EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 3
119-125
Year: 2023

STANDARDISATION AND EVALUATION OF PHYTOCHEMICALS FROM THE AERIAL PARTS OF *STROBILANTHUS ALTERNATA*

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Article Received on 26/12/2022 **Article Revised on 16/01/2023** **Article Accepted on 06/02/2023**

ABSTRACT
The present study aimed to evaluate pharmacognostical, physicochemical and phytochemical evaluation of various parts of *Strobilanthes alternata* (syn: Hemigraphis colorata). It is a tropical perennial herb chiefly grown as unornamental plant, belongs to the family Acanthaceae. In folk medicine, the leaves are ground into a paste and applied on fresh cut wounds to promote wound healing and used to treat anemia. In Kerala, the plant is popular in the name 'murikootti' because of its incredible potency to heal wounds. Macroscopical, microscopical, physico-chemical and analytical documentation of aerial part of *S. alternata* were investigated. TLC was carried out in silica gel plates using mobile phase protocols, Toluene: Ethyl Acetate: Formic acid of ratio 7.2:5:1. Phytochemical screening of the hydroalcoholic extract showed the presence of Fixed oils, Alkaloids, Flavonoids, Terpenoids, Tannins, Saponins and Phenols. In the pharmacognostical, physicochemical and analytical studies, an attempt were made to standardize the leaves, stem of *S. alternata*. The determination of these characters along with its phytochemical profiling will aid future investigators in their pharmacological analysis of this species.

KEYWORDS: Strobilanthes alternata, Pharmacognostic standardization, Physico-chemical evaluation, Analytical documentation.

INTRODUCTION
Medicine is a substance that has nutritive, curative, or preventive properties, while the term "herbal" refers to a botanical or plant-based preparation. Hence, the term "herbal medicine" is used for plant-based substances that consist of nutritive, curative, or preventive properties. Herbal medicine is an interdisciplinary branch between herbal medicine and Ayurveda as it covers all fields of herbal medicine related to botany, medicinal plant research, pharmacognosy, phytochemistry, phytotherapy, botanical medicines, Ayurveda, natural chemistry, agriculture science, Unani medicine, biotechnology, and biochemistry.

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations and are used especially for the prevention and treatment of diseases. The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, plants are being extensively explored for harboring medicinal properties. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development.[1]

Strobilanthus alternata (Acanthaceae), an exotic plant adapted to India, is a versatile tropical low-creeping perennial herb that reaches a height of 15 to 30 cm. In Kerala, the plant is popular in the name 'murikootti' or 'murian pacha' because of its incredible potency to heal wounds. The plant is known by several names such as Aluminium plant, Cemetary plant, Metal leaf, Redflame Ivy, Waffle plant, Java Ivy etc. The leaf has metallic purple luster on upper surface and a solid dark purple on ventral side.

Figure 1: *Strobilanthus alternata* (Acanthaceae).

SCIENTIFIC CLASSICATION
Kingdom: Plantae
Order: Lamiales
Family: Acanthaceae
Genus: *Strobilanthus*

Species*: alternata*
Binomial name: *Strobilanthus alternata*

Leaves are mainly used to cure for wound, gallstones. It is used as diuretic. In Java, leaves are used in treatment of bloody dysentery and hemorrhoids. The leafy decoction used to treat excessive menstruation. Externally used for skin complaints. Paste of leaves applied to fresh cut wounds to stop bleeding and promote healing and also used for anemias. The leaf buds squeezed in water and drunk for 4 days as contraceptive and to induce sterility.[2]

Figure 2: Leaves and stems of *Strobilanthus alternate.*

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

Based on literature survey done on medicinal plant of Acanthaceae, the leaves of *Strobilanthus alternata* were selected for study. Leaves of *Strobilanthus alternate* were collected from Kannur District, Kerala by adopting proper collection method. Plant identification and authentication was done by Prof. Sreeja, Department of Biology, Sir Syed College, Kannur. The collected material was shade dried, crushed and stored.

MACROSCOPIC CHARACTERIZATION OF PLANTS

Macroscopic evaluation of the selected plants was recorded as per usual observation. Organoleptic evaluation of the selected plants, color, odor, taste, size, and shapes were recorded separately.

TRANSVERSE SECTION STUDIES

Sectioning: Numerous free hand sections were taken, stained and mounted following the usual micro technique described by Brain (1975) and photographs of different magnifications were taken using electron microscope and reported the result.

