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PHYTOCHEMICAL CHARACTERISATION AND PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF *AVICENNIA ALBA*

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ABSTRACT

The history of herbal medicines is as old as civilization. For this purpose of ascertaining the phytochemical constituents, the present work was performed into the phytochemical investigation of the plant *Avicenna alba*. Separation and isolation of the constituents of interest, characterization of isolated compounds and investigation of the biosynthetic pathways of the particular pathway and quantitative evaluation. Another reason for choosing this plant is the availability of the plant. *Avicenna alba* is the important plant used from the past. It was reported to show antimicrobial activity in literature survey. So now, the time has come to perform a proper phytochemical investigation to find its proper way into medicine. Our plan of work is to identify and isolate the chemical constituents of the aerial part of *Avicennia alba* and to evaluate its pharmacological action. With the above aim, this experimental work was undertaken.

INTRODUCTION: *Avicennia alba* is a species of tropical mangrove in the family Acanthaceae. In the Malay language it is known as Api Putih, "api" meaning "fire", referring to the fact that this mangrove attracts fireflies, and "putih" meaning "white", referring to the pale-coloured underside of the leaves. It is found growing in coastal and estuarine locations in India, South East Asia, Australia.

Avicennia alba forms a low, dense bushy crown often branching near the base of the trunk. The shrub does not grow more than about 20 metres high. The roots are shallow and send up a large number of pencil-shaped pneumatophores. These aerial roots help with gas exchange and also play an important part in the exclusion of salt from the plant's vascular system. The trunk has smooth, greenish black bark which is finely fissured and does not flake. The dark green leaves, 15 cm long and 5 cm wide, have a silvery grey underside and grow in opposite pairs.

The small, orange yellow flowers, borne in a racemose inflorescence, have 4 petals and a diameter of about 4 mm when expanded.

The fruits are grayish-green capsules and conical in shape with an elongated beak up to 4 cm long.

Each contains a single seed. Traditional use include Medicinal uses include the resin for toothache, as contraceptive, an aphrodisiac.

QUICK RESPONSE CODE

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Scientific Classification:

Kingdom: Plantae
Phylum: Angiospermophyta
Class: Magnoliopsida
Order: Serophlariales
Family: Avicenniaceae

Botanical Description:

Roots: pencil like pneumatophores emerge above ground from long shallow underground roots.

Leaves: Leaves simple, over to pointed (8-10 cm long) arranged opposite one another. Shiny green above, underneath white and waxy. Leaves have gloms to secrete salt.

Flowers: small, yellow, several together, forming a cross shaped in florescence.

Fruits: Flat capsule containing one seed.

EXPERIMENTAL PART: Phytochemical Evaluation and pharmacological activity of the plant *Avicennia alba:* Our plan of work is to identify and isolate the chemical constituents of the aerial part of *Avicennia alba*. And to evaluate its pharmacological action our total experiment is categorized under two board heading namely phytochemistry part and pharmacological evaluation.

Phytochemistry study of the plant:

1. The collection of plant material.
2. Extraction by soxhletion process.
3. Phytochemical screening.
4. Isolation by the thin layer chromatography.
5. Isolation by column chromatography.

MATERIALS AND METHODS:

a. Collection of plant materials: The plant *Avicennia alba* belongs to the family of Avicenniaceae is distributed throughout India. For the present study, the plant material is collected in the month of July from Sundarban mangrove, located about 80 km from Kolkata. The plant material was washed with running water to remove the dust and soil and finally rinsed with distilled water. The steam, leaves, fruits were separated and dried insisted. The dried material then pulverized separately into coarse powder by the mechanical grinder. The resulting coarse powder was then used for extraction.

b. Preparation of extract: The coarse powder of the shade dried part [steam, fruit, and leaves] of *A. alba* was subjected to successive extraction soxhletion apparatus continuous hot extraction with various solvent increasing order of potency. The solvent used were chloroform, methanol and water.

