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Phytochemical Screening and TLC Profile of Fruits and Flowers of *Alstonia venenata* R. Br.

Thomas SK, George RE, Kunjumon M, Thankamani VI*

Department of Biotechnology, University of Kerala, Karyavattom campus, Thiruvananthapuram 695581, Kerala, India

ABSTRACT

Alstonia venenata R. Br. belonging to the family Apocynaceae is a tall evergreen shrub distributed throughout Peninsular India. Stem-bark, root-bark, fruits and leaves are used by many tribal communities and also in Ayurveda. The study investigates the phytochemical composition of hexane, butanol, methanol and water extracts of *Alstonia venenata* fruits and flowers as well as the TLC profile of hexane extracts of fruits and flowers. Quantitative data of the wet and dry weight, yields from different solvent fractions and percentage yields were noted. The phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, steroids, terpenoids, saponins, flavonoids, tannins and phenolic compounds from the various extracts. Alkaloids were present in all the fractions tested. Methanol extracts of fruits and flowers showed the presence of major phytoconstituents. TLC profile of hexane extracts of fruits and flowers were developed using anisaldehyde sulphuric acid/ceric sulphate (steroids/terpenoids) and Dragendorff's spray reagents (alkaloids). Petroleum ether: Chloroform: Methanol (5: 4.5: 0.5) showed good resolution for the hexane extracts of fruit and flower when treated with Dragendorff's spray reagent. Petroleum ether: Chloroform (1:1) was best for the hexane exacts of flowers and fruits when sprayed with ceric sulphate spray reagent.

Keywords: *Alstonia venenata*, Phytochemical, TLC, Fruits, Flowers.

INTRODUCTION

Alstonia venenata R. Br., a tall evergreen shrub growing in warm climates is a member of the family Apocynaceae. It is about six to eight metres in height, spreading, flowering in summer and requires moist humus rich soil throughout. It is distributed throughout Peninsular India mainly seen in Northern Circars (Costal regions of Andhra Pradesh and Orissa), Hills of Ganjam & Godavari up to 2000 feet, in Western Ghats, Hills of Coimbatore, Nilgiris, sparsely distributed on the Ponmudi and Annamalai Hills, Pulneys and Hills of Thirunelveli up to 4000 feet. [1] Leaves are lanceolate with wavy margins, greyish brown bark, white flowers in terminal or in sub umbellate cymes, follicular fruits tapering at both ends, bright yellow hard and woody root. The plant is known by different vernacular names as 'Analivegam' (Malayalam), 'Addasarpa' (Kannada), 'Theeppala', 'Anadana', 'Rajaadana', 'Visaghni' (Sanskrit), 'Palamunnipalai', 'Sinnappalai' (Tamil). [2] This medicinal plant is an inevitable ingredient in tri-health Ayurvedic formulations for treating gastrointestinal ailments, neurological disorders, brain and nerve functions, joint pain etc. Stem-bark, root-bark, fruits and leaves of the plant are medicinally important. The ripe fruits find use in the treatment of syphilis, insanity and epilepsy in Indian medicine. [2] Govindachari reported the isolation of twenty new alkaloids

***Corresponding author: Dr. V. Thankamani,**
Professor and Former Head, T.C 16/1669, 'Aswini', JWRA-17, Jagathy, Thiruvananthapuram-14, Kerala, India; **E-mail:** dr.thankamani@gmail.com
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belonging to the class of Yohimbine, Aspirdofractinine and Vincadifformine. [3] Fruits are used as tonic by the tribal community in the Srikakulam district of Northern Andhra Pradesh. [4] In the fruit, alkaloids like Echitoserpidine, Echitovenidine and (+)-Minovincinine, Venoterpine were reported. [5-7] The fruit alkaloid Echitovenidine is a Vincadifformine type of alkaloid which shows monoamine oxidase-inhibitory activity both *in vitro* and *in vivo* which scientifically validates the use of the plant for mental disorder. [8] The antibacterial and antifungal activity of fruits and flowers were reported in our previous work. [9-10] There is no reported data available on phytochemical constituents of the flowers of *A. venenata.* In the present study the quantitative data of wet and dry weight of the flowers and fruits, percentage yield and the phytoconstituents in different extracts of fruits and flowers were analyzed. The work forms a platform for further purification and bioactivity study of fruits and flowers of *A. venenata*.

MATERIALS AND METHODS

Plant Collection and Identification: Plant was collected from interior parts of Ponmudi Hills, Kerala, India. The plant was taxonomically identified and authenticated at the Herbarium, Department of Botany, University of Kerala, Thiruvananthapuram (Voucher specimen accession no. KUBH 5847). The flowers, green fruits and dry fruits were collected separately, washed in distilled water, shade dried, powdered and stored in dry polythene bags.