POWDER MICROSCOPIC STUDIES

Leaf powders of the selected plants were observed under microscope with distilled water, stained with Phloroglucinol and Hydrochloride and pictures were taken.[3]

QUANTITATIVE MICROSCOPY-DETERMINATION OF LEAF CONSTANTS

Leaf surfaces are studied by scrapping and by peeling of the upper and lower epidermal surfaces of the leaves and then washed with chloral hydrate and observed under microscope for its stomatal structure, epidermal pattern, veinlet pattern, vein termination pattern.[4]

PHYSICOCHEMICAL CONSTANTS

Determination of Ash Value

The ash remaining after complete ignition of the medical plant materials is determined by method known as Total Ash. Accurately weighed 3g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to 500-600oC until it is white, indicating the absence of Carbon, cool and weigh, this process repeated till constant weight is obtained.

DETERMINATION OF MOISTURE CONTENT BY LOSS ON DRYING

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead to the activation of enzymes and give suitable conditions to the proliferation of living organism. As most vegetable drugs contain all the essential food requirements for mould, insects and mites, deterioration can be very rapid, once infestation has taken place. Various methods for moisture determination are loss on drying, separation and the measurement of moisture, chemical methods, electrometric methods and spectroscopic methods.[4]

DETERMINATION OF EXTRACTIVE VALUE

The extractive value of the crude drug determines the quality as well as purity of the drug. Thus, alcohol and water soluble extractive values were determined.

EXTRACTION

ALCOHOL-WATER EXTRACTION

About 50-100g of powdered aerial part of *S.alternata* is taken. Add hydro alcohol (70:30), shake well and kept aside for 48 hours. Filter the solution. Evaporate to dryness or semisolid mass. The filtrate was taken in fresh sterilized glass tubes and stored at 4^{o}C.

PRELIMINARY PHYTOCHEMICAL SCREENING

Qualitative phytochemical analysis of *S.alternata* extract was carried out using standard procedures to identify the constituents.[5,6]

ANALYTICAL DOCUMENTATION

Chromatographic Techniques: The purpose of applying chromatography which is used as a method of quantitative analysis apart from its separation is to achieve a satisfactory separation within a suitable time interval. Various chromatography methods have been developed to that end. Some of them include column chromatography, thin-layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeation chromatography, high-pressure liquid chromatography, and affinity chromatography.[7]

Thin Layer Chromatography Experiment

Chromatographic purification TLC was carried out to isolate the principle components that were presents in most effective extracts of plants the TLC was used by different solvent systems. The stationary phase (**SILICAL GEL)** that is applied to the plate is made to dry and stabilize. To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil. Apply sample (HYDROALCOHOLIC EXTRACT OF **S.alternata)** to the marked spots. Pour the mobile phase (**Toluene: Ethyl Acetate: Formic acid** of ratio **7.2:5:1**) into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase. Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase. Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent. Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber. The movement of active constituent is expressed by Retention factor (Rf value).

RESULTS

Pharmacognostic Studies

As the crude drugs form the basis for the manufacture of wide range of medical preparations needed by people, the development of pharmacognostical research has become indispensable for procuring therapeutically potent medicine prepared from genuine drug material.

MACROSCOPICAL DESCRIPTION

Macroscopic evaluation of the selected plants was recorded as per usual observation organoleptic evaluation of the selected plants like color, odor, taste, shape, size, texture, apex, division, venation, base, petiole is given in Table No.1.

Si.No	Features	OBSERVATIONS
1	Color	Upper side: Metallic or bluish green Under side: Purplish red or wine red
2	Odour	Strong smell of Camphor
3	Taste	Sweet and lingering cool effect
4	Shape	Ovate to Cordate
5	Size	2-8cm long & 4-6cm wide
6	Texture	Slightly slippery & feel greasy
7	Apex	Broadly Acute
8	Division	Simple
9	Venation	Reticulate
10	Base	Cordate
11	Petiole	Present

MICROSCOPICALCHARACTERISATION

TRANSVERSE SECTION

Numerous free hand sections were taken, stained using safranin and mounted following the usual micro technique and found out the following characteristics.

Upper and lower surface of the leaf consists of rectangular thin walled epidermis, covered with thick cuticle and stomatas followed by mesophyll region. Palisade cell is single layered, below which spongy parenchyma with rectangular cells are seen. Midrib region shows large size vascular bundles which are covered by fibrous bundle sheath.

Transverse section of *S. alternata* stem include 3 to 4 layered outer most cork; cortex which is differentiated. Cortical vascular bundles of various shape and size are present and surrounded by sclerenchyma bundle sheath. It is composed of Xylem and Phloem vessels. The Xylem conducts water and minerals while Phloem conducts food. Endodermis is not distinct; pericycle fibres are seen. Central portion is occupied by pith consisting of collenchyma cells; most of the pith cells are pitted, some cells are filled with brown content. It allows radial transport of water and storage of food materials.

