

TABLE 16. PHYTOCHEMICAL TEST OF *A. COCHINCHINENSIS* EXTRACT

Class of chemical compounds	Ethanol extract	n-hexane extract	Methylene chloride extract	Ethyl acetate extract
Alkaloid	-	-	-	-
Phenolic	+	+	+	+
Flavonoid	-	-	-	-
Tannin	-	-	-	-
Triterpenoid	-	-	-	-
Terpenoid	+	+++	+	-
Steroid	+	+	+++	-
Saponin	-	-	-	-

Notes: + there is a positive reaction to the chemical test; - No positive reaction to the chemical test

Conclusion

The received findings show that of the 11 plants tested two plants showed breast and skin anticancer activity, namely *Coix lachrymal* L. and *Asparagus cochinchinensis* L. Extraction of *Coix lachryma* L. with various organic solvents shows that the methylene chloride extract is the most active anticancer breast and skin agent. *Asparagus cochinchinensis* L. extraction results with various organic solvents shows that the ethyl acetate extract is breast and skin anticancer active.

Further investigation should be performed to isolate and elucidate active compounds from the plants and study its interaction with their targets.

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