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### Pharmacognostical and Preliminary Phytochemical Evaluation of *Aegiceras corniculatum* (L)

J.Karthi <sup>\*1</sup>, M.Purushothaman <sup>2</sup>

<sup>1</sup>Sun Rise University, Alwar, Rajasthan – 301030, India

<sup>2</sup>Scient Institute of Pharmacy, Ibrahinpatnam, Hyderabad-501506, India.

**Abstract :** The whole plant material of *Aegiceras corniculatum* (L) was collected and powdered. The powdered material was subjected to successive soxhlet extraction with petroleum ether (40-60°), chloroform, ethanol and finally macerated with water so as to get respective extracts. Physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value were determined which were 14.38, 8.12, 10.34 and 6.09% respectively. Moisture content, foreign organic matter, crude fibre content, alcohol soluble extractive and water soluble extractive were also determined. The percentage yield of petroleum ether, chloroform, ethyl acetate, ethanol and water were 5.7, 10.0, 5.5, 4.5 and 10.5% respectively. Preliminary phytochemical analysis of different extracts was carried out. The results were positive for glycoside, carbohydrate, sterols, flavonoids and phenolic compounds in petroleum ether extract. Chloroform extract showed positive test for tannins only, ethyl acetate extract showed positive test for sterols and saponins, ethanolic extract exhibited positive test for alkaloids, flavonoids, glycosides, tannins, amino acids and saponins whereas aqueous extract was found to be positive for flavonoids, alkaloids, carbohydrates, glycosides, amino acids and saponins. These secondary metabolites are the active constituents of *Aegiceras corniculatum* (L). and may be responsible for its pharmacological activities.

**Key words :** *Aegiceras corniculatum* (L), Pharmacognostic evaluation, Phytochemical analysis and Secondary metabolites.

#### Introduction:

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances [1]. For centuries, plant and plant products have been used for treating various illnesses. Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market [2]. However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines [3]. Therefore it has become extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [4]. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy [5].

*Aegiceras corniculatum*, commonly known as Black Mangrove, River Mangrove or Khalsi, is a species of shrub or tree mangrove in the Myrsine family (or Primrose family) with a distribution in coastal and estuarine areas ranging from India through South East Asia to southern China, New Guinea and Australia. *Aegiceras corniculatum* grows as a shrub or small tree up to 7 m high, though often considerably less. Its leaves are alternate, obovate, 30–100 mm long and 15–50 mm wide, entire, leathery and minutely dotted. Its fragrant, small, white flowers are produced as umbellate clusters of 10–30, with a peduncle up to 10 mm long and with pedicels 10–18 mm long. The calyx is 2–4 mm long and corolla 4–6 mm long. The fruit is curved and cylindrical or horn-shaped, light green to pink in colour and 20–75 mm long. It grows in mud in estuaries and tidal creeks, often at the seaward edge of the mangrove zone. *Aegiceras corniculatum* extract has analgesic properties [6-10]. The present study was designed to investigate the pharmacognostic and phytochemical properties of *Aegiceras corniculatum* (L)

## Materials and Methods

### Collection of plant material and authentication:

*Aegiceras corniculatum* (L) was collected from the tribal belts of the local area of Kanniyakumari district, Tamilnadu, India. The plant was identified, confirmed and authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Andhrapradesh. After authentication the whole plant of *Aegiceras corniculatum* (L) were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder and stored in a closed air tight container for further use.

### Microscopic study

Microscopy of plant material is performed to distinguish it from the allied drugs and adulterant. The dried leaf was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtome. The thickness of section was 10-12  $\mu$ m. Very fine section was selectively subjected to staining reaction with staining reagent safranin one percent solution and light green 0.2% solution. Slides were cleaned in xylol and mounted in mountant (DPX). Photomicrographs were taken using trinocular microscope [11-14].

### Powder studies

#### Microscopic study

The shade dried plant material were mechanically pulverized to coarse powder and sifted through 40 mesh sieve. Take a pinch of powder was taken on slide and mounted with phloroglucinol, hydrochloric acid and glycerine. Slide was seen under microscope.

