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Physico-chemical, phytochemical, antioxidant and GC-MS analysis of methanolic extract of *Hemigraphis colorata* (Blume) H. G. Hallier and *Pimenta dioica* (L.) Merr.

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Abstract
The plant based bioactive compounds always played a significant role in controlling bacterial and fungal diseases in crop plants and animals. Suppression of microbial growth in plants, vegetables and fruits are also essential to reduce the health risk to man and animal. The present study was designed to evaluate physico-chemical, phytochemicals, antioxidant activity and GC - MS analysis of methanolic extracts of *Hemigraphis colorata* and *Pimenta dioica*. The results of the study showed higher ash values in *H. colorata* than in *P. dioica* but the extractive values were better in *P. dioica*. Phytochemicals such as total phenol, flavonoid and alkaloid contents were higher in *P. dioica* (771.183 ± 0.779 mg GAE/g, 64.266 ± 0.0185 mg QE/g, and 104.64 ± 0.308 mg/gm respectively). *H. colorata* showed high amount of tannin (162.18 ± 0.328 mg TAE/g). Total antioxidant activity was found high (432.333 ± 0.6466 mg/g) in *H. colorata* and low (424.49 ± 1.113mg/g) in *P. dioica.* GC-MS analysis of *P. dioica* and *H. colorata* revealed the presence of 11 and 17 major peeks respectively. The present study showed that the selected medicinal plants are the potential source of bioactive compounds and the use of these plants for pharmaceutical purpose is very promising.

Keywords: antioxidant, GC-MS analysis, *Hemigraphis colorata*, *Pimenta dioica*

Introduction
The Western Ghats of Karnataka is the most extravagant wellspring of restorative plants. The health maintenance system of the traditional community in India are prominently depended on plants and plant extracts for medicinal use. *Hemigraphis colorata* is a non-native medicinal plant belonging to the family of Acanthaceae. It shows prostrate growth and spreads on the ground with rooting stems. The leaf parts of *H. colorata* when applied to wounds promotes wound contraction and epithelization leads to wound healing [1]. It is used as a medicine for abdominal pain, glossitis, stomatitis, urolithiasis and diabetes [2]. This plant species has been used in folklore medicines for various skin diseases. The phenolic compound of *H. colorata* serves as pro-oxidants and have free radical scavenging properties [3]. The leaves of this plant accommodate flavonoids, polyphenols, tannins together with high amount of potassium and low amount of sodium. The stem of *H. colorata* is found to have saponins and tannins whereas the root is incorporated with flavonoids and polyphenols and these phytochemicals have restorative qualities [4].
Pimenta dioica belong to the family Myrtaceae commonly known as allspice. Its aromatic taste and flavour similar to a blend of cinnamon, cloves, and nutmeg. The leaves of *P. dioica* frequently used as a carminative to prevent the gas formation in the gastrointestinal tract. The extracted oil from allspice is an effective tonic to treat nervous exhaustion, chest infections, arthritis and muscle pains [5]. Allspice has been used as an herbal remedy for fever, cold, flu, diabetes, muscle cramp and heavy menstrual discharge. Extracts from allspice hold antioxidant, antiseptic and anaesthetic properties and fight against yeast and fungal infections. It is a natural source of β-carotene, vitamins A, B1, B2, C, niacin, thiamine, and riboflavin along with the minerals iron, potassium, magnesium, selenium and manganese [6]. Leaves and fruits of *P. dioica* produce essential oils, which employs a crucial role in the meat industry and for the production of beauty products [7].

Materials and Methods
Collection of Plant materials
The healthy plants *viz.*, *Hemigraphis colorata* (Blume) H.G. Hallier. and *Pimenta dioica* (L.) Merr. were collected in a polythene bag from Western Ghats of Karnataka during the month of August 2018. The plant leaves were separated and washed thoroughly with running tap water followed by distilled water to remove the dust and debris. The leaf materials were shade dried and milled in to a fine homogenous powder using a blender. The powdered samples were stored in air tight container for further study.

Physico- chemical constituents of the plant materials
Ash Values
Powdered leaf samples of *H. colorata* and *P. dioica* were used to investigate the different ash values by the following methods [8, 9].

Total Ash Value
2 gm of leaf powder sample was transferred to a silica crucible and spread a fine layer on it. The crucible was incinerated in a muffle furnace at a temperature not

exceeding 600 ºC until free from carbon. Then the crucible was cooled, weighed and the percentage of total ash content was calculated with reference to air-dried sample of the drug.