Preparation of Plant Extracts

Quantitative analysis in terms of fresh, dry, powder and moisture content were estimated. The powdered materials were extracted successively with hexane, butanol, methanol and water for 12 hours using a Soxhlet apparatus. The excess solvent in the extracts were removed by distillation and the concentrated samples were weighed, kept in screw capped bottles and stored at room temperature except water extract which was kept at 4°C. The percentage yields and other physical properties were observed.

Preliminary Phytochemical Screening

All the extracts were subjected to various phytochemical tests as per the standard procedures. The extracts were analyzed for the presence of alkaloids (Wagner's and Dragendorff's test), flavonoid glycoside (Shinoda test), tannins & phenols (Ferric chloride test), steroids and terpenoids (Liebermann- Burchard test), saponins (foam test). [11-12]

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was done to generate chromatographic profile of hexane extracts of flowers and fruits of *A. venenata*. Silica gel G was used as adsorbent for preparing TLC plates (Merck specialities Pvt. Ltd, Mumbai). Slurry was prepared by adding 25 g of Silica gel to 50 ml distilled water and coated on chromatographic glass plates of 7.5 × 2.5 cm and 20 cm × 5 cm size. The plates were air dried and heated at 110°C for thirty minutes. The different extracts were spotted using a TLC capillary tube and placed in a closed chamber and allowed to run until the solvent front reached the top. The solvent systems developed were first observed under the U.V light and fluorescence spots were noted. Later the plates were sprayed with (a) Anisaldehyde-sulphuric acid reagent (b) Ceric sulphate and (c) Dragendorff's reagent. The R_f values were calculated using the formula. [13]

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

RESULT AND DISCUSSION

The quantitative data from fruits and flowers of *Alstonia venenata* are listed in Table 1. Total dry weight after shade drying was found to be 73 g of flower, 130.40 g of green fruit and 52.50 g dry fruit. The moisture content of the flower was 81.98% and that of green-fruit was 77%. Shade drying of the flower and green fruit resulted in the reduction of initial weight to 18.02% and 23% respectively.

The yields from the fruits and flowers were expressed as total dry weight, total solids, weight of fractions and percentage yield. The results are given in the Table 2. In case of flower and dry fruit maximum yield was noted in the methanol extract, while from green fruit the maximum yield was obtained in water extract. The total solids obtained in various solvent extracts were 38.23 g from 73 g of flower (52.37%), 47.79 g from 130.40 g of green fruit (36.65%) and 14.54 g from 52.50 g of dry fruit (27.70%).

Table 1: Quantitative data- *Alstonia venenata* fruits and flowers

S. No	Plant parts	Wet wt. (g)	Dry wt. (g)	Dry wt. (%)	% Moisture
1.	Flower	405	73	18.02	81.98
2.	Green fruit	567	130.40	23	77
3.	Dry fruit	-	52.50	-	-

Table 2: Solvent extract yields of fruits and flowers of *A. venenata*

S. No	Solvents	Yield in grams and (%)		
		Flower	Green fruit	Dry fruit
1.	Hexane	4.11 (5.63)	10.7 (8.20)	2.88 (5.48)
2.	Butanol	6.72 (9.02)	7.63 (5.85)	1.25 (2.38)
3.	Methanol	20.48 (28.05)	11.74 (9.40)	6.06 (11.54)
4.	Water	6.92 (9.47)	17.72 (13.59)	4.35 (8.28)
Total yield from each part and (%)		38.23 (52.37)	47.79 (36.65)	14.54 (27.70)

Phytochemical analysis of the extracts revealed the presence of alkaloids, steroids, terpenoids, saponins, flavonoids, sugars, tannins and phenolic compounds from the various extracts as given in Table 3. The flowers and fruits extracts of *Alstonia venenata* showed the presence of alkaloids in almost all the extracts which points out the fact that the plant is a rich source of alkaloids. Methanol fractions from all the plant parts showed the presence of all major phytochemical constituents followed by butanol, water and hexane extracts.

Table 3: Phytochemicals in Organic Solvent Extracts of *A. venenata*

S. No	Plant Name	Solvents	Steroids	Terpenoids	Alkaloids	Flavonoids	Carbohydrates	Tannins & Phenolic	Saponins
1.	Flower	Hexane	+	+	+	-	-	-	-
		Butanol	-	+	+	+	++	+	-
		Methanol	+	+	+	+	+	+	+
		Water	-	-	-	+	++	+	++
2.	Dry fruit	Hexane	-	+	++	-	-	-	-
		Butanol	+	+	++	-	+	-	-
		Methanol	+	+	+	+	+	+	+
		Water	-	-	+	+	++	+	++
3.	Green fruit	Hexane	-	+	+	-	-	-	-
		Butanol	+	+	+	+	+	-	-
		Methanol	+	+	++	-	++	+	+
		Water	-		+	-	++	+	+

(+) = Present; (-) =Absent

Table 4: Thin layer chromatogram (TLC) of hexane extracts of *A. venenata*- fruits and flowers

