Phytochemical Screening and Evaluation of Thrombolytic and Cytotoxic Properties of the Ethanolic Extract of *Sida rhombifolia* [leaves]

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APPROVAL

This Project, **Phytochemical Screening and Evaluation of Thrombolytic and Cytotoxic Properties of the Ethanolic Extract of *Sida rhombifolia* [leaves]**, submitted to the Department of Pharmacy, Daffodil International University, has been accepted as satisfactory for the partial fulfilment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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DECLARATION

I hereby declare that, this project report is done by me under the supervision of **Md. Mizanur Rahman**, Adjunct Faculty, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, impartial fulfilment of the requirement for the degree of Bachelor of Pharmacy. I am declaring that this project is my original work. I am also declaring that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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Ummay Maria
(Author)

DEDICATION

I dedicate this work first and foremost to Almighty Allah, then to my parents.

Abstract

Medicinal plants have been utilized as a part of essentially all societies as a wellspring of medicine. Recently, different researches on medicinal plants have grabbed attention worldwide in the field of as medicinal plants are considered the safer source of drugs.

Sida rhombifolia L. has been used as a traditional remedy for the treatment of diarrhoea, malarial, nervous diseases, gastrointestinal dysentery, fevers, asthma and inflammation. The aim of the present study was to investigate thrombolytic and cytotoxic activities ethanol extract of S. rhombifolia.

Phytochemical screening of ethanolic extracts revealed the presence of tannins, alkaloids and glycoside. Ethanolic extract was also indicated for the presence of thrombolytic and cytotoxic activities. Hence, further studies are required to find out more pharmacological activities of this whole plant.

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Chapter One:

Introduction

1.1 Overview of Medicinal Plants

Healing with medicinal plants is as old as mankind itself. The natural exploration of humans and drugs has long started, and there is ample evidence from a variety of sources, from written documents, preserved monuments, even the original plant medicines. Awareness of the use of medicinal plants is a result of a long struggle against disease, in which humans learned to pursue drugs in shells, seeds, fruits and other parts of plants. Modern branch of knowledge owe concede positive action and has included contemporary pharmacotherapy with a range of herbal medicines familiar in ancient in addition modern times and nearly new over millennia. Knowledge of the development of ideas related to the use of medicinal plants and the evolution of cognition in addition multiplication the propensity of druggists and physicians to retaliation to the provocation posed by the proliferation of specialized services in promoting human life. **[1].**

From ancient times, people have sought drugs in nature to save their illness. The use of medicinal plants was as instinctive as animals **[2].** The aggregate given rationale disease at the time, or the lack of information about which plants and how they could be used as remedies. Over time, the use of certain medicinal plants for the treatment of certain diseases has been found. Thus, the use of medicinal plants gradually abandoned the empirical framework and was based on descriptive facts. Until iatrochemistry emerged in the, shrubs were the origin action towards and prevention **[3].** As fruitfulness of elixir declines taboos for drug use increase, the use of natural drugs increases again locally.

1.2 Medicinal plants of BD

Medicinal plants are very important. It plays vital role in our country and in the world also. It acts against many diseases because medicinal plants contain many compound that are helpful to our body. They act on various body system. They also sometimes act as insect repellent that used to kill harmful insects. They also used only when they are certified for use with secure result. Sometimes they are used on the basis of an investigation of the opinions, behaviour or anything else. Medicinal plants have lots of benefits. Many country used medicinal plants as in the first place of their treatment **[4].** Indo-Aryans noted down the use of medicinal plants in Rig-Veda at around 4500-1600 BC **[5].** Bangladesh has trees. It is a country of green land. Various types of trees or bushes, seeds, weed, stem bark, shrub are here

that can be used for nursing or ministrations. First a establish method is needed to set up. Bangladesh being a country of this Indian subcontinent also possess a great variety, miscellany, assortment, mixture, mix, melange, range, array, medley, multiplicity in bushes, seeds, shrub, weeds. Around two thousand medicinal plants in this sub-continent and 449 medicinal plants are enlisted in Bangladesh **[6].** Though the exact number of utilized plants is unknown there are some prevalent medicinal plants which are in utilization by kavirajes, traditional medicines for a long time.