Acid- Insoluble Ash Value
Total ash obtained from the sample was allowed to boil in a 25 mL of 2N HCl for 5 min. The insoluble ash was gathered on an ashless filter paper and rinsed with hot water. Then the filter paper containing residue was positioned on a silica crucible and ignited in a muffle furnace at the temperature not exceeding 600 ºC to obtain constant weight. Percentage of acid insoluble ash was computed in accordance with air-dried drug.

Water-Soluble Ash Value
Total ash obtained was diluted by adding 25 mL of distilled water and kept it for boiling for 5 min. Insoluble matter was collected on an ashless filter paper, washed with hot water and subsequently ignited and weighed. Water-soluble ash content was expressed in percentage with reference to the air-dried drug.

Sulphated Ash Value
The total ash obtained was dampened in 1 mL of conc. H_2SO_4, heated gently until no further white fumes, ignited and weighed. Then the sulphated ash value was figured out in percentage with reference to the air-dried drug.

Extractive Values
Water Soluble Extractive Value
5 gm of air-dried powdered plant sample was weighed and transferred to a conical flask containing 300 mL of water and shaken vigorously. The contents were boiled gently for 6 hrs followed by cooling and rapid filtration. The filterate was evaporated to dryness and final weight was noted and calculated as % (w/w) with reference to air-dried drug.

Alcohol Soluble Extractive Value
5 gm of finely powdered air-dried plant sample was weighed in a glass-stopper conical flask. To this 100 mL of alcohol (90% v/v) was added and shaken intermediately for 24 hours and filtered. The filtrate was transferred to a pre weighed porcelain dish and evaporated to dryness by placing it on the boiling water bath and remeasured the weight of the flask [10]. The extractive value (%) was calculated using the following formula

Extractive value (%) = Weight of dried extract/ Weight of plant material X 100

Fluorescence analysis
The powdered leaf samples of *H. colorata* and *P. dioica* homogenized with various organic reagents such as 1N sodium hydroxide, 1N hydrochloric acid, distilled water, acetic acid, picric acid, sulphuric acid, $FeCl_3$ and alcohol and their extracts were observed under UV light (280 nm) and normal daylight [11].

Qualitative phytochemical analysis
Phytochemicals such as tannins, saponins, alkaloids, flavonoids, terpenoids, glycosides, steroids, phenols, carbohydrates, protein, phlobatanins and coumarins were analysed with crude methanolic extract of dry powdered samples by following the standard methods [12].

Quantitative phytochemical analysis
Total Flavonoid
0.5 mL of each extract was taken in the test tubes and 1.5 mL methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water were added to each test tubes. Then the tubes containing the reaction mixture was incubated at room temperature for 30 min and the absorbance was measured at 415 nm. The total flavonoid content was expressed as (µg) equivalents of quercetin/mg of the sample [13].

Total Tannin
Tannin content of the extracts was estimated by the following method with slight modifications. 20 µl of the sample was diluted with 980 µl of distilled water and to this, 500 µl of 1% potassium ferricyanide ($K_3Fe(CN)_6$) and 100 µl of 1% ferric chloride ($FeCl_3$) solutions were added. The content was mixed thoroughly and made up to 3mL by adding distilled water. Then the reaction mixture was incubated for 10 minutes at room temperature and the optical density was measured at 720 nm using UV spectrophotometer. Tannin content was calculated as µg of tannic acid equivalents/mg [14].

Total Alkaloid
Alkaloid content of the crude extracts was estimated by the standard procedure [15]. 2.5 g of the sample was mixed with 200 mL of 10% acetic acid (prepared in ethanol) and allowed to stand for 4 hrs. The filtered reaction mixture was concentrated on a water bath to get one-quarter of the original volume. To this, conc. ammonium hydroxide (NH_4OH) was added dropwise until the precipitation was completed. The precipitate was collected, washed using dilute ammonium hydroxide and filtered. The filtered residue was dried and weighed. Percentage of the total alkaloid content was calculated using the formula:

Percentage of alkaloid = (final weight of the sample/initial weight of the extract) × 100

Total Phenol
0.1 mL of the extract was made up to 0.25 mL with distilled water, stirred thoroughly. To this mixture,0.25 mL of FC reagent (Folin-Ciocalteu) and 0.5mL of 20% Na_2CO_3 was added and made up to 5 mL with distilled water. Then the reaction mixture was incubated at room temperature for 30 minutes. Optical density of the sample was measured spectrophotometrically at 760 nm and the results were expressed as µg of gallic acid equivalents/mg of extract [16].

