DIU Journal of Health and Life Sciences Volume 01 , No.01 & 02, 2014
https://doi.org/10.36481/diuhls.v01i1-2.1wy25j71

PHYTOCHEMICAL AND PHARMACOLOGICAL [LEALHALITY & FREE RADICAL SCAVENGING] SCREENING OF THE PLANT *CLEOME VISCOSA*

KH Ahammad Uz Zaman[1], Sharifa Sultana[1], Kanij Nahar Deepa[1]

[1]Department of Pharmacy, Daffodil International University

Abstract: *Phytochemical screening of the ethanolic extract of leaves of Cleome viscosa indicates the presence of glycosides, alkaloids, steroids and gums. Pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine tends to investigate for possible cytotoxic and free radical scavenging activities. Traditionally this plant is used to relieve earache and the bruised leaves are applied to the skin as a counter-irritant. As it has a great antibacterial effect and antiviral effect, it must have some toxic effect to biological organ. Moreover, every toxic substance is a drug at lower doses. The ethanolic extract of leaves of Cleome viscosa showed significant lethality (cytotoxic activity) against the brine shrimp nauplii as well as free radical scavenging activity. The obtained results provide a support for the use of this plant in traditional medicine.*

Keywords: *Cleome viscose. Free radical, Brine Shrimp Lethality Bioassay.*

Introduction

Since Bangladesh is a country of low economic growth, scientific exploration and standardization of potential crude drugs is an urgent need to revolutionize our drug sector. Plant secondary metabolites have been used for mankind as remedies since the beginning of civilization. Now a day they still play an important role in the health care for about 80% of the world population[1]. Divers bioactive metabolites like steroids, terpenoids, flavonoids, alkaloids, glycosides, etc. in plants have formed the therapeutic basis of herbal medication. Thus emphasis is given on the biological screening of medicinal plants for further exploration of their active constituents[2]. The present project work was designed to investigate the scientific basis of the traditional use of *Cleome viscosa* for its cyto-toxic and free radical scavenging activity.

Haldi Hurhurey (*Cleome viscosa*) is a medicinal plant which posses important active principals that have special medicinal value and various parts of plants are traditionally use against a variety of diseases by the rural people. The plant is an annual herb with very small flowers, fruits, grows in tropical and warm areas of Bangladesh[3].

Botanical feature

Botanical name: *Cleome viscosa*.
Local name: Haldi Hurhurey *etc.*
Family: Capparidaceae.

Genus: *Cleome.*
Species: *viscosa*.
Synonyms: *Polanisia viscosa.*
Accession Number: *30.169*
Parts used: Mainly leaf, root and seeds. Sometimes whole plant is also used.
Traditional use: The juice of the leaves of *cleome viscosa* is traditionally used to relieve earache and the bruised leaves are applied to the skin as a counter-irritant. The seeds are used as anthalmintic and curminative. They are also given occasionally in fever and diarrhea[2].

Table 1: Worldwide ethno medical uses

WORLDWIDE ETHNOMEDICAL USES [3]	
Indo-China	The Root: - Stimulant and Anti-scorbutic. The whole Plant (burnished) **:-** Counter-irritant and blistering.
La-Rennion	The Plant **:-** Astringent and anti-spasmodic
Australia	The Plant: - To relieve headache.
U.S.A.	The Root: - Used as vermifuge.
Sri-Lanka	The Root and Seeds: - Used as cardiac stimulant **.**They are given internally in case of snake bite (Roberts).

Figure 1: *Cleome viscosa* (whole plant)

Figure 2: Fruits and Flowers

Materials and methods

Phytochemical Screening: The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plant and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function[3]. In all these operations, methods are needed for separation, purification and identification of many different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. As a result of modern extraction, and isolation techniques and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations[5]. For this present investigation the *Cleome viscosa* was collected from Kushtia district, Bangladesh. The collected plant parts were separated from undesirable materials or plants or plant parts. They were sun-dried for one week after cutting into small pieces. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced[3].