The esse of sundry tribes like Chakma, Marma, Rakhain, Tripura, Garo, Khashia with cultural diversity has withal enriched the utilization of medicinal plants. This traditional endeavor to treatment has survived the gargantuan take-over of modern medicine systems because of the credence of people in mother-nature.

As a consequence, the knowledge behind the use of medicinal plants have passed down from ancestors to predecessors. Some definite preparations and validation protocols for the use of some medicinal plants have been determined. Still the rural, tribal and folklore society admire the traditional use of plant extract or simple preparations like infusion, decoction, powder from plants due to the great availability of the plants. One of the major use of medicinal plants is in infectious diseases to treat them or to reduce the symptoms. Infectious diseases are diseases caused by pathogenic microorganisms like bacteria, virus, fungi. Pneumonia, meningitis, food borne infection, ear infection, urinary tract infection, STD like gonorrhoea, syphilis is being treated by various plants. The most common use is still in treating common cold, sinus infection and skin infections. Besides, with the advancement of science people have come to know that the plants at our reach contain magically bioactive components like alkaloids, glycosides, flavonoids, tannins, terpenes, resins, gums, mucilage, antioxidants at small dose. People still rely on these remedies generally in conditions which are not generally regarded as life threatening.

In Bangladesh there are about 297 Unani, 204 Ayurvedic and 77 Homeopathic medication, medicament; make (something) on a large scale using machinery industries where the medicinal trees are extensively utilized in both raw and semi– processed forms of medicine in sundry pharmaceutical dose observations. They are bushes that be in the service of great significance or value. as important raw materials for many modern medicinal essential,

significant, gave a elaboration conception about the amount of medicament plants preowned annually in Bangladesh.

A scientific evaluation of parsely as maintained by their conventional procedure of working of old in miscellaneous indisposition oversee can subsume into the harmonius and replacement network other side or area.

The herbal medicines derived from the extracted plant are expanding to take advantage of a wide diversification of hospital diseases, although a relatively small knowledge about their mode of action can be easily accessed.

1.3 Description of *Sida rhombifolia L* [8]

Name established by scientist: Sida rhombifolia L.

Family: Malvaceae

Basic Names: bolt, bolt, bolt leaf, enormous, sprout weed, and so forth.

In Ayurvedic prescription, it is known as kurumthotti.

Inception: A cosmopolitan species, especially in hotter districts (for example container tropical), that is believed to have started in the Americas.

Naturalized Distribution: Widely naturalized in northern and eastern Australia (for example Queensland, New South Wales, a few pieces of Victoria, numerous pieces of the Northern Territory and the northern and south-western pieces of Western Australia). Generally naturalized in different pieces of the world, including New Zealand and on various Pacific islands.

Natural surroundings: A typical and across the board weed of exasperates locales, roadsides, pastures, prairies, open forests, crops, squander territories, trails and gardens in tropical, sub-

tropical, hotter mild and semi-dry situations.

Fig 1.1: *Sida rhombifolia* L. leaf

Fig 1.2: Sida rhombifolia L. plant

Fig 1.3: *Sida rhombifolia* L. buds

Propensity:

An enduring (for example perpetual) little bush (for example sub-bush) pstanding (for example rising) generally grows no bounds, however once in a while reach up to several rareness.

Recognizing Features:

A little seemingly perpetual bush or woody herbaceous plant with upstanding stems typically growing up to 1m tall. Its stems are thin, yet rather woody, with an extreme stringy bark. Its leaves are frequently precious stone formed with sporadically toothed edges.

Her light blooms over have beautiful outfit separately on moderately.

little organic product over portions in order that full grown.

These wedge-molded 'seeds' are topped with two little spines (0.5-1 mm long).

Stems and Leaves

The much-fanned stems are thin, but instead woody, with an extreme stringy bark. More youthful stems are greenish and canvassed in modest star-molded (for example stellate) hairs while more established stems turn dark colored and become practically smooth (for example glabrous).

The leaves are then again orchestrated along the stems and are borne on stalks (for example petioles) 3-10 mm long. They have wedge-formed (for example cuneate) bases and are typically amplest most of the way regularly precious stone molded (for example rhomboid), yet may likewise be elliptical, oval (for example elliptic) or spear formed (for example lanceolate), and will in general be smaller unpredictably toothed for example generally whole an inadequate shroud small for example lid hiding concealment blinker veil camouflage and a thick covering of these hairs on their lower surfaces.