Antioxidant Activity
Total Antioxidant Activity
Total antioxidant activity of the selected plant extracts was assessed according to the method described [17]. To 0.3 mL extract, 3 mL of phosphomolybdenum reagent was added and mixed thoroughly and the reaction mixture was incubated at 95°C for 90 min. Then it was cooled to room temperature and the optical density was measured at 695 nm using UV-VIS spectrophotometer against a blank (0.3 mL methanol). Total antioxidant activity of the sample was calculated as the number of gram equivalent of ascorbic acid.

DPPH Radical Scavenging Assay

DPPH free radical scavenging assay was performed by the following method [18] with slight modifications. Methanolic leaf extracts of selected plants were prepared in different concentrations (20-100 µg/ml). To each test tube, 3 mL of 0.1 mM DPPH solution was added, gently shaken and incubated in dark for 30 minutes at room temperature. Optical density was measured at 517 nm against a reagent blank and ascorbic acid was used as the control. DPPH radical scavenging activity was calculated using the formula:

Percentage of inhibition (%) = (Ac – As) / As X 100

Where Ac =Absorbance of control, As = Absorbance of sample

Reducing Power Assay

To different concentration of extracts, 2.5 mL of 0.2 M phosphate and 2.5 mL of 1% of freshly prepared potassium ferricyanide (K_3Fe $(CN)_6$) solutions were added mixed. Then the reaction mixture was incubated for 20 minutes at 50 °C. After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) solution was added and centrifuged at 3000 rpm for 30 min. To 2.5 mL supernatant, 2.5 mL of methanol and 0.5 mL of 0.1% $FeCl_3$ solutions were added and mixed thoroughly. The optical density was measured at 700 nm. Reducing power value of the extracts was calculated in mg/g equivalents of ascorbic acid [19].

GC–MS Analysis

Methanolic extract of plant samples was subjected to GC-MS analysis [(GC-MS model (GCMS-TQ8040NX, Japan, 30 m long, 0.25 mm thick SH-Rxi-5Sil MS column and internal diameter of 0.25 mm (5% diphenyl / 95% dimethylpolysiloxane)]. The column oven temperature was set to 60.0 °C and the injector temperature was 260.00 °C and helium (99.999%) was acted as carrier gas at a flow rate of 1.20 mL/min. 1.0 µL of each extract was injected by the split less injection technique and split ratio of 10.0 was used for the analysis. Mass ranges were scanned at a rate of 3333 scans / 0.200 seconds from 40 to 650 m / z and the total GC run time was maintained to 40 min. Software of the GCMS solution was used to manage the data. The mass spectra of the compounds found in the extracts were matched with the National Institute of Standards and Technology (NIST)17 libraries [20].

Results

Physico - chemical parameters

Ash Values

Ash values are used to determine the authenticity and purity of the sample. Ash values such as total ash, acid insoluble ash, water soluble ash and sulphated ash values of *H. colorata* were found to be 26.025%, 11.05%, 16.89%, 31.14% respectively. *P. dioica* showed higher total ash value (15.43 %) followed by sulphated ash (19.71%), water soluble ash (8.64 %) and acid insoluble ash (7.38%) (Table 1). This percentage values indicates that the leaves of *H. colorata* and *P. dioica* are the best sources for drug action and effects.

Extractive Values

The quality and purity of the drug are determined based on the extractive value of the crude drug. Alcohol soluble extracts of both plants showed higher values than the water-soluble extracts The results showed that more compounds from the samples can be extracted with alcohol than water (Table 1).