Cold extraction (Ethanol extraction): About 400 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 1300 ml of 80% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a water- bath until dried. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol.

Chemical Group Tests: Testing of different chemical groups present in extract represent the preliminary phytochemical studies. The chemical group test, which are performed as follows[6]. In each test 10% (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test. The following reagents were used for the different chemical group test[6].

Mayer's reagent: 1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

Dragendroff's Reagent: 1.7 gm basic bismuth nitrate and 20 gm tartaric acid ware dissolved in 80 ml water. This solution was mixed with a solution contains 16 gm potassium iodide and 40 ml water.

Fehling's solution A: 34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

Fehling's solution B: 176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml. Equal volume of above solution were mixed at the time of use.

Benedicts Reagent: 1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

Molish Reagent: 2.5 gm of pure α-naphthol was dissolved in 25 ml of ethanol.

The following **t**ests were performed for identifying different chemical groups[6].

Test for alkaloids

Mayer's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow color precipitate was formed and that was indicated as the presence of alkaloids.

Dragendroff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added. Orange brown precipitate was formed and that was indicated as the presence of alkaloids.

Tests for Glycosides

A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color was considered as an indication for the presence of glycosides.

A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution. Brick-red precipitate was considered as an indication for the presence of glycosides.

Test for Steroids

Sulphuric acid test: 1 ml solution of chloroform extract was taken and then added1ml Sulphuric acid. Red color indicates the presence of steroid.

Test for gums

5 ml solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indica te the presence of gums and carbohydrate

Tests for reducing sugar

Benedict's test: 0.5 ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar.

Fehling's Test (Standard Test): 2ml of an aqueous extract of the plant material was added 1ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for few minutes. A red or brick red color precipitate was formed in the presence of a reducing sugar.

Alpha Naphthol Solution test: 5 ml solution of extract added with 2 drops of 5% alpha-Naphthol solution (Freshly prepared) and added 1 ml of sulfuric acid on the sides of the

test tube. Violet colored ring was formed at the junction of two liquids in the presence of reducing sugars.

Tests for tannins

Ferric Chloride Test: 5 ml solution of the extract was taken in a test tube. Then 1 ml of 5%Ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins.

Test for Flavonoids

Added a few drops of concentrated hydrochloric acid to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids

Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

Table 2: Different chemical group tests performed and the results are mentioned

Sample	Test solution	Observation	Inference
Test for Alkaloids: #2 ml solution of the extract and 0.2ml of dilute hydrochloric acid	0.1 ml of Mayer's reagent.	Yellowish buff colored precipitate was obtained.	Presence of alkaloid.
#2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid.	0.1 ml of Dragendroff's reagent.	Orange brown precipitate was observed.	Presence of alkaloid.
Test For Glycosides: #A small amount of an alcoholic extract was taken in 1ml of water.	A few drops of aqueous NaOH were added.	A yellow color was found.	Presence of glycosides.
# A small amount of an alcoholic extract was taken in water and alcohol.	Boiled with Fehling's solution.	Brick-red precipitate was found.	Presence of glycosides.
Test for Steroids: # 10 mg extract dissolved in 1 ml chloroform.	1 ml sulfuric acid.	Chloroform layer Acquired reddish brown color and acid layer showed green fluorescence.	Presence of steroid.
Tests for Gums : # 5 ml solution of extract.	Molish reagent and sulfuric acid.	Red-violet ring produced at the junction of two liquids.	Presence of gums.

