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Preliminary phytochemical and biochemical analysis of *Hemigraphis colorata* H.G. Hallier

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Abstract
The traditional and folk medicine system uses the plant products for the treatment of various infectious diseases. Studies by various researchers have proved that plants are one of the major sources for drug. The curative properties of medicinal plants are due to the presence of various chemical substances of different composition which occur as secondary metabolites.
Hemigraphis colorata is traditional medicinal plant mainly used to treat cut and wounds. The present work revealed that the phytochemical analysis of ethanolic extract of *Hemigraphis colorata* contain carbohydrate, protein, flavonoid, saponin, tannin, steroid and glycoside. The chloroform extract shows the presence of carbohydrate, protein, aminoacid, alkaloid, tannin, steroid and glycoside. The benzene extract indicate the presence of tannin, terpenoid, gum, carbohydrate, protein, steroid and glycoside. The moisture content in dry leaves of *Hemigraphis colorata* is 4.5%, total ash value (12.5%). The physicochemical parameters such as ash value and determination of moisture loss was used to determine the quality and purity of a crude drug. The biochemical analysis of *Hemigraphis colorata* revealed that 28.12 mg of carbohydrate, 5.6 mg of protein and 25.30 mg of starch. *Hemigraphis colorata* possessed considerable level of bioactive compounds and therefor, these species can be used as a potential source of drugs. The plant has immense power to cure fresh wound, ulcers, inflammations and skin complaints.

Keywords: *Hemigraphis colorata,* acanthaceae, phytochemical analysis, biochemical analysis

Introduction
Medicinal plants are widely used for medicinal purposes. Various plant part including root, leaf, flower, fruit and bark are used to cure disease. Medicinal plants are the oldest form of healthcare known to mankind. From the ancient time herbs and plants are used as the remedy for various disease.
The plant *Hemigraphis colorata* is a versatile tropical low-creeping perennial herb. It is a prostrate growing plant with spreading rooting stems. The leaves are opposite, ovate to cordate, bearing well-defined veins, slender and lance shaped with toothed, scalloped margins. They are green stained with red purple above and darker pruple beneath. Flowers are small five lobed, bell shaped, white in colour and are held in spikes on the stem tip. Seed are small, flat and white in colour (Saravanan *et al.,* 2010) [5]. The plant is known by several name such as Red flame ivy, Aluminium plant, Metal leaf, Waffle plant. etc. In Kerala the plant is popularly known as 'Murikootti' or 'Murianpacha' (Ramnivas *et al.,* 2016) [4].
H. colorata, possesses medicinal properties. It is used as a traditional medicine for wound healing in southern part of India. Leaf paste of *H. colorata* was shown to have anti-inflammatory effect on the carrageenan induced paw edema model (Subramoniam *et al.,* 2001) [6]. Leaf extracts were also shown to have anti-bacterial properties (Anitha *et al*., 2012) [1]. The leaves are ground in to a paste and applied on fresh cut wounds used to promote urination, check hemorrhage, Stop dysentery, Treat veneral diseases and to heal hemorrhoids. Traditional uses, the whole plant of *Hemigraphis colorata* is ground in to a paste with water and plant has immense power to fresh wound, cuts, ulcers, Inflammation and skin complaints.
Phytochemical analysis in *Hemigraphis colorata*, reveals that they have been used as drugs, dyes and food supplements. The phytoconstituents present in *H. colorata* are phenols, saponins, flavonoids, terpenoids, carbohydrates, carboxylic acid, xanthoprotein, tannin, protein, alkaloids, steroids and sterol.
Biochemical analysis is the study of chemical substance and vital processes occurring in living organisms. It is performed for testing the presence of phenolics, flavonoids, saponins, glycosides and proteins.

Material and Methods
Study Area
Hemigraphis colorata leaves were collected from Nilambur, Malappuram district of Kerala. Nilambur gets about of rainfall 2400 mm and situated at a height of approximately 183 ft. above sea level.

Sample Collection
Fresh leaves of the selected plant materials were collected during August. The leaves were washed in running tap water. The leaves are shade dried and ground to fine powder and stored in air tight container for further analysis.