Table 1: Physico-chemical study of *H. colorata* and *P. dioica*

Parameters	*H. colorata*	*P. dioica*
Total ash value	26.025 %	15.43 %
Acid insoluble ash value	11.05 %	7.38 %
Water soluble ash value	16.89 %	8.64 %
Sulphated ash value	31.14 %	19.71 %
Alcohol soluble extractive value	4 %	10.5 %
Water soluble extractive value	3.5 %	7 %

Fluorescence analysis

The usage of powdered herbal drug is very easy and the adulteration of the drug can be detected by observing the herbal powder under UV light. Table 2 represents the fluorescence analysis of the powdered form of leaves of *H. colorata* and *P. dioica.*

Table 2: Fluorescence analysis of *H. colorata* and *P. dioica*

Solvents used	*H. colorata*	*P. dioica*
	Under UV light (280 nm)	
Powder + Distilled water	Slightly fluorescent	No fluorescence
Powder + 1N HCL	No fluorescence	No fluorescence
Powder +Alcohol	Pale orange colour	Slightly fluorescent
Powder + Picric acid	Yellow colour	Yellow colour
Powder + Conc. H_2SO_4	Greenish yellow	Dark green
Powder + $FeCl_3$	Dark green	Dark brown
Powder +1N Na_2CO_3	Greenish colour	Brownish green

Phytochemical analysis

H. colorata extracts revealed the existence of all the analysed phytochemicals. But terpenoids, phlobatannins and coumarins were absent in *P. dioica* extract (Table 3).

Table 3: Phytochemical screening of *H. colorata* and *P. dioica*

Phytochemicals	*H. colorata*	*P. dioica*
Tannins	+	+
Flavonoids	+	+
Terpenoids	+	-
Saponins	+	+
Steroids	+	+
Phlobatannins	+	-
Carbohydrates	+	+
Glycosides	+	+
Coumarins	+	-
Alkaloids	+	+
Proteins	+	+
Phenol	+	+

(+) = Present, (-) = Absent

Quantitative analysis

Total Phenol, Flavonoid, Tannin and Alkaloid

High number of total phenols (771.183 ± 0.779 mg GAE/g) and low alkaloid (104.64 ± 0.308 mg/gm), tannin (128.633 ± 0.0166 mg TAE/g) and flavonoid (64.266 ± 0.0185 mg QE/g) content was found in *P. dioica. H. colorata* showed a higher amount of tannin (162.18 ± 0.328 mg TAE/g) followed by total phenol (53.59 ± 0.7937 mg GAE/g), flavonoid (15.463 ± 0.0033 mg QE/g) and alkaloid (15.3533 ± 0.1510 mg/g) content (Fig 1).

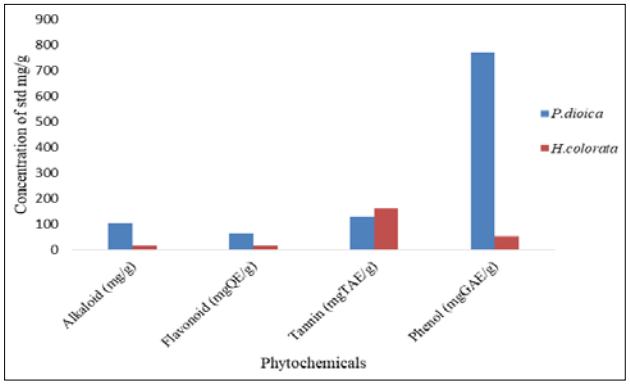

Fig.1: Quantitative analysis of phytochemicals

Total Antioxidant activity

The total antioxidant activity was found high (432.333 ± 0.6466 mg/g) in *H. colorata* and low (424.49 ± 1.113 mg/g) in *P. dioica*. Reducing power assay of leaf extracts of *H. colorata* was 75.9100 ± 0.005 mg/g and 88.1533 ± 0.033 mg/g in *P. dioica*. Results of DPPH free radical scavenging activity was represented as percentage of inhibition. High percentage (90.036%) inhibition of DPPH radical scavenging activity was observed in *P. dioica* leaf extract and it was low (78.620%) in the leaf extract of *H. colorata* (Table 4). The values of quantitative analysis were expressed as mean ± Standard Error Means (SEM) of triplicates and $p < 0.05$ was considered as statistically significant.