Tests for Flavonoids: # 1 ml solution of ethanolic extract.	Few drops of conc. HCl was added to the extract	Immediate red color was not formed.	Absence of Flavonoids.
Tests for Saponins: # 1 ml solution of the extract was diluted with distilled water to 20 ml.	Shaken in a graduated cylinder for 15 minutes.	No centimeter layer of foam.	Absence of Saponins.
Tests for Reducing sugars: # 5 ml solution of extract.	5 ml Fehling's A and B solution boiled for 5 minutes on a boiling water bath.	Brick red colored precipitate was not obtained	Absence of reducing sugars
# 5 ml solution of extract.	5 ml Benedict's reagent and boiled for 5 minutes on a boiling water bath	Brick red color is not precipitate.	Absence of reducing sugars
Tests for Tannins: # 5 ml solution of extract.	1 ml of 10% Lead acetate solution.	Yellow precipitate was not obtained	Absence of tannins.

Brine Shrimp Lethality Bioassay: Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, pesticidal, etc[7]. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus, in-vivo lethality of a simple zoological organism (brine shrimp nauplii) can be used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products [8].*Artemia salina* Leach (brine shrimp eggs form store), table salt, pure NaCl, small tank (glass jar) to grow shrimp, cover and lamp to attract shrimp, pipettes(5 ml,1 ml), micro-pipette (10 µl, 200 µl adjustable), test tube(15 ml), DMSO (Dimethyl sulfoxide), electric water blower to produce current, electric bulb to produce heat , stand to hold the bulb ,petri dish, test tube stand, beaker (1 liter) were used in this test.

Preparation of stock solution: 500 mg of dried ethanolic extract was taken in 10 ml volumetric flask and volume was adjusted by DMSO. The concentration of this solution was 50 µg/µl.

Preparation of sea water: 38g sea salt (pure NaCl 20g and table salt 18g) was weighed accurately, dissolved in distilled water to make one liter and then filtered off to get a clear solution.

Hatching of brine shrimp: Sea water was taken in the small tank and shrimp eggs were added to the one side of the divided tank and the side was covered. The shrimps were

allowed for 30 hours to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and they were taken for bioassay. Application of test solution and brine shrimp nauplii to the test tubes: 24 clean test tubes were taken, 12 of which were for the samples in six concentrations (two test tubes for each concentration) and 12 for control test. Then 5ml of seawater was given to each of the test tubes. Then with the help of the micropipette specific volumes (0.5, 1, 2, 4 & 8 µl) of samples were transferred from the stock solutions to the test tubes to get final sample concentrations of 5,10,20,40 and 80µg/ml respectively. The concentration of DMSO in these test tubes did not exceed 40 µl/4ml. For the control, same volumes of DMSO (as in the sample test tubes) were taken in the rest of the 12 test tubes. Finally with the help of a Pasteur pipette 10 living shrimps were kept to each of the test tubes[6].

Counting of nauplii: After 24 hrs the test tubes were observed and the number of survived nauplii in each test tube was counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

Free radical Scavenging Activity of *Cleome viscose:* Free radicals are metastable chemical species which, after being generated *in vivo* as byproducts of various biochemical reactions, tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses. These radicals may be envisaged as molecular sharks, which if not scavenged effectively on time, are capable of damaging crucial bio-molecules including those present in cell membranes, mitochondria, DNA etc. and thus predisposing various pathophysiological states. The role of free radicals, especially of the so called 'reactive oxygen species' (ROS), has been well-established in the pathogenesis of many disease conditions such as rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, some metabolic disorders, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, AIDS. ROS is a collective term, which includes not only the oxygen radicals ($O_2^{\cdot-}$, and $^{\cdot}OH$) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3)[9]. In recent years one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of Phytochemical have been found to have quite prominent antioxidant activity. The objective of the present study was to investigate the antioxidant activity of the crude extract of *Cleome viscosa.*The anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquot of the different concentrations (1-500 µg/ml) of the extract was added to 3 ml of a 0.004% EtOH solution of DPPH. Absorbance at 517 nm was determined after 30 min,

and IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals[10].

Apparatus: Test tubes, Beakers, Magnetic stirrer, Thermometer, Pipette, UV spectrophotometer (single beam), Electronic balance Reagents: Ethanol, 0.004% DPPH (Aldrich, USA), Ascorbic acid (Loba, India) were used in this experiment.