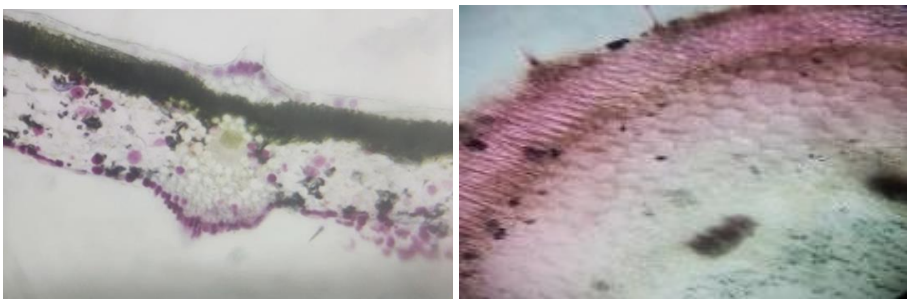

Figure 3: Transverse section of leaf and stem.

POWDER MICROSCOPY

To evaluate the behavior of powdered drug with different chemical reagents, a pinch of powder was placed in test tubes containing different reagents. Significant difference in the behavior of powder was observed and found out the presence of Epidermis, Bundle of Fibers, Parenchymal Cells. The powder plant material is greenish in color, showing fragments of parenchyma, palisade cells, fragments of epidermal cells along with stomata lignified fibers and vessels having simple pits.

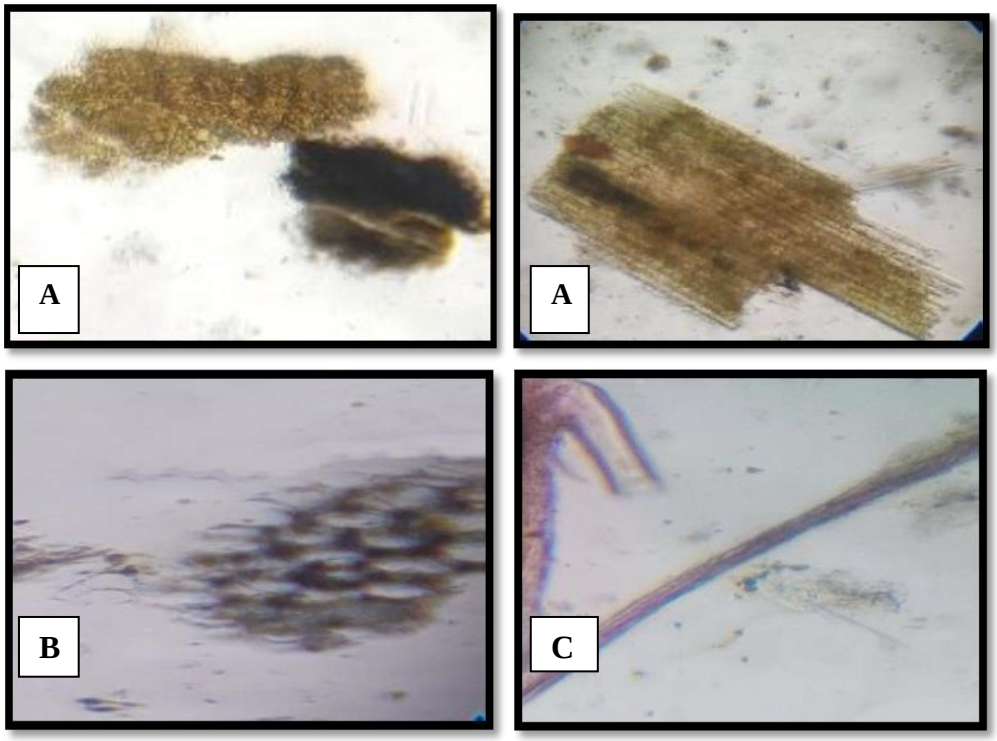

Figure 4: Powder showing the fragments of bundle of fibres (A), parenchyma cells (B) and Epidermis (C).

Vein Termination Number& Vein Islet Number

Vein termination number is defined as the number veinlet terminations per square mm of the leaf surface, midway between midrib of the leaf and its margin.The vein islet number is the average number of vein islets per square mm of a leaf surface.

The Vein Termination number was found to be 9.

The Vein islet number was found to be 11.

