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Preliminary phytochemical and anatomical studies of *Staurogyne spatulata* (bi.) Koord

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Abstract
Staurogyne spatulata (Blume) koord, belongs to the family Acanthaceae is a small pubescent herb. Scientific information on their pharmacognosy and phytochemistry is very scant. Hence the current study describes some pharmacognostical, physiochemical and phytochemical investigations undertaken on the stem, root and leaves of species. The herbs were subjected for morphological, microscopical, physiochemical, phytochemical and fluorescence analysis methods for standardization. The parameters from the above were recorded with an objective of drawing attention on the species as well as a reference for further scientific investigations.

Keywords: *Staurogyne spatulata*, pharmacognostic study, phytochemistry, anatomy

1. Introduction
Plants are important sources of natural products. There are thousands of plant species, but only a few have been investigated both phytochemically and pharmacologically. Vast wealth of plants are unexplored. *Staurogyne spatulata* belongs to the family acanthaceae. It is widely distributed throughout Indonesia, Malaysia, Africa, Brazil and Central America. *Staurogyne spatulata* is an herb and it grows up to 15cm height. Many of the members of the Acanthaceae family are medicinally important as they contain biologically active compounds, have lots of medicinal properties like anti-inflammatory, antifungal, anticancer, hepatoprotective, antioxidant, cytotoxic, antiviral potential, immunomodulatory and antiplatelet aggregation [1]. The genus staurogyne includes 150 species. Plant have simple, opposite, decussated leaves with entire margins and without stipules. The flowers are perfect, zygomorphic. Mainly two stamens are present and anthers are bithecous with the thecae equally inserted on the filament in all genera. The theca is glabrous, in which they are pubescent with glandular trichomes. Furthermore, literature on *Staurogyne spatulata* revealed that there is no study till now phytochemically. The aim of this study is to determine the phytochemical composition and anatomical characteristics of *Staurogyne spatulata*.

2. Materials and Methods
2.1 Pharmacognostical Studies
The whole plant of *Staurogyne spatulata* were collected from valapad, Thrisur District, Kerala. The plant materials were dried under shade at room temperature, finally dried in oven at 55°c for 2 hours. This process continued daily till the parts get dried well. The stem, leaf and root were grinded separately by using grinding machine. The powdered materials were stored in separate bottles. The materials were kept in clean and airtight bottles till use. The samples were used for cold extraction by using distilled water and allowed for further phytochemical analysis.

2.2 Powder Analysis
Powder microscopy is a quality control method. Dried and powdered plant materials are used. A pinch of fine powdered plant materials were well mixed with water and kept for 1 hour. After staining for 5 minutes, observed under fluorescent microscope.

2.3 Determination of physicochemical parameters
The various physiochemical parameters such as total ash and water soluble ash were determined. About 10 g of powder after accurate weighing was placed in a tared evaporated dish. Weighed accurately and noted the initial weight of the drug. Then again dried at 100°c. The resulting ash was cooled and weighed. The procedure was repeated to obtain a constant weight. The percentage of total ash with reference to the air dried drug was finally calculated [2].
A small quantity of dried and finely powdered crude drug was placed on a clean watch glass. The powder first mixed with water, it kept as control. After the drug treated with 1-2 drops of the freshly prepared reagent solutions separately, that is 1 N sodium hydroxide in methanol, 1 N sodium hydroxide, 50% sulphuric acid, 50% picric acid, ethanol, 1 N HCl etc. The added reagents were mixed gently and waited for 1-2 min. Then these are subjected to visible light. Then placed in laminar air flow chamber and viewed in natural and ultra violet light. The colors observed by application of different reagents in both visible and ultra violet lights were recorded.

2.4 Preliminary phytochemical analysis
Shade dried and powdered whole plant samples were successively extracted with distilled water. The extracts were filtered using whatsman No.1 filter paper and subjected to qualitative tests for the identification of various phytochemical

constituents as per the standard procedure.