Table 4: Total antioxidant, DPPH and reducing power assay

Method used	*H. colorata*	*P. dioica*
Total antioxidant (mg/g)	432.333 ± 0.6466	424.49 ± 1.113
Reducing power (mg/g)	75.9100 ± 0.005	88.1533 ± 0.033
DPPH (%)	78.620 ± 0.2010	90.036 ± 0.5385

Results are expressed as Mean ± SEM, n=3, $p < 0.05$ considered as significant

GC MS Analysis

The GC–MS chromatogram of *P. dioica* showed 11 major peeks (Fig. 2). Among them Eugenol (22.97%) Phenol, 4-(2-propenyl) (16.97%) and Lupeol (12.36%) showed a higher peak area. *H. colorata* extracts showed 17 major peeks (Fig. 3) with different retention time. Eugenol (17.28%), stigmasterol (7.13%), gamma. Sitosterol (6.01%) and phenol, 4-(2-propenyl) - (6.08%) were obtained in *H. colorata* with higher peak area percentage. The identified bioactive compounds were presented in table 5 and 6 with their peak area percentage, retention time, molecular formula, molecular weight and biological activity.

Discussion

Majority of the crude drugs utilized by Indian systems of medicine are of plant origin. According to the World Health Organization [WHO], a detailed study of bioactive components should be performed before considering the plant for medicinal purposes [21]. *P. dioica* and *H. colorata* are the two important medicinal plants extensively utilized in conventional medicines by virtue of its several biological activities. The powdered form of the drug has been used for physicochemical studies which help to evaluate the purity and quality of the drug from plant origin. Ash value of a drug helps to determine numerous impurities like oxalate, carbonate and silicate. Evaluation of various inorganic substances present in the drugs was employed by the help of water-soluble ash, which is a water-soluble part of the total ash. The acid insoluble ash mainly involves silica which designate the deterioration with earthy matter [22]. The extractive values of the sample help to determine the chemical constituents of the crude drug and also assists to evaluate the specific constituents soluble in a particular solvent [23]. Alcohol and water extractive values were calculated in each of the chosen plants and better values were obtained using alcohol. Variability in extractive values may be due to specific compound, condition of soil, change in atmosphere and water content present in the sample [24]. Alkaloids, flavonoids and tannins were reported in the phytochemical analysis of aqueous and alcoholic extracts of *H. colorata* [25]. Such compounds from plants are mainly applicable for drug formulation because of their low toxicity to the host cell [26, 27]. Flavonoids are group of secondary metabolites naturally occurring in plants which are effective in scavenging oxidizing molecules. Derivatives of flavonoid compounds shows antimicrobial, anticancer, anti-allergic properties hence purified compounds can be used for the treatment of several diseases [28, 29]. Alkaloids have been held with medicinal practices for decades and one of their frequent features is their cytotoxicity [30]. Tannins are the class of astringent; polyphenolic biomolecules possess anti-diarrhoeal and antimicrobial activities [31, 32]. Phenolic compound deals with modifying colour, taste, aroma and flavour thus improve the quality and nutritional value. Phenols act as repelling and attracting agents to organisms and also shows defence mechanism against reactive oxygen species (ROS) so a high concentration act as protective agents against fungal and bacterial pathogens. Surplus production of ROS accelerates to oxidative stress and diseases [33]. Intake of rich antioxidant containing diet is necessary to neutralize the free radicals from the body. Plant based antioxidant compounds helps to replace the use of synthetic antioxidants [34, 35]. *In vitro* experiments were used to determine the antioxidant activity; however, investigators have reported that there is no uniform approach to estimate the antioxidant activity quantitatively and precisely [36]. Hence three different methods are used to evaluate antioxidant activity. *H. colorata* and *P. dioica* showed high antioxidant activity and other secondary metabolites quantified during this study may be responsible for antioxidant properties.

GC-MS is an excellent tool for the extraction, separation and identification of compounds in pure form. Eugenol is a common compound identified in both the selected plants. Eugenol essential oil extracted from plant source possess antifungal activity against *Fusarium oxysporum* [37] and synergestic effect on *Candida albicans* and *Laetiporus sulphureus* [38]. Eugenol contribute a wide range of application in food industry such as enhance the shelf life of food [39] suppress the growth of bacteria and virus [40, 41]. Lupeol has been reported to have anti- inflammatory, anti-protozoal and anti-tumour activity [42]. Phytol was found to be present in *H. colorata.* The effectiveness of phytol in different stages of arthritis has been reported [43]. All the other bioactive compounds revealed through this study justifies the use of *H. colorata* and *P. dioica* for various ailments.