STOMATAL INDEX

It is the percentage, which number of stomata formed to the total number of epidermic cells; each stoma being considered as one cell. Stomatal index is calculated by taking section of leaf and stained using safranin and observed under Camera Lucida and result is given in S.I = S/E + S * 100

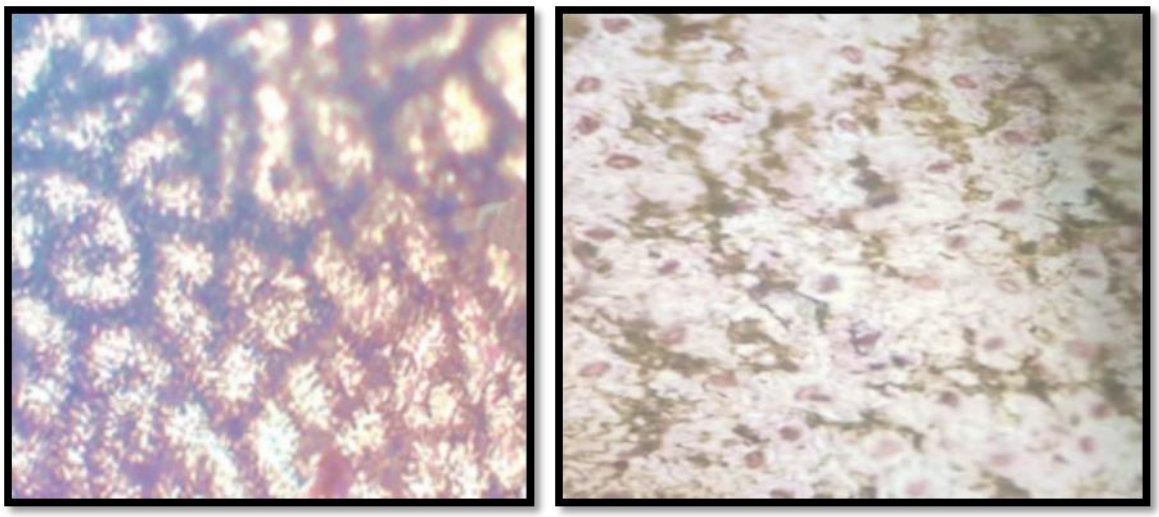

Figure 5: Vein islets and terminations, Stomata and epidermal cells.

CHEMICAL TEST FOR THE DETECTION OF ORGANIC CONSTITUENTS

The chemical test for the identification of chemical constituents were done hydro alcoholic extract of the S. alternata and founded the presence of Fixed oils, Alkaloids, Flavonoids, Terpenoids, Tannins, Saponins and Phenols.

PHYSICOCHEMICAL CONSTANTS

Physicochemical analysis of leaf and stem bark powder *viz.* foreign matter, loss on drying, swelling index, ash value and extractive value are presented in Table 2.

SI.NO	PARAMETERS	AVERAGE VALUE
1	Total Ash Value	11.8
2	Moisture Content by Loss on Drying	6.6%w/w
5	Extractive Value	14%w/w

THIN LAYER CHROMATOGRAPHY

By analysing these two compounds, the sample (Hydroalcoholic extract) and standard (Quercetin) they give almost same Rf values with same solvent therefore the two compounds are most likely identical. TLC profile confirmed the presence of Phenolic compounds which is given in Table 3.

Various literature revealed that the phenolic compounds have significant antioxidant capacity which helps to control wound oxidative stress and thereby accelerate wound healing.

FRACTION	Rf VALUE
QUERCETIN(STANDARD)	0.9
SAMPLE	0.74

WOUND HEALING OINTMENT FROM HYDROALCOHOLIC EXTRACT OF *STROBILANTHUS ALTERNATA*

Wounds are the disruption of functional continuity and anatomical structure of cells and tissues at the site of injury. They can be caused by insults to the tissue by physical, chemical, microbiological or immunological processes. It has been estimated that 14 million people suffer from wounds and burns annually with over 80% of these living in low-and middle-income countries.[8] *Strobilanthus alternata* is a prostate herb with rooting branches. The leaves are 6-10cm long and shimmering silvery violet underneath red purple. It is claimed in folk medicine that the plant has very good wound healing activity. The leaves are ground into a paste and applied on fresh cut wounds.

Table 4: Ingredients for preparation of ointments.