#### Determination of physicochemical parameters [15-18]:

The dried plant material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content, pet ether soluble, chloroform soluble, ethyl acetate soluble, alcohol soluble extractive and water soluble extractive, Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, and Haemolytic value.

#### Extraction of powdered plant material:

The shade dried powdered plant material was subjected to sequential soxhlet extraction using the solvents of different polarity such as petroleum ether (40-600), chloroform, ethyl acetate, ethanol and finally macerated with water so as to get respective extracts. Cold maceration was also done using ethanol and water. The extracts were filtered individually, evaporated to dryness and the percent yields of all the extracts were determined. All the extracts were then stored in a refrigerator till further analysis.

**Preliminary phytochemical analysis:**

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional protocols.

**Results and Discussion:****Microscopic features:****Anatomy of the Leaf:**

The leaf is smooth and even on both surfaces the midrib is alternate, obovate, 30–100 mm long and 15–50 mm wide, entire, leathery and minutely dotted. The midrib has a thick adaxial epidermis layer of circular cells with thick warty cuticle.

These are one or two layers of the epidermal cells are also circular, highly thick walled compact. A thin layer of palisade mesophyll cells is horizontally transverse along the adaxial side of the midrib. The adaxial epidermal cells are comparatively smaller but they are highly thick walled and possess thick, undulate cuticular surface

The ground tissue includes one or two subepidermal layer of collenchyma cells and the remaining tissue includes large angular, thin walled and compact parenchyma cells

The vascular strand is single and shallow arch shaped. It is collateral and consists of several short rows of xylem elements. The xylem cells are wide angular and thick walled. Phloem occurs in small or three celled limits along in lower surface of the xylem arch. The vascular strand is 300µm wide and 150µm thick

**Epidermis of the Lamina**

The adaxial epidermis of the lamina consists of polyhedral cells with straight, fairly thick walls, The Epidermis is Aponomatic (without Stomata)

**Adaxial epidermis**

The Adaxial epidermis is Stomatiferous. The Stomata are dense and are random in distribution. The Stomata are exclusively paracytic type. Each stomata has a pair of parallel subsidiary cells, one either side of the guard cells

The guard cells are Elliptical in shape and are 10 x 20 µm in size. The anticlinal walls of the epidermal cells are thick and straight. The cuticular striations are feebly visible on the surface epidermal cells

**Powder Characteristics****Venation Pattern:**

The leaflet exhibits well defined reticulate venation. The Veinlets are thickly coated and straight branches of veins.

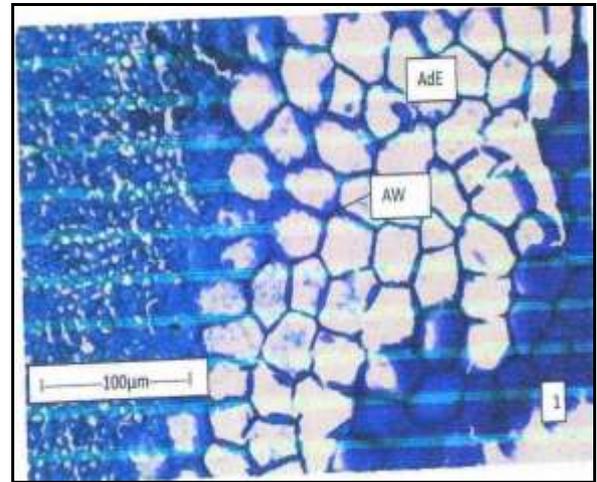
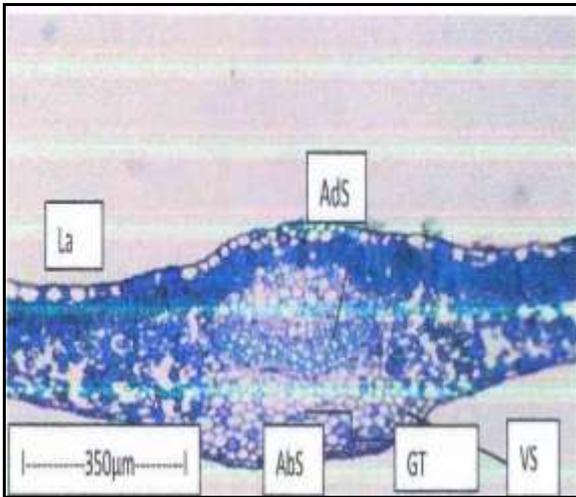
The veinlets are rectangular, squarish or polygonal in outline. The Vein-Termination are well expressed and prominent. Almost all veinlets have one or occasionally two terminations are dendroid in outline

