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Phytochemical screening of *Hemigraphis colorata* (Blume) H.G. Hallier

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Abstract
Hemigraphis colorata (Blume) H.G. Hallier (syn: *Hemigraphis alternata*, family: Acanthaceae), is an exotic plant adapted to India. It is a versatile low-creeping perennial herb mostly valued as an ornamental plant. Since remote past, the leaves are ground into a paste and applied on fresh cut wounds to promote wound healing. Clinical studies also highlight its significant antimicrobial, anti-diabetic and anti oxidant activities. The present investigation present study was focused to evaluate the pharmacognostic and preliminary phytochemical properties of *H. colorata* leaves. The morphological and anatomical characters, quantitative microscopy, powder microscopy and the behavior of powdered sample with different reagents were recorded. Pharmacognostic study of crude drug would be essential for any advanced pharmaceutical research on this plant.

Keywords: *Hemigraphis colorata*, Leaf, Phytochemical

Introduction
Hemigraphis colorata is an excellent indoor and outdoor plant, chiefly grown because of its attractive and vivid foliage. It prostrates and spreads with rooting stems when grown on ground, and on hanging baskets it cascades over beautifully. It is also used to decorate aquariums and goldfish bowls. The plant is well adapted to live in tropical climate. The plant is known by several vernacular names such as Aluminium plant, Cemetary plant, Metal leaf, Red flame Ivy, Waffle plant, Java Ivy etc. In Kerala it is known as 'murikootti' or 'murian pacha' (Fig 1).
Hemigraphis is a versatile low-creeping perennial herb that reaches a height of 15 to 30 cm. The leaf has metallic purple lustre on upper surface and a solid dark purple on ventral side. The leaves are opposite, ovate to cordate, serrate-crenate, about 2 to 8 cm long and 4 to 6 cm wide, bearing well-defined veins. It blooms irregularly throughout the year in the tropics.
Flowers are small (1 to 1.5 cm diameter), five lobed, bell shaped with imbricate bracts. These are white in colour with faint purple marks within and appear in terminal 2 to 10 cm long spikes. Capsules are small, slender, oval, linear and light green in colour. Seeds are small, flat and white in colour [1-3].

Fig 1: Habit of *H. colorata*

Pharmacology and Phytochemistry

Hemigraphis is a therapeutic plant with immense power to cure vitiated pitta, fresh wound, cuts, ulcers, inflammations and skin complaints. Traditionally, the leaves are consumed to mend gall stones and excessive menstruation. In Vanuatu, sap of leaf buds are squeezed in water and drunk at dawn for 4 days as contraceptive and to induce sterility [4]. In Java, leaves are used to treat bloody dysentery and piles. It is also has diuretic ability. In folk medicine, the leaf juice is applied directly on open wound to stop bleeding [5, 6] and is used internally to cure anemia.

Phytochemicals are variety of secondary metabolites which provide the curative property. The phenolic compounds in the benzene extract of *H. colorata* leaves have showed its activity against *Acinetobacter* species and *Streptococcus aureus* [7]. The phenolic acids such as chlorogenate, cinnamate, coumarate, gallate and ferulate present in the plant acts as pro-oxidants and exhibits free radical scavenging activity [8]. The steroids and coumarins present in the extract provide anti-diabetes activity [9]. The crude leaf paste promotes excision wound healing [10, 11]. In mice, the leaf paste provides faster wound contraction and epithelialisation but oral administration is seen ineffective [12]. The excision and incision wound model studies revealed that methanolic extract is comparable to standard reference Vokadine [13]. The herbal scaffold made from chitosan was highly haemostatic and can be effectively applied for infectious wounds [14].

Materials and Methods

The plants were collected from the natural habitat, Erumeli, Kottayam, Kerala and was positively identified and confirmed by the herbarium in the Department of Botany, University of Kerala.

Pharmacognostic Studies

Morphological studies were performed by physical evaluation. For micro-characterization, free hand sections of about 10-20 µm thickness of leaves were made and stained with safranine (0.5%). The micro slides preparation was further observed in an image analyzer (Olympus BX 51) and the anatomical peculiarities were photo documented.

As a part of quantitative microscopy, stomatal number, stomatal index and vein islet number were determined by using fresh leaves of the plant (Wallis 1985) [15]. Stomatal type and various leaf constants such as stomatal number, stomatal index, vein islet number, and palisade ratio [16] (Chase and Pratt 1949) were determined by using fresh leaves. Organoleptic character like total ash, acid insoluble ash and watersoluble ash values were also determined (Kokashi *et al*., 1958; Daniel, 1991; Harborne, 1998) [17-19]. Behaviour of powder with different reagents was also noticed.