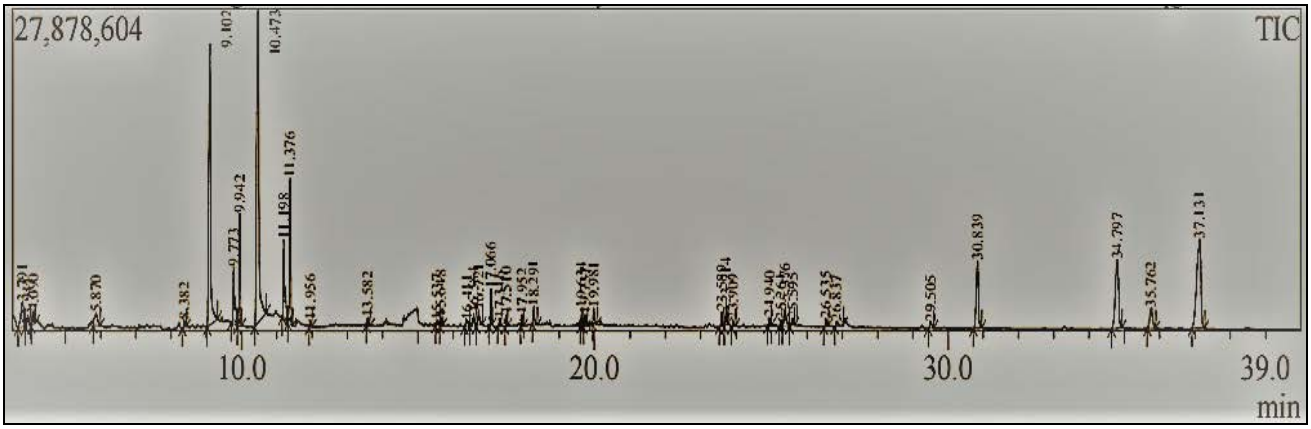

Fig. 2: GC–MS chromatogram of methanolic extract of *P. dioica* leaves

Table 5: Phytoconstituents identified from the methanolic extract of *P. dioica* leaves by GC–MS analysis.

Sl. No.	R. time	Compound name	Peek area %	Molecular formula	Molecular weight	Biological activity
1	3.791	Acetic acid	2.20	$C_2H_4O_2$	60.05 g/mol	Antibacterial
2	5.870	Beta-myrcene	1.25	$C_{10}H_{16}$	136.23 g/mol	Antioxidant, antibacterial
3	9.102	Phenol, 4-(2-propenyl)	16.97	$C_9H_{10}O$	134.17 g/mol	Antioxidant, Anti-microbial, Anti inflammatory
4	9.942	Phenol,4-(2-propenyl)-acetate	4.10	$C_{11}H_{12}O_2$	176.21 g/mol	Antimicrobial
5	10.473	Eugenol	22.97	$C_{10}H_{12}O_2$	164.2 g/mol	Antiseptic, food flavouring agent
6	11.198	3-Allyl-6-methoxyphenol	3.82	$C_{12}H_{14}O_3$	164.20 g/mol	Antiseptic, Antioxidant
7	25.376	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	1.20	$C_{21}H_{40}O_4$	356.53 g/mol	Antimicrobial
8	30.839	Alpha-tocopherol-beta-d-mannoside	4.92	$C_{35}H_{60}O_7$	592.8 g/mol	Enzyme inhibitory, anti-cancer
9	34.797	Gamma-sitosterol	7.28	$C_{29}H_{50}O$	414.7 g/mol	Anti-cancer
10	35.762	Beta-amyrin	2.03	$C_{30}H_{50}O$	426.7 g/mol	Anti-inflammatory
11	37.131	Lupeol	12.36	$C_{30}H_{50}O$	426.7g/mol	Anti-inflammatory, anticancer

Table 6: Phytoconstituents identified in the methanolic extract of *H. colorata* leaves by GC-MS analysis