**Epidermal Trichomes:**

Non Granular covering type of Epidermal Trichome are abundant along the leaf margin and scarcely distributed on the lamina the Trichomes are unicellular and un-branched. They are acicular in shape and are mostly lopsided or curved horizontally. The trichomes measure 350µm in length and 15 µm thickness

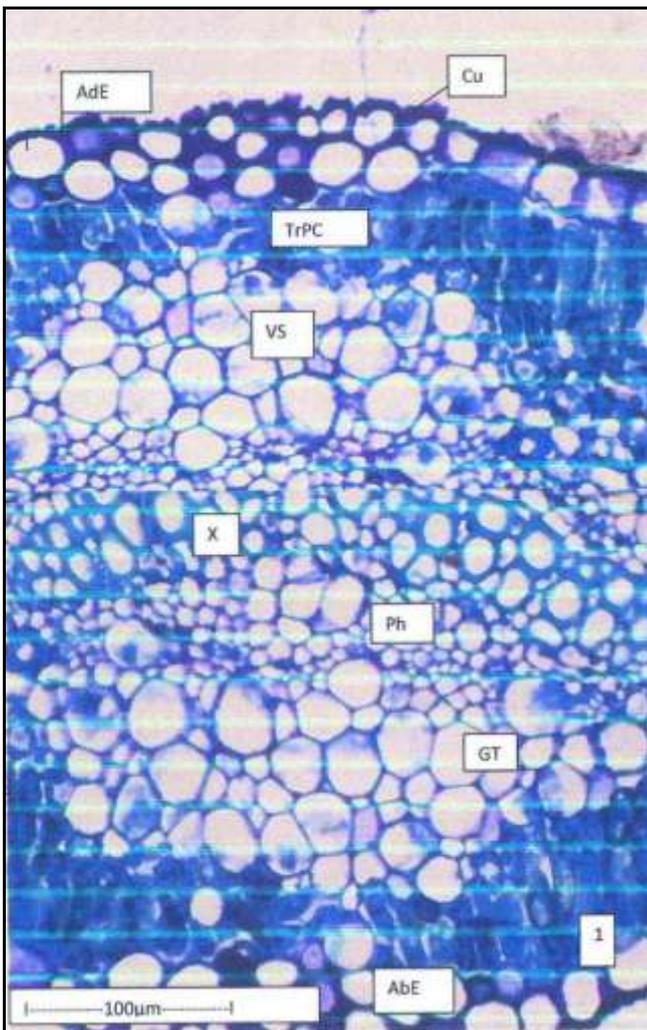
**Crystal Distribution:**

Calcium oxalate crystals are fairly common in the lamina. They are restricted to the cells fording the veins. The crystals are pragmatic type; they are eufoidal or rhom foidal in shape