Preliminary Phytochemical Studies

For preliminary phytochemical studies extraction of crude drug with different solvents was carried out by direct extraction by cold maceration method using solvents *viz*. acetone, methanol and distilled water as per increasing order of their polarity. Presence of various phytoconstituents *viz.*, alkaloids (Dragendorff's test, Mayer's test, Wagner's test), aminoacids (Ninhydrin test), anthocyanin, anthraquinones (Borntrager's test, Modified Borntrager's test), carbohydrates (Benedict's reagent test, Fehling solution test, Molisch's test), coumarins (Sodium hydroxide test), flavonoids (Shinoda test, Ammonia test, Sodium Hydroxide test), flavones, glycosides (Anthrone test, Benedict's reagent test, Fehling solution test), phenols (Ferric chloride test, Phosphomolybdic acid test), phlobatannins (Hydrochloric acid test), protein (Biurete test, Millon's test, Xanthoprotein test), quinines (Sodium hydroxide test),quinines (Sulphuric acid test), reducing sugar (Benedict's test, Fehling test), resins (Turbidity test), saponins (Foam test), steroids and terpenoids (Lieberman burchard test, Salkowski test, Hirshonn reaction) and tannins (Braemer's test, Bromine water test) were tested.

Result and Discussion

The plants are playing an important role in the development of novel drugs and well defined pharmacognostic parameters and standards must be established before the inclusion of any crude drug in herbal pharmacopoeia. Physical evaluation is the major step employed in the identification and standardization of crude drugs. It helps in the determination of adulterants and confirms the genuineness of crude drug.

Table 1: Quantitative microscopic examination of *Hemigraphis colorata* leaf

Determinations	Value
Palisade ratio	2.75
Stomatal Index (Upper epidermis)	8.65
Stomatal Index (Lower epidermis)	22.34
Vein islet number	2.3

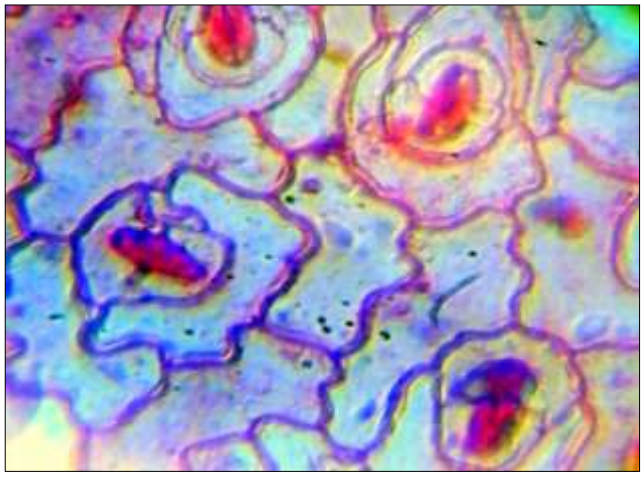

Fig 2: Stomatal type of *H. colorata*

The leaves have metallic purple coloured dorsal surface and a solid purple colour on ventral surface. They are opposite, ovate to heart-shaped, with scalloped edges. The leaves show an area ranges between 17.1- 22.5cm^2, length 5.1-7.6 cm and width vary from 3.9 cm to 4.8 cm. On upper surface of the leaves trichomes are present. Leaf peeling from upper and lower side showed diacytic stomata, in which cross walls are transverse to the long axis of the stoma (Fig- 2). The stomatal index on upper epidermis is 8.65 and lower is 22.34. The palisade ratio is calculated as 2.75. The vein islet number and vein termination number are 2.3 respectively (Table 1). Vein islet can be used for proper identification of plants and standardization of crude drug to prevent from adulteration of drug in powdered form.