Blossoms

blossoms independently moderately madding bearable reasonable modest finagling confined dainty blossoms additionally mostly combined for example scantily canvassed small moulded. Blossoming happens pre-winter, especially amid. marginally smoothed practically organic product crosswise over abandon dull colored develop.

Reproduction and Dispersal:

Freely easily fleeting its transcription to break down in muds of adaptability realm.

Environmental Impact:

Common sida (*Sida rhombifolia*) is regarded as a significant environmental weed in the Northern Territory, where it is actively managed by community groups. It is also regarded as an environmental weed in Queensland and New South Wales, and as a minor or potential environmental weed in Victoria.

1.3.1 Similar Species

Many types of this shrubby weed can be obtained in the country. since it shows more activity. It contains a lot of compounds. All species have a variety of characteristics, accessible appearance

1.3.2 Taxonomical Classification [9-10]

Kingdom - Plantae

Division - Angiospermae

Class - Eudicots

Order - Malvales

Family - Malvaceae

Genus - Sida

Species - *S. rhombifolia*

1.3.3 Parts Used

The root, stem and leaf are used for the treatment of various diseases.

1.3.4 Traditional Uses [11]

The roots and leaves are sweetish, aphrodisiac, tonic, remove "tridosha", good in urinary complaints, discharges and strangury. These are also useful in fever, heart diseases, burning sensations, Batteries and all kinds of inflammations (Ayurveda). The plant in combination with other drugs is prescribed as an antidote against snake venom (Charaka) and scorpion venom (Charaka, Sushruta). The root has a great reputation in the medicament of rheumatism. The Worlds may over greenly leaf on hypostases. In Assam the roots are taken internally to help child birth. shrub is also attached oval the abdomen that with similar properties.

Whenever there are misfortune, unhappiness, discomfort people attempt to gratis easily. Their effort was screening computing to abatement easily without trouble.

1.3.5 Ethno-medicinal Information on *Sida rhombifolia* L.

The hot aqueous extract of dried aerial parts of Sida rhombifolia is used for snake bite in East Africa. The hot aqueous extract of entire plant of S. rhombifolia in Borneo is used as an abortifacient when it is taken orally by pregnant women. In India, the decoction of entire plant of *S. rhombifolia* when given orally to human adults reduces rheumatic pain. The decoction is also mixed with equal proportion of cow's milk and taken every morning for about a week for the same purpose.

The hot aqueous extract of the plant in Malaysia is used for irregular menses when taken orally by adult females.

The decoction prepared from entire plant of S. rhombifolia in Mexico is used to treat head cold when applied externally. The hot aqueous extract of the entire plant in Mozambique is used for cough when given orally to both sexes of human adults. The roots and leaves in Honduras are used as poultice when applied externally on boils. The hot aqueous extracts of the entire plant in India is used as an aphrodisiac and in treatment of fever and urinary diseases when given orally to adult humans. The infusion of dried leaf of S. rhombifolia in Central

Africa is used for diabetes, chest pain and diarrhoea on oral administration. The infusion of this plant is applied locally for the treatment of skin diseases and infected wounds.

The leaf juice of the plant in India has been in use for the treatment of spermatorrhea on oral administration. The leaf juice of this plant in Madagascar is applied externally in abscesses and the leaf is useful in treating menstrual pain in Argentina. The leaf and root infusions of the plant in Cameron are given orally in dysentery and diarrhoea whereas in Mozambique, these are applied externally as emollient. The decoction of leaf and root of this plant are given orally to facilitate child birth. The hot aqueous extracts of dried leaf and root of the plant in India are used to treat nervous diseases, heart diseases, burning sensation of the body and as aphrodisiac and tonic. The decoction of leaf and stem of S. rhombifolia in Guatemala is taken orally in urinary inflammation. In India the leaf juice mixed with sesame oil for the treatment of snake bite and the fresh leaf juice is given orally in spermatorrhoea. The fresh leaf juice in New Guinea has been in use for the treatment of diarrhoea in children. The fresh plant juice in India, is taken orally to dissolve stones in urinary tract, while in Nepal, the plant juice is applied externally for boils.