Test for carbohydrates-Benedicts test: - 5 ml of Benedict's reagent was taken and plant extract was added to it. Boiled for 2 minutes and cooled for some time. Color change observed.
Test for protein-Nitric acid test**: -**3ml of concentrated nitric acid was taken in a test tube. The aqueous extract was slowly added along the sides of the test tube. Protein will give a dark ring at the zone of contact between nitric acid and the extract.
Test for amino acid-Ninnhydin test: -1ml sample was taken in a test tube. Afew drops of ninhydrin reagent was added into it. Blue color indicated the presence of amino acid.
Test for saponin-Froath test: - 1ml sample was taken in a test tube. 2ml of distilled water was added and shaken well. Appearance of froth on shaking of the mixture showed presence of saponins.
Test for alkaloid-Wagner's test: - To little of the extract 3-5 drops of wagner's reagent was added. Brown flocculent precipitate indicated the presence of alkaloid.
Test for terpenoids- Acetic anhydride test: - 2ml of extract was added to 2ml of acetic anhydride and concentrated H_2SO_4. Appearance of blue green rings will indicate the presence of terpenoid.
Test for phenol-Fecl$_2$ test: - 3 drops of $FeCl_2$ (5%) was added to 5 drops of sample solution taken in a test tube. Dark green color will indicate presence of phenol.
Test for coumarin-NaOH test: - To 1ml extract 1ml of 20% NaOH was added. Yellow colour change was noted.
Test for flavonoids-NaOH test: - To 1ml sample 3ml of dilute NaOH was added, the sample turned yellow color. Then dilute HCl was added and the yellow color disappeared. Indicated the presence of flavonoid.
Test for tannin-Lead acetate reaction: - To 2ml of extract few drops of 1% lead acetate was added. Yellow precipitate developed.
Test for diterpene-Copper acetate test: - 1ml of extract mixed with few drops of copper acetate. Emeralde green colour appeared shows the presence of diterpene.
Test for Glycoside- Salkowski test: - Crude extract was mixed with 2 ml of chloroform then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. Reddish brown colour developed.
Test for steroids-Chloroform test:- crude extract was mixed with 2ml of chloroform. Then concentrated H_2SO_4 was added along the side of test tube. A red color produced in lower chloroform layer which indicate the presence of steroid.
Test for anthocyanin-To 2ml of extract 2ml of 2N HCl was added, red colouration developed.

3. Microscopic Evaluation

The fresh plant materials were washed thoroughly with normal tap water followed by distilled water. Then took the section of each part and allowed for double staining and mounted in DPX. The mounted sections were observed under the magnus MLX-TR microscope for identifying and understanding the characters.

4. Results & Discussion

T.S of leaf: T.S of leaf is dorsiventral with fairly prominent midrib and thin lamina. The midrib consisted of short wide adaxial hump and wide, short abaxial semicircular part. The midrib possess thick epidermal layer on upper and lower sides. Upper and lower epidermis consists of radially elongated, broad rectangular cells embedded with diacytic stomata covered with thick cuticle and bearing simple, secretory and glandular trichomes. Simple trichomes are multicellular, uniseriate and are very long. Glandular trichomes are sessile with multicellular globular head and few with unicellular stalk. Mesophyll condain3-4 rows of small sized compactly placed palisade cells, are located between upper and lower epidermis. Palisade cells are densely filled with chloroplast. Vascular bundles are not prominent. 3-5 rows of spongy parenchyma embedded around vascular bundle. Different types of calcium crystals are embedded throughout the parenchyma cells. The plant has a thick cuticle on the stem and leaf help to protect the plant from excess water loss through transpiration.
T.S of stem: The stem is bright green in color. The stem covered with different types of trichomes. Conical shaped and base broaded trichomes are present around the stem. Stem covered with thick cuticle. Epidermis is single layered with barrel shaped cells. Epidermis is followed by the zone of cortex consisting of 2-3 layers of elongated collenchymatous cells and four layers of paraenchymatous cells. Single layer of endodermis and pericycle present inner to the cortex. It consists of xylem, phloem, secondary xylem etc. Phloem consists of 2-3 layers of polyhedral paraenchymatous cells. Stem shows secondary growth. Primary xylem was found towards the inside. Vessels are arranged in radial rows. Pith is made up of polygonal, rounded paraenchymatous cells with lots of Ca crystals.
The arrangement of diacytic stomata on the abaxial surface of the leaf also a technique to preserve water. Parenchyma around the vascular tissue likely protect against water loss as well. The long, thin, fibrous roots maximize their surface area to volume ratio which also aids in rapid water absorption. The stem has thick layer of collenchyma tissue allow the stem to be strong yet flexible. This feature is ideal for a herb that needs support against the wind and protection from pests. Additionally, the thick band of secondary xylem form a rather strong ring structure that not only transport water, but also helps to keep the stem from breaking under stress, in almost all sections of the root and stem. Large amount of secondary xylem is an adaptation in order to maximize the transfer of water from the root to the rest of the plant when water is present.
T.s of root: The epidermis is single layered thick and the cells are oval shaped. The epidermis followed by aerenchymatous cells. Single layered endodermis is present. Root has more xylem vessels for water conduction. Development of protoxylem towards the pith and the metaxylem towards the outer region. Pith region is very small compared to stem. Pith is made with circular parenchyma.
The detailed anatomical and morphological studies provide the clear picture about the adaptation towards the environmental conditions and also interpreted the taxonomical relationship between related species. The plant has a thick cuticle on the stem and leaf help to protect the plant from excess water loss through transpiration. Trichomes on the stem and leaf help to protect against direct sun exposure,

reduce loss of water and insulate the plant from wind [3]. The herb is very close to the ground. It is more susceptible to damage from ground herbivores in terms of defensive strategies. Trichomes prevent small animals and insects from reaching the plant surface.However [4].