Sl. no.	R. time	Compound name	Peek area %	Molecular formula	Molecular weight	Biological activity
1	3.771	Glycerin	3.88	$C_3H_8O_3$	92 g/mol	Antimicrobial, flavoring agent
2	3.886	Benzylmethyl ketone	1.52	$C_9H_{10}O$	134.17 g/mol	Precursor, manufacture of amphetamine
3	9.083	Phenol,4-(2-propenyl)-	6.08	$C_9H_{10}O$	134.17 g/mol	Antioxidant, anti-microbial and anti-inflammatory
4	10.452	Eugenol	17.28	$C_{10}H_{12}O_2$	164.2 g/mol	Antiseptic, food flavoring agent
5	11.214	3-Allyl-6-methoxyphenol	4.81	$C_{10}H_{12}O$	164.2 g/mol	Anticancer activity
6	17.077	Neophytadiene	3.18	$C_{20}H_{38}$	278.5 g/mol	Anti-inflammatory, antimicrobial
7	17.965	Hexadecanoic acid, methyl ester	1.55	$C_{17}H_{34}O$	270.45 g/mol	Antifungal
8	18.314	n-Hexadecanoic acid	2.67	$C_{16}H_{32}O_2$	256.4 g/mol	Anti-inflammatory, antioxidant, insecticidal, cytotoxic
9	19.594	(Z)-18-Octadec-9-enolide	2.10	$C_{18}H_{32}O$	280.44 g/mol	Insecticidal
10	19.650	9,12,15-Octadecatrienoic acid, methyl ester	2.91	$C_{19}H_{32}O$	292.45 g/mol	Antimicrobial
11	19.756	Phytol	3.71	$C_{20}H_{40}O$	296.53 g/mol	Precursor for synthetic vitamin E and K1
12	20.017	9,12,15-Octadecatrienoicc acid, (Z, Z, Z)-	5.81	$C_{18}H_{30}O_2$	278.42g/mol	Antibacterial, Antifungal
13	26.563	Squalene	3.14	$C_{30}H_{50}$	410.73 g/mol	Antioxidant, antitumor, pesticide
14	28.115	Desmosterol	1.44	$C_{27}H_{44}O$	384.64 g/mol	Phagocytotic activity
15	32.786	Campesterol	2.59	$C_{28}H_{48}O$	400.68 g/mol	Food additive
16	33.396	Stigmasterol	7.13	$C_{29}H_{48}O$	412.69 g/mol	Hypoglycemic, Anti ostioarthritic, antioxidant
17	34.858	Gamma. Sitosterol	6.01	$C_{29}H_{50}O$	414.7 g/mol	Anticancer

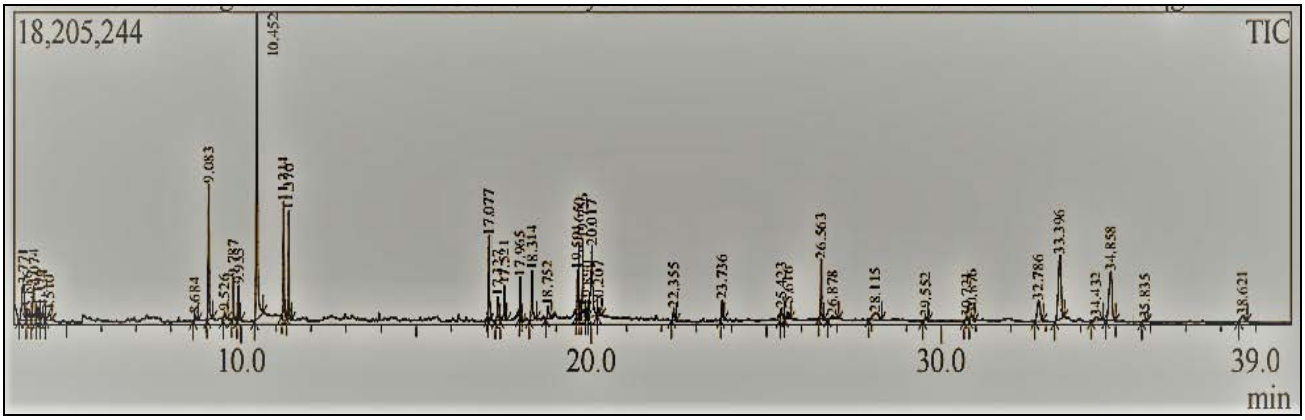

Fig 3: GC–MS chromatogram of the methanolic extract of *H. colorata* leaves

Conclusion

The present study concluded that the selected plants *H. colorata* and *P. dioica* are the source of secondary metabolites such as alkaloids, flavonoids, tannins, phenols and antioxidants. The biomolecules of these plants need to be characterized and encouraged to use against various diseases because plant natural products help to reduce the side effects. This study holds prominence in exploiting these medicinal plants as an alternative source to cure many diseases and various processing operations are urged in future. Hence, isolation, purification and characterization of active compounds of these plants need to be investigated.