Habit and Fresh Leavess

Fig 1

Physicochemical Analysis
The leaf was evaluated for its physicochemical parameters like total ash, determination of moisture loss using standard procedures (Pulok Mukherjee, 2002 [2]; Anonymous, 2007, Kokate *et al*., 1994)

Moisture content

$$\text{Moisture content\%} = \frac{\text{Fresh wt} - \text{Dry wt x100}}{\text{Fresh wt}}$$

Total ash

$$\text{Ash content \%} = \frac{\text{Fresh wt} - \text{Dry wt x 100}}{\text{Fresh wt}}$$

Preliminary Phytochemical Analysis
(Raaman, 2006; Karpagam *et al*., 2008; Kokate *et al*., 2001) [2, 3].

Extraction
The powdered leaf was collected and 15g of it were measured and introduced into 100ml of ethanol, chloroform and benzene. Extraction is carried out by shaker system for 48hrs.The nature and yield of the extract were noted. The extracts were stored in a refrigerator at 4˚c for further studies.

Test for Carbohydrates
To 2 ml test solution add 2 drops of the molish reagent. The solution is the poured slowly into test tube containing 2ml of concentrated sulphuric acid. So that two layers form. The formation of a purple product at the interface of the 2 layersindicated the presence of carbohydrates.

Test for Protein
It is used to determine the presence of peptide bounds in protein. To 3ml of test sample add 3% sodium hydroxide and few drops of 1% copper sulphate. The solution turns from blue to violet (purple) indicated the presence of protein.

Test for Starch
Mix 3ml test solutions. A few drops of dilute iodine solutions. Blue colour appear. It disappears on boiling and reappears on cooling.

Test for Steroids
To 2ml of extract add 2ml chloroform & add 2ml concentrated sulphuric acid. Shake well; chloroform layer appear red and acid layer show greenish yellow fluroscence which indicated the presence of steroid.

Test for amino acid
To 5ml of test sample solution add a few drops of 40% sodium hydroxide &10% lead acetate boiled the solution formation of black precipitate indicated the presence of amino acid.

Test for Glycosides
To the extract add Glacial acetic acid, few drops of 5% ferric chloride and concentrated sulphuric acid are added and observed for a reddish brown coloration at the junction of the two layers, and bluish green colour in the upper layer which indicated the presence of glycoside

Test for Flavonoid
To 2ml of extract add few drops of ammonia solution. A yellow coloration was observed for the presence of flavonoid

Test for Alkaloid
To 0.5g of each extract add 5ml of 1% aqueous hydrochloric acid and kept in water bath, 1ml of filtrate is to be treated with Mayer's reagent. Formation of a yellow coloured precipitate indicated the presence of alkaloids

Test for Tannin
To 0.5ml of extract 1ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannin and green black for catecholic tannin.

Test for Saponins
To 1ml extract add 2ml distilled water and shake it persistent foam indicated the presence of saponin.

Test for Terpenoid
2ml of extract was mixed with 2ml chloroform in a test tube. To this 3ml concentrated sulphuric acid was carefully added alone the wall of the test tube, an interface with reddish brown colouration confirmed the presence of terpenoid

Test for Gums
To 1ml of extract add 3ml of dil.hydrochloric acid, fehlings solution is added drop by drop, till red coloration visualize the presence of gums.

Biochemical Analysis

The biochemical analysis is performed on *Hemigraphis colorata*. The powder of selected plant leaves where tested for estimation of carbohydrate (Anthrone method), protein (Lowry's method) Sadasivam and Manikam (2008) [7], starch.

Estimation of Carbohydrate by Anthorne Method

100mg of dried powdered leaves wash hydrolysed in a boiling water bath for 30 minutes with 80%ethanol in water and centrifuge 8000g for 15 minutes and preserved 4ml of supernatant. From it 1ml of the supernatant dried and dissolved in 50 ml distilled H2O. Anthrone reagent is prepared by mixing 300gm anthrone with 150ml ice cold H_2SO4.0.2ml of sample made up to 1ml with distilled H2O add 4ml of Anthrone reagent and rapidly cooled in ice bath. OD values which was read at 630nm using Bovine Serum Albumin (BSA).