Procedure: At first 6 test tubes were taken to make aliquots of 6 conc. (1, 5, 10, 50, 100 and 500 µg/ml). Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the concentration by dilution technique. Here ascorbic acid was taken as standard. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations 3 ml of 0.004% DPPH solution was applied on each test tube by pipette. The room temperature was recorded and kept the test tubes for 30 minutes in light to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. After 30 minutes, absorbance of each test tube was determined by UV spectrophotometer. IC_{50} was determined from % inhibition *vs* concentration graph.

Result

Table 3: Results of different group tests are given bellow [+ = Presence; - = Absence]

Extract	Alkaloid	Glycoside	Steroid	Gums	Reducing sugars	tannins	flavonoids	Saponins
Ethanol	+	+	+	+	-	-	-	-

Phytochemical studies showed that glycosides, alkaloids, steroids and gums are present in the ethanolic extract.

Brine Shrimp Lethality Bioassay: In this bioassay, the crude extract showed lethality indicating the biological activity of the compound present in the extract. Test sample showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus log concentration on the graph paper produced an

approximate linear correlation between them. From the graph (figure) the concentrations at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. The values were found to be 40µg/ml for the crude extract. The 90% mortality (LC_{90}) values were 121.40 µg/ml respectively. Table is given below:

Table 4: Result of Brine shrimp lethality bioassay of 95% ethanolic extract

Test sample	Conc. (µg/ml)	Log (Conc.)	No. of alive shrimp	Percent (%) mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
95% ethanolic extract	0.5	0.3	8	20	40	121.40
	10	1.0	7	30		
	20	1.3	6	40		
	40	1.6	5	50		
	80	1.9	2	80		
	160	2.2	0	100		

Table 5: Absorbance of Ascorbic acid.

Concentration (µg/ml)	Absorbance-1(nm)	Absorbance-2(nm)	Average (nm)	SD
500	0.013	0.011	0.012	0.001
100	0.020	0.026	0.023	0.003
50	0.092	0.094	0.093	0.001
10	0.192	0.194	0.193	0.001
5	0.269	0.267	0.268	0.001
1	0.340	0.342	0.341	0.001

Table 6: Absorbance of extract of *Cleome viscosa*

Concentration (µg/ml)	Absorbance-1(nm)	Absorbance-2(nm)	Average(nm)	SD
500	0.540	0.536	0.079	0.003
100	0.573	0.560	0.104	0.004
50	0.604	0.598	0.231	0.006
10	0.607	0.604	0.375	0.003
5	0.483\615	0.477\609	0.480	0.003
1	0.491\640	0.496\620	0.493	0.003

Table 7: Evaluation of antioxidant activity of extract of *Cleome viscose*

Sample	**Concentration(μg/ml)**	**% inhibition**	**IC_{50}(μg/ml)**
Et. extract of . *Cleome viscosa*	1	36.07±0.003	44.88
	5	37.82±0.003	
	10	51.42±0.003	
	50	70.07±0.006	
	100	86.59±0.004	
	500	89.76±0.003	
Ascorbic acid	1	55.83±0.001	0.9
	5	65.16±0.004	
	10	75.00±0.001	
	50	87.95±0.001	
	100	97.02±0.003	
	500	98.43±0.001	