Table 2: Physico-chemical characteristics

Ash value	Leaf
Total Ash (%)	11.8
Acid insoluble ash (%)	1.18
Water insoluble ash (%)	10.1
Water soluble ash (%)	2.22

Table 3: Organoleptic characters

Character	Leaf Powder
Colour	Ash green
Odour	Mouldy
Size	Coarse
Taste	Bitter
Texture	Coarse

C.S. of leaf showed single layered epidermis covered with cuticle. The upper region of the leaf surface also contains multicellular non-glandular trichomes. Mesophyll is differentiated into upper radially elongated and compactly packed single layered palisade tissue and loosely arranged spongy tissue. In between palisade and spongy tissues many layered compactly packed thin walled parenchymatous cells (Fig-3). Well define bundle sheath cells without chloroplast is seen surrounding vascular tissue. Xylem is composed of trachieds, vessels, fibres and parenchyma. Phloem is present along with xylem and is composed of non-lignified cells. Polygonal cells of chlorenchyma present below upper epidermis and above lower epidermis.

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As the part of physic-chemical studies, ash value determination was carried out and complied (Table 2). The official ash values are of key requisite in determination of the purity of powdered drugs. Organoleptic characters refer to evaluation of drugs with the use of sense organs and were recorded (Table 3). Detection of colour variation of the powdered samples under day light is also a way to identify its purity (Table 4).

Table 4: Behaviour of the *H. colorata* powdered leaf drug with various reagents

Reagents	Colour formation
Powder+ Distilled Water	Bluish grey
Powder+ 5% $FeCl_3$	Lemon yellow
Powder+ l% Acetic acid	Magenta Pink
Powder+ 5% KOH	Royal Ivory
Powder+ 5% NaOH	Solemn yellow
Powder+ Conc. HCl	Rouge red
Powder+ Conc. H_2SO_4	Reddish brown
Powder+ Conc. HNO_3	Happy celebration
Powder+ N/10 Iodine soln.	Yellowish red
Powder+ Ammonia soln.	Light reddish green

The pharmacological action of the crude drug is largely depends on the metabolites present in it. The qualitative screening by using various extracts revealed the presence of a wide range of phyto-constituents (Table 5). The test was positive to alkaloids, carbohydrates, flavonoids, phenols, proteins, resins steroids and terpenoids. This is in conformity with earlier work [20, 21].

Conclusion

The world is now focused more towards the utilization of plants and plant related compounds to safe guard human health, which is attributed mainly by their phyto-compounds. The present study is a preliminary attempt to provide detailed information on the biological, biochemical and physical properties of leaves of *Hemigraphis colorata*. The quantitative determination of pharmacognostic parameters can be used to differentiate the *H. colorata* from closely related species and also to become aware of adulteration.

Table 5: Phtochemical characterization of crude drug of *H. colorata* leaf powder

Phytochemical	Name of Test	Acetone	Methanol	Water
Alkaloids	Dragendorff's test	Present	Present	Present
	Mayer's test	Present	Present	Present
	Wagner's test	Present	Present	Present
Aminoacid	Ninhydrin test	Absent	Absent	Absent
Anthocyanin	Sodium hydroxide test	Absent	Absent	Absent
	Sulphuric acid test	Absent	Absent	Absent
Anthraquinones	Borntrager's test	Absent	Absent	Absent
	Modified Borntrager's test	Absent	Absent	Absent
Carbohydrate	Bendict's reagent test	Present	Present	Present
	Fehling solution test	Present	Present	Present
	Molisch's test	Present	Present	Present
Coumarins	Sodium hydroxide test	Absent	Absent	Absent
Flavonoids	Ammonia test	Absent	Present	Present
	Lead acetate test	Absent	Absent	Present
	Shinoda test	Absent	Absent	Present
Flavones	Sodium hydroxide test	Absent	Absent	Present
	Sulphuric acid test	Absent	Absent	Present
Glycosides	Anthrone test	Absent	Absent	Absent
	Bendict's reagent test	Absent	Absent	Absent
	Fehling's test	Present	Present	Present
Phenols	Ferric chloride test	Present	Present	Present
	Phosphomolybdic acid test	Present	Present	Present
Phlobatannins	Hydrochloric acid test	Absent	Present	Present
Proteins	Biuret test	Absent	Absent	Present
	Million's test	Absent	Absent	Present
	Xanthoprotein test	Absent	Absent	Present

Quinine	Sodium hydroxide test	Present	Present	Present
Quinone	Suphuric acid test	Present	Absent	Absent
Resin	Turbidity test	Absent	Absent	Present
Saponins	Foam test	Absent	Absent	Absent
Steroids/ Terpenoids	Lieberman Burchard test	Absent	Absent	Present
	Salkowski test	Absent	Present	Present
	Hirshonn reaction	Absent	Absent	Absent
Tannins	Braemer's test	Absent	Absent	Absent
	Bromine water test	Absent	Absent	Absent

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