S. No	Plant parts	Solvent system	Reagent	R*f* values
1.	Green-fruit	PE: CH (1:1)	CS	0.008, 0.19, 0.27, 0.35, 0.43, 0.49, 0.54, 0.66, 0.93
2.	Green-fruit	PE: CH: M (5:4.5:0.5)	D	0.22, 0.82
3.	Dry-fruit	PE: CH (1:1)	CS	0.06, 0.1, 0.16, 0.2, 0.25, 0.32, 0.39, 0.5, 0.88
4.	Dry-fruit	PE: CH: M (5: 4.5: 0.5)	D	0.22, 0.83
5.	Flower	PE: CH (1: 1)	CS	0.05, 0.14, 0.2, 0.24, 0.3, 0.4, 0.35, 0.43, 0.53, 0.54, 0.96
6.	Flower	PE: CH: M (5: 4.5: 0.5)	D	0.21, 0.87

CS= Ceric sulphate, D= Dragendorff's, PE= Petroleum ether, CH=Chloroform M=Methanol

Fig. 1

Fig. 2

Fig. 3

Fig. 1-3: Chromatogram (TLC) of hexane extracts of *A. venenata*- fruits and flowers

Fig. 1 and 2: Lane 1. Green fruit hexane, Lane 2. Dry fruit hexane, Lane 3. Flower hexane, [Petroleum ether: Chloroform (1: 1)]; Fig. 1 Ceric sulphate spray, Fig. 2 Anisaldehyde Sulphuric acid spray. **Fig. 3:** Lane 1. Green fruit hexane, Lane 2. Dry fruit hexane, Lane 3. Flower hexane [Petroleum ether: Chloroform: Methanol (5: 4.5: 0.5)]; Dragendorff's spray.

The hexane extract of flower contained steroids, terpenoids and alkaloids whereas the dry fruit and green fruit hexane extract contained only terpenoids and alkaloids.

Chemical constituents of different extracts from fruits and flowers of *Alstonia venenata* were obtained by Thin Layer Chromatography (TLC). The solvent system selected for TLC analysis of the hexane fractions of fruits and flower extracts and the R*f* values obtained for different extracts using three different spray reagents are listed in Table 4. The TLC plates were also displayed in Fig 1, 2 and 3. Petroleum ether: Chloroform (1:1) showed good resolution of the hexane exacts of flowers and fruits when sprayed with ceric sulphate than anisaldehyde spray reagent for steroids and terpenoids. Pink and light blue coloured bands were prominently visible after the plates were heated at 105°C for 10 minutes. 9 spots were observed for dry fruit and green fruit hexane extracts, and 11 spots were observed for flower hexane extract using the solvent system as given in Table 4. Petroleum ether: Chloroform: Methanol (5: 4.5: 0.5) showed good resolution for the hexane extracts of fruits and flowers when treated with Dragendorff's spray reagent for alkaloids. The hexane extract of green fruit, dry fruit and flower showed 2 spots using this solvent system.

Alkaloids, terpenoids, steroids, tannins, saponins, flavonoid, quinines, antraquinones, phenols and glycosides were also reported from crude stem-bark and leaf extracts of *A. venenata* in a similar work. [14] Thankamani *et al.*, have reported alkaloids, carbohydrates, amino acids, phenols, tannins, cardiac glycosides, saponins, flavonoids, steroids, fixed oils and fats from *A. scholaris* flower extracts. [15] The fruit and flower extracts of *A. venenata* also showed similar compounds as reported in *A. scholaris* which also contained alkaloids, phenolic compounds, terpenoids, and flavonoids. [15-16] In addition to *Alstonia scholaris*, *Alstonia macrophylla, Alstonia boonei, Alstonia congensis,* were also reported to contain alkaloids, steroids, terpenoids and phenolic compounds. [17]

Presence of these secondary metabolites makes the plant useful for the treatment of various ailments. Terpenoids are generally antiseptic, diuretic, antihelmintic, stimulant, analgesic and counter-irritant in nature. [18] Tannin containing drugs are used as astringent for the treatment of burns [19] as healing agents in inflammation, piles, antidote, gonorrhoea and

leucorrhoea. [20] Saponins are pharmaceutically important compounds due to their relationship to sex hormones, cortisones, diuretic steroids and vitamin D. [12] For the treatment of diseases such as rheumatoid arthritis, collagen disorders, allergy and asthma synthetic steroids are prepared from plant sapogenins. [21] Alkaloids are also responsible for the pharmacological properties of many medicinal plants. Due to a great variety of chemical structures alkaloids stand as a class of major importance in developing new drugs. [22] For further separation and isolation of compounds from fruits and flowers of this plant the TLC chromatogram can be used. Based on this preliminary phytochemical analysis purification of different molecules from the crude extracts could be further carried out.

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