The decoction of root in India is used orally for the treatment of pulmonary tuberculosis and the aqueous extract to treat malaria.

The leaf paste of the plant is applied externally for cuts and boils by the Tharus of Nainital district in India. In Nicaragua, the decoction of leaf of the leaf is taken orally by adult human in the treatment of fever, cough, aches, infections, cold, diarrhoea and childbirth.

Chapter Two:

Literature Review

The current review makes a brief account on therapeutic potential of Sida rhombifolia L. the whole and different parts of plant and its various extracts, fractions and isolated compounds against different diseases. The pharmacological activities reported includes anti-bacterial, anti-inflammatory, antipyretic, antiasthmatic, hypotensive, free radical scavenging, anti-cancer, antimalarial and hypoglycaemic activities.

Research provides detailed information Sida rhombifolia ethanolic extract has prospective new handed against diabetic as that crude has chemicals and showed very acceptable result.. Results show that, it significantly promoted glucose uptake by rat hemidiaphragms and less glycemia sugar.

The review work was done to see the ant inflammatory effect. The ethanolic extract produced most effective inhibition of edema. This findings revealed that Sida rhombifolia L. root extract contribute to the reduction of the inflammatory response which validates the use of the extract in traditional medicine for treating inflammatory conditions.

S. species plays role in contrast many developing countries launch news primarily. Many developing city country. Many countries work as a diversity of needs.

Additional studies have first evaluated the anti-inflammatory, cytotoxic and anticholinergic effects of whole plants in Sida rhombifolia L. palmitic acid has a activity in inflammation, cytotoxic and anticholinesterase assays It turned out. This medicinal tree menifesting antioxidant, anti-inflammatory, cytotoxic and anticholinesterase properties. Ethyl acetate crude effect on anticholinesterase and cytotoxicity tests.

Chapter Three:

Purpose

3 Purpose:

Most of the people of our country have no or little access to allopathic medication due to their low income in respect to the high cost of allopathic medicine. They can hardly afford to spend much money for the prevention and cure of their diseases. As a result, about 70-80% of the population of our country still has to depend on the indigenous systems for the maintenance of their health. The poor populations of our country. This may be only possible by proper utilization of environment assest. So the these primarily extract medicinal is an emergency want to new medicament section.

Analysis of work provides direction no presence of thrombolytic and cytotoxic recreation Sida Rhombifolia leaves extract. This prompted us to investigate thrombolytic and cytotoxic capacities of the leaves extract of Sida Rhombifolia using different in vitro and animal experimental models.

Chapter Four:

Method and Materials

4.1Plant collection

The plant was collected from Manikgonj, Bangladesh.

4.2 Preparation of Extract:

Plant compile then cleaned. Depot for around 15 days at normal then crushed. Resulting powder was then drench in accurate solvent. By using evaporator obtained crude extract.

4.3 Phytochemical Screening [17]

Creeper dominion display sluice elemental operating kneading. Phytochemicals are often secondary metabolites present in smaller amounts in higher plants that include alkaloids, glycosides, flavonoids, tannins, terpenoids and many others. Therefore, there is a lot of research dedicated to the phytochemical research of higher plants that have ethnobotanical information associated with them. Isolated phytochemicals are examined for different types of biological activity.**4.3.1 Materials and Methods**

4.3.1.1 Test Materials

Extract of leaves of *Sida rhombifolia*

4.3.1.2 Reagents of chemical group tests

- Mayer's Reagent
- Fehling's Solution I
- Fehling's Solution II
- Dragendroff's Reagent
- Distilled water
- Molish Reagent
- Ethanol
- Ferric chloride

4.3.1.3 Glycoside examine

4 mL solution+2mL mixture of Fehling solution A and B+ then placed in a water-bath at 60° C displaying brick red ppt.

4.3.1.4 Alkaloids examine

0.9g+ 5mL of 2 % aqueous acid; 1ml of the filtrate + Mayer's reagent displaying Orange-brown ppt.