Protein estimation (Lowry *et al.*, 1951)

1gm of the sample weighed and grained well with a pestle and motor in 1ml of the buffer. Add 5% of TCA and kept in cooled for 1hour. Centrifuge at 3500rpm for 20 minutes. Dissolved precipitated protein in 0.1N NaOH (Reagent A). 0.5% Cuso4 in 1% potassium sodium tartarate (Reagent B). 50ml of reagent A and B was mixed prior to use and reagent C was obtained. Which was immediately added in to the test tube was mixed well and allowed to stand for 10 minutes. 0.5ml of reagent D (folin ciocalteau reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes to develop blue colour. OD values read at 660 nm using glucose as standard and calculate the amount of protein (Lowry *et al.*, 1951)

Estimation of starch (Hedge and Hotreiter, 1962)

The total soluble carbohydrates from the selected sample were extract and estimated by the Anthrone reagent method (Hedge and Hotreiter, 1962). Using glucose as standard at 620nm in a spectrophotometer the OD values were expressed as mg/100gm on dry weight basis.

Result

The present investigation was carried to find out the phytochemical and biochemical constituents present in *Hemigraphis colorata.*

The phytochemical analysis in ethanolic extract of *Hemigraphis colorata* contain carbohydrate, protein, flavonoid, saponin, tannin, steroid and glycoside. The chloroform extract shows the presence of carbohydrate, protein, aminoacid, alkaloid, tannin, steroid and glycoside. The Benzene extract indicate the presence of tannin, terpenoid, gum, carbohydrate, protein, steroid and glycoside.

The Physicochemical analysis of *Hemigraphis colorata* were the moisture content is 4.5%, total ash value 12.5%. The biochemical analysis on *Hemigraphis colorata* revealed 28.12 mg of carbohydrate, 5.6 mg of protein and 25.30 mg of starch.

Table 1: Phytochemical constituents in *Hemigraphis colorata*

Sl. No	Phytochemicals	Ethanol extract	Chloroform Extract	Benzene Extract
1	Alkaloids	_	+	_
2	Flavonoids	+	_	_
3	Carbohydrates	+	+	+
4	Proteins	+	_	+
5	Starch	_	_	_
6	Amino acid	_	_	_
7	Steroids	+	+	+
8	Tannins	+	+	+
9	Saponins	+	_	_
10	Terpenoids	_	_	+
11	Glycoside	+	+	+
12	Gum	_	_	_

+ indicate= present, _indicate =absent

Table 2: Physicochemical analysis in dry leaf of *Hemigraphis colorata*

SL.NO	Parameter Analysed	Sample (%)
1	Moisture content	4.5%
2	Total ash	12.5%

Table 3: The content of total carbohydrates, proteins, starch in the leaf of *Hemigraphis colorata*

SL. No	Biochemical constituents	Sample (mg/g)
1	Carbohydrate	28.12mg/g
2	Protein	5.6mg/g
3	Starch	25.30mg/g