	SI.NO.	INGREDIENTS	OFFICIAL FROMULA	WORKING FORMULA
SEMISOLID ABSORBTION TYPE OINTMENT BASE	1	Wool Fat	5g	0.5
	2	Hard Paraffin	5g	0.5
	3	White Soft Paraffin	85g	8.5
	4	Hydroalcoholic extract of S. alternate	1g	qs

PROCEDURE: All ingredients were weighed using an electronic balance. Simple ointment was first prepared. Hard soft paraffin was placed into an evaporating dish and melted over a water bath. The dish was removed from heat and the other ingredients were added in descending order of melting point until all were melted in (the order being Wool fat and then the White soft paraffin).the mixture was continuously stirred to ensure homogeneity, but at the same time gently to avoid incorporation of excess air. Hydroalcoholic extract of *S. alternata* were weighed and mixed together before a sufficient amount of Simple Ointment BP was added to make up to the required quantity while stirring.[9]

PACKAGING: The ointment was packaged in small clear plastic containers of 10g net weight.

WEIGHT VARIATION: The filled containers were weighed, and the average weight was calculated. This was compared with the expected average weight from theoretical calculation based on knowledge of weight of empty containers.

LABELLING: The labels that were placed on the container of the ointment contain of the ointment contain the following information: Name of the product, Net weight, Ingredients-medical and non-medical, indication, direction for use, caution, date of manufacture, expiry date, batch number, manufacturer and their address.

APPEARANCE: The general appearance of the formulation ointment was observed and recorded. Qualities included color, granular or lumpy surface.

CONSISTENCY: A small amount of ointment was slowly rubbed between the thumb and fore finger to gauge consistency of the ointment.

SPREADABILITY: A small amount of ointment was rubbed on the back of the hand and the ease with which it is spread over the skin was noticed.

WASHABILITY: A small amount of ointment was rubbed on the back of the hand, after which it was washed off with warm water.

PARAMETERS	OBSERVATIONS
Appearance	Buff Color
Consistency	Smooth
Spread ability	Evenly Spread
Washability	Up to limit

Figure 6: Wound healing ointment prepared from *S .alternata.*

DISCUSSION

The herbal raw material often shows a natural variability due to many external influences such as climate, soil quality, harvesting and drying conditions, with the consequence that the qualitative and quantitative composition varies from batch to batch from harvest to harvest, in particular. Plant identity can be achieved by macroscopical like Color, Odour, Taste, Size, Shape etc. and microscopical examination like Vein islet, Vein termination, Stomatal Index etc. Microscopical evaluation is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs and detection of foreign matter and adulterants. Hence, pharmacognostical studies were carried out with a focus on bringing out diagnostic character will be of immense help in proper identification, which play an important role in the standardization of plant materials.[1]

The powder microscopy was also studied and identified the presence of Epidermis, Bundle of fibers, Parenchymal cells and Fibre. Determination of physicochemical constants is important for the purpose of evaluation of crude drugs. Preliminary phytochemical analysis is used for the purpose of evaluation of crude drugs. These findings are useful to supplement the existing information with regard to identification and standardization of *S. alternata* even in the powdered form of the plant drug to distinguish it from drug and adulterant.

Preliminary phytochemical screening revealed the presence of Alkaloids, Phenolic Compounds, Flavonoids, Terpenoids, Tannins and Saponins. The physicochemical studies include the Total Ash value, Moisture content, and Extractive value were calculated and recorded.

TLC is a valuable quality assessment tool for the identification and quantification of chemical constituents present in plant drugs. In the present study, the Rf values of individual compounds appearing as spots vertically have been noted and compared with the standard. TLC profile revealed or confirmed the presence of Phenolic compounds, and the Wound Healing property may be due to the presence of Phenolic compounds(the less polar compounds moving higher up the plates resulting in higher Rf values), which may thus be used as a quality control profile for this drug.

SUMMARY AND CONCLUSION

Strobilanthus alternata is an ethno-medicinal plant which possesses considerable level of bioactive compounds and therefore, these species can be used as a potential source of drugs. It is having the properties such as antibacterial, antidiabetic, wound healing, and antioxidant activities. This herb is a promising wound healing promoter, but a proper phytochemical and pharmacological study is the needed which provides new pharmacological avenues for this plant. This plant can be easily available and cultivated, therefore cannot be a shortage of raw materials for the phytochemical investigation.

The plant under the study has shown the presence of Phenolic compounds. The TLC profile confirmed the presence of Phenolic compounds. Numerous literatures revealed the Antioxidant property of Phenolic compounds which accelerate the wound healing property. The evidence of the study suggests that the compound isolation is possible. Separation and spectral characterization of many other constituents can be undertaken. Effort can put to discover the potency of individual separated compounds for its wound healing capacity so as to learn therapeutic function of each

ingredient. In future we can anticipate the pharmacological studies. Also, an additional focus can be made to expand various types of formulation and assess their efficacy.

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