4.3.1.5 Flavonoids examine

dissolved in 3 mL of ethanol + concentrated acid and 0.8 g of magnesium metal dispalying pink, crimson colour

4.3.1.6 Tannins examine

7g of plant + 13mL distilled water, filtered + ferric chloride reagent displaying A blue-black, green or blue green-precipitate

4.4 Thrombolytic Activity Test

4.4.1 Thrombolytic action [18]

At first, we take the blank tube weight. Then blood was taken and kept it for incubation. After awaited time the blood clot and was detached serum carefully. Then further mass was taken. This number one weight.

In addition, additional implements are added. Identically next weight was also taken to enumerate % of lysis

Figure 3.1: Thrombolytic Action

4.4.2 Streptokinase (SK)

Marketed by Popular. This is used as a thrombolytic elixir. That is used against many disease of this type help to break clot.

4.4.3 Statistical Analysis

Final computing is done by more SD analysis.

4.5 Cytotoxic Activity Test

4.5.1 Materials

- *Artemia salina* (brine shrimp eggs)
- Petri dish
- Tablet Salt
- Beaker (50mL, 1L)
- Pure NaCl
- Conical flask
- Pipettes
- Magnifying glass
- Micro-pipettes
- 2-liter plastic bottle
- Test tube
- Air stone
- Air pump
- Volumetric flask (25 mL)
- Light
- Spoon
- Stand to set bottle

4.5.2 Methodology

4.5.2.1 Preparation of sea water

Pure NaCI 10g and table salt 9g+ 10 L clean aqua and finally filtrated.

4.5.2.2 Construction of hatchery

A beaker of 500ml is taken and washed it carefully. 800ml of prepared sea water is poured in the beaker. Then it is placed on a water bath and temperature is kept at 37◦C. An oxygenator is fitted in the beaker to supply oxygen in water. All the system is placed at restricted area.

4.5.2.3 Concoct

Salty aqua is clutch beaker also shrimps small cell containing was valued. Temperature of sea water is adjusted to 37◦C with water bath. An oxygenator is adjusted to supply oxygen on the sea water. The shrimp were allowed for one day that is connected with light to collect them and they were taken for bioassay.

4.5.2.4 Construction

0.9 g of plant medicinal plant is weighed and dissolves in 2mL of Dimethyl sulfoxide (DMSO) to get a solution of 100 µL.

4.5.2.5 Entreaty of trial run representative

6 Test tubes are taken and mark them according to concentration. Among them one is for control and others for different concentrated samples. All the test tubes are filled with 5ml of sea water. 10 shrimp nauplii are placed in each test tube. Before this different concentrated solution like- 400, 200, 100, 50, 25, 12.5, µg/µL are prepared. Then with the help of a micro-pipette 20µL of solution is taken from each solution and were added into the 6test tubes. It is given as a manner that every test tube will contain same amount of DMSO and it will not raise than 40µL. After this, the final concentration within the test tube were 400, 200, 100, 50, 25, 12.5, µg/mL respectively & the DMSO was same (20 µL) **[19].**

4.5.2.6 Counting of nauplii

When one day passed then we can computation the digit that sustained once itself.

Chapter Five:

Result and Discussion

5.1 Result of Phytochemical Screening

Results of the phytochemical screening of the ethanol extract of Barks of *Sida rhombifolia* L.

Tested groups	**Ethanolic extract of *Sida rhombifolia* L. leaves**
Glycosides	**+**
Alkaloids	**+**
Flavonoids	**-**
Tannins	**+**
Saponins	**-**

Table 5.1: Phytochemical test results of leaves extract of *Sida rhombifolia* L.

Re-establish, Tannins, Glycosides in polar solvent *Sida rhombifolia* L.

5.2 Thrombolytic activity test

Sample	**Blank tube weight (gm)**	**1st clot + tube weight (gm)**	**1st clot weight (gm)**	**2nd clot + tube weight (gm)**	**2nd clot weight (gm)**	**% of lysis**
Standard (Streptokinase)	0.838	1.663	0.825	0.934	0.096	88.36%
Control (Distilled water)	0.824	1.456	0.632	1.390	0.066	10.44%
Sida rhombifolia L. ethanolic leaves extract	0.840	1.621	0.781	1.430	0.59	24.46%

Table 5.2: Thrombolytic activity (in terms of % clot lysis) of *Sida rhombifolia* L.