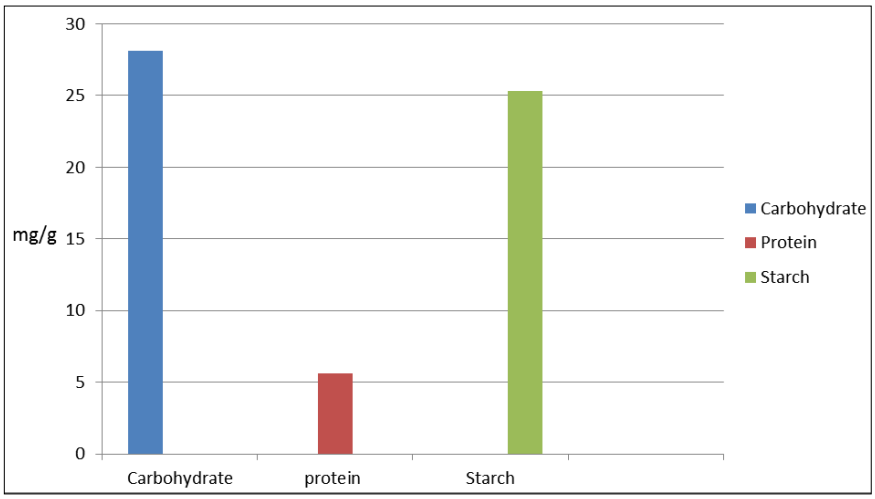

Fig 2

Discussion

The phytochemical constituents are variety of secondary metabolites, with curative property. The phytochemical constituents on *Hemigraphis colorata* were identified by examining the crude extracts of its leaves using various solvents. The phytoconstituents are phenols, saponins, flavonoids, terpenoids, carbohydrates, carboxylic acid, xanthoprotein, tannins, proteins, alkaloids, steroids and sterol.

The ethanolic extract of *Hemigraphis colorata* contains carbohydrate, flavonoid, saponin, tannin, steroid and glycoside, similar results were observed in Niya Pathallookaran *et al.,*(2017), Asha Gangadharan *et al*., (2013) and Akhil and Prabhu (2013). Flavonoids are used in anti-oxidant, anti-inflammation and anti-malarial activity. Saponin are used in hyperglycemia, anti-cancer and weight loss. The ethanolic extract of *Hemigraphis colorata* contain saponin in similar result were observed in Biju *et al.*, (2015).

The cholorofom extract of *Hemigraphis colorata* contains carbohydrates, aminoacids, alkaloids, tannin, steroid, and glycoside in similar results were observed in Joyson *et al.*, (2017). Steroids are responsible for anti-diabetic effects, they have anti-bacterial properties, analgesic properties and act on central nervous activities. Glycoside are responsible for anti-oxidant effect. Akhil and Prabhu (2013) reported the steroids and glycoside are absent in this chloroform extract of plant.

Tannin have various physiological effects like anti-irritant, anti-microbial and anti-parasitic effect. Joyson *et al*., (2017) and Radhika *et al.*, (2017) reports that the tannin are absent in the chloroform extract of this plant. The chloroform extract of *Hemigraphis colorata* contain alkaloid are responsible for anti-malarial, anti-cancer, analgesic and anti-bacterial activity.

The benzene exract of *Hemigraphis colorata* contain tannin, terpenoid, gum, protein, carbohydrate, steroid and glycoside in similar results were observed in Biju *et al.,*(2015), Terpenoids display a wide range of biological activity against cancer, malaria, inflammation and a variety of infectious diseases.

The physicochemical analysis of *Hemigraphis colorata* showed the moisture content (4.5%).It was higher than the same plant species (2.25%) reported by (Radhika *et al*., 2017). *Hemigraphis colorata* contain total ash content (12.5%).It was lower than that same plant (13.0%) of ash content (Radhika *et al.,* 2017)

The biochemical analysis of *Hemigraphis colorata* revealed 28.12 mg of carbohydrate, 5.6 mg of protein and 25.30 mg of starch.

Protieins are one of the building block of the body tissues, and can also serve as a fuel source. They provide structure and strength to cells and tissues, controlling the biochemical reaction and aiding the immune system.

Carbohydrate are responsible for the immune system, they provide fuel for the central nervous system, and they supply energy. Starch is the most important carbohydrate in the human diet. starch are responsible for the anti-coagulant activity, as a anti-tumor agents and anti- diabetic agents.

Summary

The traditional and folk medicine system uses the plant products for the treatment of various infectious diseases. The curative properties of medicinal plants are due to the presence of various chemical substances of different composition which occur as secondary metabolites.

Hemigraphis colorata is traditional medicinal plant mainly used to treat cut and wounds. The present work revealed that the phytochemical analysis of ethanolic extract of *Hemigraphis colorata* contain carbohydrate, protein, flavonoid, saponin, tannin, steroid and glycoside.The chloroform extract shows the presence of carbohydrate, protein, aminoacid, alkaloid, tannin, steroid and glycoside.The benzene extract indicate the presence of tannin, terpenoid, gum, carbohydrate, protein, steroid and glycoside.

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