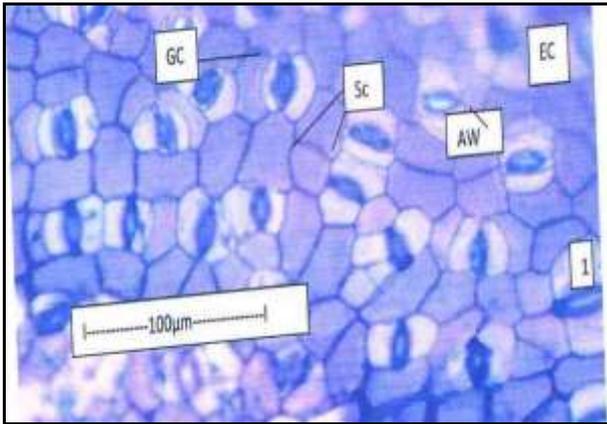


**Fig.No 1: T.S of Leaf through midrib Adaxial epidermis**

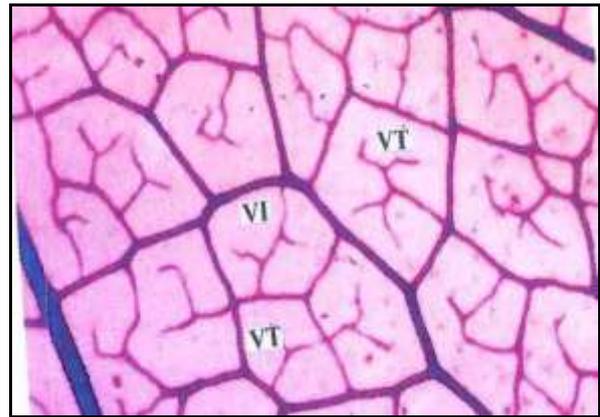
**Fig.No 2: Adaxial epidermis**



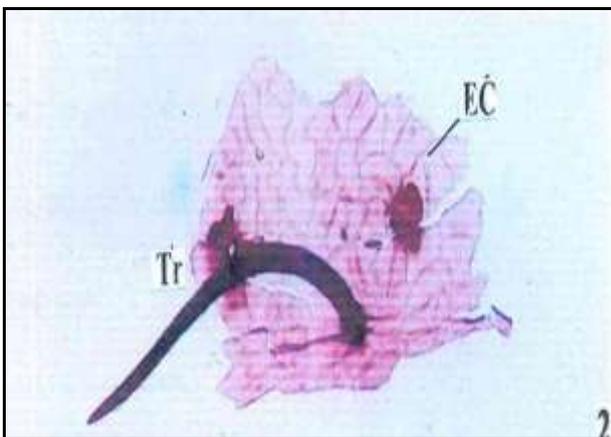
**Fig.No 3: Epidermis of the Lamina**



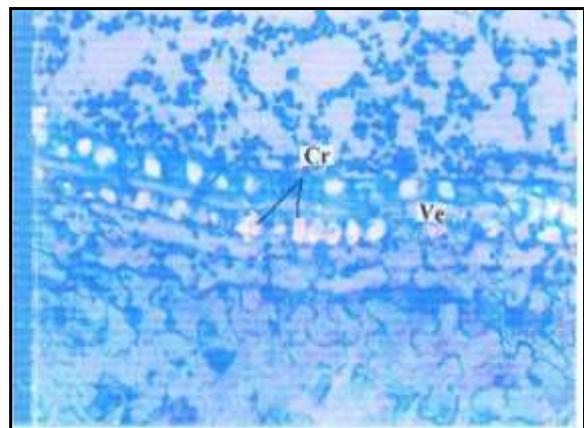
**Fig.No 4: The Epidermis Stomata**



**Fig.No 5: Venation Pattern islet**



**Fig.No 6: Epidermal Trichome**



**Fig.No 7: Calcium Oxalate**

Abe: abaxiul epidermis; Ade: adaxial epidermis; Ads: adexial side; Ep: epidermis; Gt: ground tissue; La: lamina; Mr: midrib; Ph: phloem; Lv: latrial vein; Pm:palisade mesophyll; Sm: spongy mesophyll; Sc: sclerenchyma; X: xylem AW- Arlicliral walls, EC- Epidermal Cell, GC –Guard Cells, SC- Subsidiary Cells