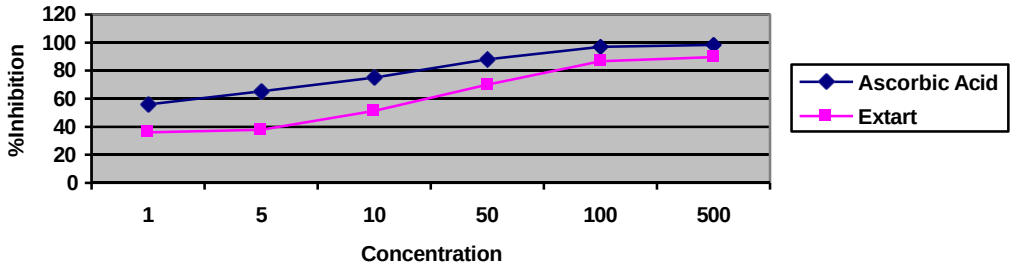

Figure 7: DPPH Scavenging Assay of Cleome *viscosa* compared with ascorbic acid

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of the barks of *Cleome viscosa* showed potential free-radical scavenging activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine. Most of the phenolic compounds may be responsible for antioxidant properties of many plants[11]. IC_{50} of the *Cleome viscosa* is 44.88 μg/ml which indicated the potential antioxidant activity of the plant extract and the activity may be due to the presence of phenolic compounds (tannins) present in the extract[12].

Discussion

Phytochemical studies showed that glycosides, alkaloids, steroids and gums are present in the ethanolic extract.

Brine Shrimp Lethality Bioassay: In this bioassay, the crude extract showed lethality indicating the biological activity of the compound present in the extract. Test sample

showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. From the graph (figure) the concentrations at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. The values were found to be 40µg/ml for the crude extract. The 90% mortality (LC_{90}) values were 121.40 µg/ml respectively.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of the barks of *Cleome viscosa* showed potential free-radical scavenging activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine. Most of the phenolic compounds may be responsible for antioxidant properties of many plants[11]. IC_{50} of the *Cleome viscosa* is 44.88 µg/ml which indicated the potential antioxidant activity of the plant extract and the activity may be due to the presence of phenolic compounds (tannins) present in the extract[12].

Conclusion

The crude extracts were found to show strong lethality against the brine shrimp nauplii and strong free radical scavenging property. These results tend to suggest its possible _antitumor, antibacterial or pesticidal as well as antioxidant activities. However, further researches are necessary particularly with its purified fraction.

References

1. *Ghani A, Medicinal Plants of Bangladesh: The Asiatic Society of Bangladesh, 3rd edition, 1998.*
2. *Yusuf M., Chowdhury J.,Wahab MA. & Begum J. Medicinal Plants of Bangladesh, BCSIR, Dhaka, Bangladesh, 1994.*
3. *Mali RG, "Cleome viscosa (wild mustard): a review on ethnobotany, phytochemistry and pharmacology", Pharmaceutical Biology, Jan 2010, Vol. 48(1), pp. 105-12*
4. *Evans WC..Trease and Evan's Text book of Pharmacognosy. University Press, Cambridge.13th edition. 1989.*
5. *Myers Phytochemical methods (a guide to modern techniques to plant analysis). Champan and Hall. 3rd edition. 1982.*
6. *Anderson JE, Chang CJ, McLaughlin JL., "Bioactive components of Allamanda schottii", Journal of Natural Product, 1988, Vol. 51(2), pp. 307-8.*
7. *Hui YH, Chang CJ, Smith DL, McLaughlin JL, "16 alpha-hydroxy-(-)-kauranoic acid: a selectively cytotoxic diterpene from Annona bullata", Pharm Res, 1990, Vol. 7(4), pp. 376-8.*
8. *Halliwell B, Gutteridge J M C., In: Free radicals, ageing, and disease, Free radicals in Biology and Medicine. Clarendron Press, Oxford. 2nd edition. 1985.*
9. *Gupta M, Mazumdar UK, Sivakumar T, Vamis MLM, Karkis S, Sambathkumar R, Mainkandan L, "Antioxidant and Anti-inflammatory activities of Acalypha Fruticasa", Nigerian Journal of Natural Products, 2003, Med : 25-29.*
10. *Larson R A., "The antioxidants of higher plants", Phytochemistry, 1988. Vol. 27(4), pp. 969–978.*
11. *Sadhu SK, Okuyama E, Fujimoto H, Ishibashi M., "Separation of Leucas aspera, a Medicinal Plant of Bangladesh, Guided by Prostaglandin Inhibitory and Antioxidant Activities", Chem Pharm Bull 51 (5), 2003, pp. 595-598.*