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The plant has adapted many features at the cellular level that help it to survive in its natural environment. The whole plant showed the presence of starch, resin, calcium crystals in cell. Calcium crystals accumulated over plant tissue in some taxonomical levels. They have different sizes and shapes. They occur as simple, double triple or even joined together forming chains and aggregates of varying shapes. The shape may be round, oval, star, rod, bean and shaped. Major function is calcium regulation and protection against herbivores. These features can be conveniently employed in taxonomic distinctions [5].

Powder Microscopy

Leaf: Powder of leaf is greenish in color having characteristics odour and astringent bitter taste. Plenty of simple and glandular trichomes of various shape and sizes scattered throughout or attached with the paraenchymatous cells of the epidermis. Simple unicellular trichomes are short, warty and conical. While the multicellular trichomes are long, straight or slightly thick walled. Glanduar trichomes are mostly sessile, long unicellular stalk and have broad base. Fragments of spiral, annular vessels are present. The stomata was abundant and diacytic. Spongy parenchyma embedded with prismatic and circular crystals of Calcium oxalates are present.

Stem: Powder of stem is pale green in color having characteristics odour and slightly bitter taste. Stem consists of lots of fibres and vessels. Fibres are long cells with tapering ends. Some of the fibres are narrow, thick and with thin lumen. Some other fibres are wider, short and with wider lumen. The vessel elements are wide long and cylindrical cells. The stem consists of different types of Calcium crystals like round, star and cylinder shaped. Starch grains are also present in the stem cells.

Root: Powder is pale yellow in color, with some specific odour and slightly bitter in taste. Root consists of more stone cells compared to stem and leaf. Have tracheids, phloem fibres, vessels and many starch grains.

Physicochemical and fluorescence analysis

Ash test: Moisture content of *Staurogyne spatulala* was found to be 50.58% W/W. Powdered drug under UV and ordinary light when treated with different reagent showed various color radiation which help in identifying the drug in powder form. When the organic contents is lower which indicate the impurity of the drug. The drug contains more inorganic salts it may cause side effects. Fluorescent analysis under UV and visible light the crude drug showed colour differences, which indicate the colours have their own drug characters. Pharamacognostic parameter encompasses all possible informations regarding the chemical constituents present in the herbal drug. The major bioactive compounds present in these crude preparations are the coumarins, flavonones, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition and under short UV radiation. Flavonones which are light yellow in aqueous condition, under UV light, turns to bright yellow under alkaline conditions. Terpenoids, especially sapogenins, exhibit yellow green fluorescence under short UV light.

Phytochemical Screening

Quantitative analysis in aqueous extract showed the presence of protein, saponin, alkaloid, coumarin, flavonoids, diterpene, tannin, carbohydrates, and steroids in stem, leaf and root. These phytochemicals play important role in various plant defense mechanisms against herbivores, fungi and bacteria. In future, these secondary metabolites can be extracted from this plant and may be used as medicine. Studies on quantitative analysis, antibacterial and antioxidant activity can also be conducted for future references.

Table 1: Showing results of phytochemical analysis.

	Name of the test	Name of the reaction	Leaf	Stem	Root
1	Test for carbohydrates	Benedict's test	+	-	-
2	Test for protein	Nitric acid test	+	+	+
3	Test for aminoacid	Ninnhydrin test	-	-	-
4	Test for alkaloid	Wagner's test	+	+	+
5	Test for terpenoid	Acetic anhydride test	-	-	-
6	Test for saponin	Froath test	+	+	+
7	Test for phenol	$FeCl_3$	-	-	-
8	Test for coumarin	NaOH test	+	+	+
9	Test for tannin	Lead acetate test	+	+	+
10	Test for flavonoid	NaOH test	+	+	+
11	Test for diterpene	Copper acetate test	+	+	+
12	Test for anthocyanin	HCl reaction	-	-	-
13	Test for steroid	Chloroform test	+	-	+
14	Test for glycoside	Salkowski test	-	-	-

Table 2: Showing results of florescence analysis.

Sl. No	Treatment	Visible Light	UV light
1	Powder + water (control)	Greyish colour	No colour
2	Powder + NaOH	Brown	Yellowish green
3	Powder+NaOH in ethanol	Light green	Light green
4	Powder + ethanol	Dark green	Yellow colouration
5	Powder + HNO_3+ NH_3	Orange	Suspended with yellow colour
6	Powder + 50% HNO_3	Brownish orange	Reddish yellow
7	Powder +HCl	Found immiscible	Green
8	Powder + H_2SO_4	Dark	Black
9	Powder + Picric acid	No precipitate	Yellowish green
10	Powder+ Glacial acetic acid	Light brownish	Green
11	Powder+con. HNO_3	Reddish (suspension)	Yellow

5. Conclusion

The present work encompasses macroscopy, microscopy, and physiochemical, preliminary and phytochemical analysis of *Staurogyne spatulala*. The phytochemical analysis revealed the presence of alkaloids, steroids, flavanoids, tannin, coumarin, diterpenes, carbohydrates, saponins and protein. The objective of the present investigation is the ease of identification of the species both in whole and powdered form and this can rectify the authenticity of the drug for future investigation.

6. References

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