1. **Petroleum ether extraction:** 250 gm of powder (Ariel parts) was packed separately into the extraction chamber of soxhletion apparatus and washed extracted with petroleum ether 40-60ºc for 4 days. After extraction the liquid extract was collected and concentrate under vaccum.20 gm of solid yellowish green extracted isolated.
2. **Chloroform extraction:** The marc left over after pet extraction was dried and re-extracted with 1lit of chloroform for 4 days. After the extraction the chloroform liquid extract was concentrate in vacuum.
3. **Methanol extraction:** The marc left over after chloroform extraction was dried and re extracted with 1lit of methanol for 5 days. After the extraction the liquid extraction was collected and concentrates under vacuum. 80 gm of highly viscous yellowish brown extract is obtaining.
4. **Aqueous extraction:** The dried marc left after methanol extraction was boiled with distilled water of 100ºc. The aqueous extract then filters and concentrates to dryness.10 gm of solid dark brown extracted is obtain.

The extractive value of each extract was determined and tabulated in **table 1**.

TABLE 1: DATA SHOWING PERCENTAGE YIELD OF DIFFERENT EXTRACTS OF *AVICENNIA ALBA*

SL. NO.	Extract	Percentage yield of aerial part
1.	Petroleum Ether	20/250*100= 8%
2.	Chloroform	20/250*100= 8%
3.	Methanol	80/250*100=32%
4.	Water	10/250*100= 4%

Phytochemical Screening: The freshly prepared extract of *Avicennia alba* is qualitatively tested for the presence of the chemical constituents. Phytochemical screening of the extract is performed using the following reagent and chemicals and corresponding observation are noted (**table 2**).

TABLE 2: LIST OF PHYTOCHEMICAL SCREENING TESTS PERFORMED

SL NO	EXPERIMENT	ETHER	CHLOROFORM	METHANOL	WATER
1)	**Test for alkaloids**				
	• Mayer's reagent	-	-	+	-
	• Wagner's reagent	-	-	+	+
2)	**Test for amino acid**				
	• Millon's test	-	-	+	-
	• Ninhydrine test	-	-	-	-
3)	**Test for carbohydrate**				
	• Molish test	-	-	-	+
	• Felling 1 + felling 2	-	+	+	+
4)	**Test for fat and fixed oil**				
	• Saponification test	-	-	-	-
5)	**Test for flavonoids**				
	• Shinoda test	-	-	-	-
	• Zinc hydrochloride test	-	-	-	-
6)	**Test for glycosides**				
	• Bronte reagent	+	-	+	+
	• Keller-Killiani	+	+	+	-
	• Froth formation test	-	-	-	+
7)	**Test for tannins**				
	• Ferric chloride test	-	-	+	+
	• Gelatin test	-	-	-	-
	• Lead acetate test	-	-	+	+
8)	**Test for steroids**				
	• Salkowski test	+	+	+	+
9)	**Test for Napthoquinone**				
	• Jug lone test	-	-	-	-
	• Darn-karrer test	-	-	-	-

Inference from Tests: From the above Phytochemical screening, it is observed that the methanolic extract of *Avicennia alba* (aerial parts) contains Alkaloids, amino acids, carbohydrate, glycosides, tannins and steroids

Isolation by Thin Layer Chromatography:_ The next step of our experiment is to isolate the chemical constituents present in the aerial part of *Avicennia alba* by thin layer chromatography. The proper solvent of the experiment is chosen. Thin layer chromatography is a chromatography technique used to separate mixture.

Thin layer chromatography is performing on a seat of glass, plastic or aluminium foil. Which is coated with a thin layer of absorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate via capillary. Because different analysts ascend the TLC plate at different state, separation is achieved. It can be calculated by means of Retention factor (Rf) as follows:-

Rf = (Distance travelled by the solute from origin/ Distance travelled by the solvent from origin)

Preparation of TLC plate: First a thin plate (0.2 mm) is taken and washed and cleaned carefully. Then required amount of silica gel is collected is to make slurry in suitable solvent in a beaker with continuous starring. Amass used amount of slurry put on a giving size plate which is kept on the level surface.

Chromatography technique: By the help of capillary tube take the spot of extract at the bottom of the plate and air dried. A suitable solvent system is prepared in a TLC chamber and kept it for half an hour. Now the plate is being put inside the TLC chamber at 45° angle and allows the solvent system to run. After running the ¾th of the plate, taken it out, air dried and the spot is detected by using the detecting agent and Rf value is calculated.

By the trial and error method, we selected the solvent Butanol, ethyl acetate, formic acid and benzene with the ratio of 4:2:2:1.