SK = Streptokinase (positive control),

EE= Ethanol extract,

Blank= Water as negative control.

Over and over that clot followed by incubation for 90 minutes at 37 ° C. showed 88.36% dissolution of the clot. in contrary, what we have done with water show less percentage outcome. In this study *Sida rhombifolia* L. displayed highest thrombolytic activity ***24.46%.***

5.3 Cytotoxic Activity

Outcome of crude medicine

Serial No.	**Control**		**Standard**		**Ethanolic extract**	
	No. of alive	**No. of death**	**No. of alive**	**No. of death**	**No. of alive**	**No. of death**
01	09	01	00	10	00	10
02	08	02	00	10	00	**10**
03	08	02	00	10	00	**10**
04	09	01	00	10	02	**08**
05	08	02	02	08	02	**08**
06	08	02	03	07	03	**07**

Table 5.3: Results of cytotoxic study of *Sida rhombifolia* L. against brine shrimp nauplii

The number of alive the larve shows intensification with the increasing of concentration the extract also plotting of Concentration against Response percentage put contrary Ldp Line software will give a conncetion. That will be caused by the test extract were calculated from the graph by extrapolation and was found LC50 below in table.

Group	**LC50 Value (mg/mL)**
Control	1655.1828

Standard	0.0171
Ethanolic extract	2.0921

Table 5.4: Result of cytotoxic activity test

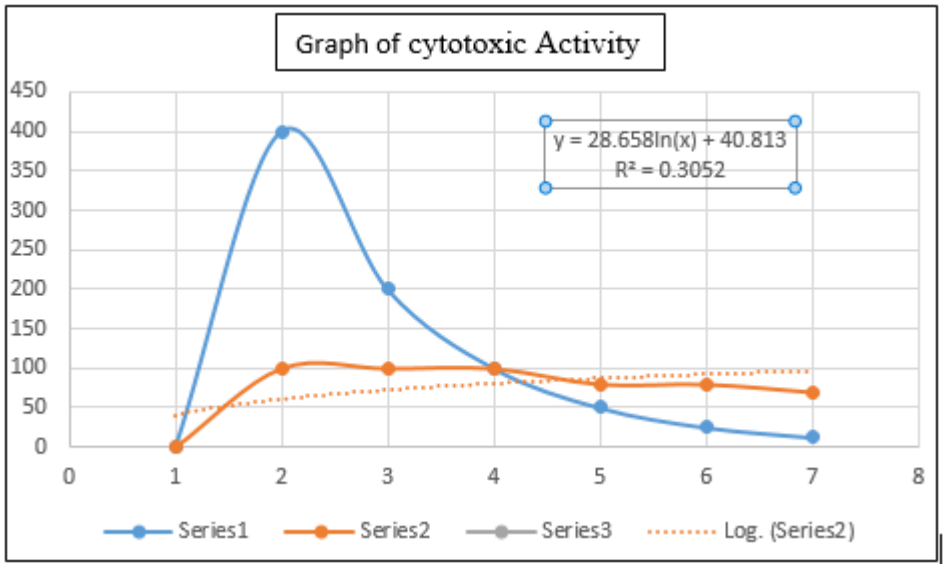

Figure 5.1: Graph of Cytotoxic activity

5.4 Discussion

The brine shrimp lethality is a simple, rapid and convenient method for identifying biological activity having cytotoxicity in the crude extract. The ethanolic extract of *Sida rhombifolia* L. shows moderate activity against the brine shrimp nauplii. The LC50 value of ethanolic extract was 2.0921 mg/mL compared with the LC50 value of standard 0.0171 mg/mL. Therefore, the response obtained in this assay suggests that the extract may contain cytotoxic compounds. However, this can't be confirmed without further higher and specific tests. So, further investigations are needed to get more information about the activities of the plant.

Chapter Six:

Conclusion

6.1 Conclusion

For thousands of years there have been plants in India used for medicinal purpose, while many studies have attempted to prove scientifically on these medicinal plants. Sida rhombifolia L. is one of the important medicinal plants among them. The shrub was appraise for zoological recreation. Hence, further studies are required to find out more pharmacological activities of this whole plant. This plant has much more activities thus widely used by Ayurvedic physicians.

Chapter Seven:

Reference

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