**Table No 1: Total ash, water soluble ash, acid insoluble ash and sulphated ash of aerial plant parts of the powder**

S. No.	Ash	Percentage in AC
1	Total ash	14.38
2	Water soluble ash	8.12
3	Acid insoluble ash	10.34
4	Sulphated ash	6.09

**Table No 2: Extractive values of roots powder with various solvents**

S. No.	Solvent	Extraction period (h)	AC Extractive values (%) w/w
1.	Petroleum ether (60-80°C)	24	5.7
2.	Chloroform	24	10.0
3.	Ethyl acetate	24	5.5
4.	Ethanol	24	4.5
5.	Aqueous	24	10.5

**Table No 3: Crude fiber content, Loss on drying, Swelling index, Foaming index, Tanins contents, Bitterness value, Haemolytic value.**

S. No.	Parameter	Observation in AC
1.	Crude fiber content	9.45 %
2.	Loss on drying	14 %
3.	Swelling index	No significant result
4.	Foaming index	No significant result
5.	Tannins	22
6.	Bitterness value	1.9 unit / g
7.	Haemolytic activity	23.45 %

The Total ash; water insoluble ash; sulphated ash; and acid insoluble ash (14.38%, 8.12%, 10.34%, and 6.09%). Extracting values, i.e. petroleum ether (PE); chloroform (CF); ethyl acetate (EA); ethanol (ET) and aqueous extract (5.7%, 10.0%, 5.5%, 4.5%, and 10.5%). The fiber content was found to be 9.45%. Plant bitterness was found to be 1.9 unit / g. The plant also has hemolytic potential. The tannin content was found to be 22.

**Table 5.2: Preliminary phytochemical screening of *Aegicras corniculatum* (L)**

Sr. No	Plant Constituents Test / Reagent	Pet. Ether extract	Ethyl acetate extract	Chloroform extract	Ethanol extract	Aqueous extract
1.	<b>ALKALOIDS</b> Mayer's reagent Dragendroff's reagent Wagner's reagent	+ + +	- - +	+ + +	+ + +	- - -
2.	<b>GLYCOSIDES</b> Killer-Killani test Sodium nitropruside test	- - -	- - +	- - -	- - +	- - -
3.	<b>CARBOHYDRATES</b> Molisch's reagent Fehling solution	- -	- -	- -	+ +	+ +
4.	<b>STEROLS</b> Liebermann-Burchard's test Salkowski test Hesses reaction Hersch reaction	- - + - -	- - - - -	- - - - -	+ - + - -	- - - - -
5.	<b>SAPONINS</b> Foam test Sodium bicarbonate test	- -	- -	- -	+ +	- -
6.	<b>PHENOLIC COMPOUNDS &amp; TANNINS</b> Ferric chloride solution Lead acetate solution	- -	- -	- -	- -	- -
7.	<b>PROTEINS &amp; AMINO ACIDS</b> Biuret test Millon's reagent Ninhydrin reagent	- - -	- - -	- - +	+ + +	- - -
8.	<b>FLAVANOIDS</b> Shinoda/Pew test Ammonia test	- -	- -	- -	+ +	- -

+ve : Detected; -ve : absent

The preliminary phytochemical investigations of various extracts of *Aegiceras corniculatum* (L) were studied. The results were positive for glycoside, carbohydrate, sterols, flavonoids and phenolic compounds in petroleum ether extract. Chloroform extract showed positive test for tannins only, ethyl acetate extract showed positive test for sterols and saponins, ethanolic extract exhibited positive test for alkaloids, flavonoids, glycosides, tannins, amino acids and saponins whereas aqueous extract was found to be positive for flavonoids, alkaloids, carbohydrates, glycosides, amino acids and saponins. These secondary metabolites are the active constituents of *Aegiceras corniculatum* (L) and may be responsible for its pharmacological activities.

### Conclusion:

*Aegiceras corniculatum* (L) powder was subjected for preliminary Pharmacognostic standardization including phytochemical screening. The present investigation adds to the existing knowledge of *Aegiceras corniculatum* (L) and will be quite useful for development of a formulation for treating various ailments.

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