Constituents	Solvent system	Distance travelled by the solute	Distance travelled by the solvent	Rf value observed
Alkaloids	Butanol, Ethyl acetate, Formic acid, Benzene 4:2:2:1.	2.2 cm	5cm	0.44

Inference: From the literature survey the Rf value was match with the practical value of Rf of alkaloids for that we conclude that the sample has content of alkaloids.

Isolation by Column Chromatography: The next step of the experiment was to isolate alkaloid present in the plant *Avicennia alba* by the column chromatography. The solvent is used here is same as thin layer chromatography.

Preparation of column: The classical preparative chromatography column is a glass tube with a diameter from 50mm and height of 50 cm to 1 mt. with a top at the bottom. Slurry is prepared at the eluent with the stationary phase powdered and then carefully poured into the column. Care must be known to avoid air bubble. A solution of the organic material is pipette on top of the stationary phase. The layer is usually tapped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance to organic material. Often spherical eluent reservoirs or an eluent fluid and Stoppard separating funnel is put on the top on the column.

ELUENT SAMPLE

Pharmacological evaluation studies of the Methanolic Extract of Crude Drug:

Oral Acute Toxicity Study: The acute toxicity study was performed as described by Turner (1971) [6]. Adult albino mice wt (20-25gm) divided into four groups containing 6 mice in a group the mice were fasted for 18 hour with water adding. The three suspension prepared as above were administrated orally at four different dose of 1000, 2000, 4000, 6000mg/kg body weight respectively. And including control mice received vehicle (tween 80 1% p.o.) only.

Observation: The animals were observed for 72 hours any behavioural change and mortality.

Result: No mortality and change in the behaviour were observed in all the treated and controlled gr. of mice up to a case of 6000mg/kg body weight. From the review of literature we know that *Avicennia* has analgesic properties. Here the aim is to re-establish analgesic activity of the methanolic extract of the crude drug through tail flick method.

Tail Flick Test: It is one of the important methods developed to evaluate the analgesic action.

Animal Required: Mice (wt- 25 to 30gm)

Drug required: Normal saline (0.9%w/v), Nimuselide (10 mg/kg body wt), Methanolic extracts of Avicennia alba (250mg/kg body wt).

Procedure: A total of 18 mice in three groups namely controlled, standard and test are taken and weighed properly. Basal reaction time of each of them is noted. Drugs are administrated in oral route. Reaction time noted between ten minutes interval. Result, calculation, and inference are drawn (**Table 3**).

Observation table

TABLE 3: TAIL FLICK TEST FOR OBSERVATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *AVICENNIA ALBA*

Group	Body wt (gm)	Drug used	Basal reaction time	Reaction time (min)							
				0	10	20	30	40	50	60	Avg
X1	20	Control (normal Saline)	2	2	3	2	2	1	2	1	1.86
X2	22		2	2	2	1	2	1	2	1	1.57
X3	20		1	1	2	1	1	2	1	2	1.43
X4	20		3	3	3	2	2	1	2	2	2.14
X5	21		3	3	3	2	3	3	2	3	2.71
X6	20		1	1	2	1	2	1	1	1	1.29
Y1	22	Nimesulide (10mg/kg body wt)	2	2	3	4	5	6	5	5	4.29
Y2	23		3	3	4	4	5	6	5	4	4.43
Y3	23		2	2	4	5	5	4	3	2	3.57
Y4	21		3	3	4	5	5	4	2	1	3.43
Y5	23		3	3	3	4	5	5	2	2	3.43
Y6	23		2	3	4	5	4	4	3	2	3.43
Z1	25	Methanolic Extracts of *Avicennia alba* (aerial parts)	1	2	3	4	2	2	3	1	2.43
Z2	24		2	2	4	5	4	3	3	2	3.29
Z3	25		3	3	2	3	3	2	2	1	2.29
Z4	26		2	2	2	4	2	3	2	2	2.43
Z5	25		2	2	3	3	4	2	2	4	2.86
Z6	27		1	3	3	2	4	2	2	1	2.43

RESULT & DISCUSSIONS: From the above experiment we have concluded that the methanolic extract of Avicennia alba has analgesic properties although less effective than the drug nimesulide (10 mg/kg) used as control. The result was statistically significant ($p < 0.05$). This reaffirms the fact that the crude drug *Avicennia alba* possesses definite analgesic action.

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