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Conflict of interest

We declare that we have no conflict of interest.

References

1. Anitha VT, Marimuthu J, Jeeva S. Anti–bacterial studies on *Hemigraphis colorata* (Blume) HG Hallier and *Elephantopus scaber* L. Asian Pacific Journal of Tropical Medicine,2012:5(1):52-7.
2. Sushma MS, Lahari SL, Naidu MJ, Kavitha GK. Biological effects of Hemigraphis alternate–a review. International Journal of Indigenous Herbs and Drugs, 2020,27-30.
3. Priya MD. Review on pharmacological activity of *Hemigraphis colorata* (Blume) HG Hallier. Molecular Medicine,2012:3:1-3.
4. Sharannya Mohan, Josna George, Kannika Parameswari, Dinesh MD. A short review on Traditional wound healer *Hemigraphis colorata*. International Journal of Engineering Science Invention, 2017:6 (11):56-57.
5. Rao PS, Navinchandra S, Jayaveera KN. An important spice, *Pimenta dioica* (Linn.) Merill: A review. International Current Pharmaceutical Journal,2012: 1(8):221-5.
6. Badmanaban R, David Banji, Dhrubo Jyoti Sen, Goutham Retheesh. Nice holistic flora allspice needs not to pay much more price in spice. World Journal of Pharmaceutical and Life Sciences,2018:4(1):63-71.
7. Rao PS, Navinchandra S, Jayaveera KN. HPTLC analysis of the essential oil from *Pimenta dioica* leaf. Journal of Applied Pharmaceutical Science, 2012:2(12):141.
8. Chaudhari Shalavadi MH. Physicochemical and phytochemical screening of *Convolvulus pluricaulis* collected from Bagalkot, Karnataka. International Journal of Green Pharmacy (IJGP),2018, 12(03).
9. World Health Organization, (WHO). Final text for revision of the International Pharmacopoeia,2012; 1-2.
10. Chaudhari RK, Girase NO. Determination of soluble extractives and physicochemical studies of bark of *Sesbania sesban* (L) Merr. Journal of Chemical and Pharmaceutical Research,2015:7(8):657-60.
11. Gayathri V, Kiruba D. Fluorescence analysis of two medicinal plants-*Psidium guajava* L and *Citrus aurantium*. International Journal of Pharmaceutical Sciences and Research,2015:6(3):1279.
12. Harborne JB. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall,1973, 49-188.
13. Sandip P, Shreewardhan R, Sandeephan M, Abhay C, Debjani D. Phytochemical evaluation and free radical scavenging potential of *Hugonia Mystax* (L) leaf extract. Bionano Frontier,2014:7(2):20.
14. Price ML, Butler LG. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. Journal of Agricultural and food chemistry,1977:25(6):1268-73.
15. Laveena KB, Chandra M. Evaluation of bioactive compounds, antioxidant, and antibacterial properties of medicinal plants *Sauropus androgynus* L. and *Erythrina variegata* L. Asian Journal of Pharmaceutical and Clinical Research,2018:11:313-7.
16. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food chemistry,2003:51(8):2144-55.
17. Prieto P, Pineda M, Anguilar M. Analytical Biochemistry,1999, 269, 337.
18. Desmarchelier C, Novoa Bermudez MJ, Coussio J, Ciccia G, Boveris A. Antioxidant and prooxidant activities in aqueous extracts of Argentine plants. International Journal of Pharmacognosy, 1997:35(2):116-20.
19. Oyaizu M. Studies on products of browning reaction antioxidant activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics,1986:44(6):307-15.

20. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research,2012:5(2):90-4.
21. Vaghasiya Y, Nair R, Chanda S. Antibacterial and preliminary phytochemical and physico-chemical analysis of *Eucalyptus citriodora* Hk leaf. Natural Product Research,2008:22(9):754-62.
22. Musa KY, Katsayal AU, Ahmed A, Mohammed Z, Danmalam UH. Pharmacognostic investigation of the leaves of *Gisekia pharnacioides*. African Journal of Biotechnology,2006, 5(10).
23. Shah R, Shah R, Chanda S. Pharmacognostical and preliminary phytochemical investigation of *Tephrosia purpurea* (Linn.) Pers. root from Gujarat region. International Journal of Pharmaceutical Research,2011:3(2):49-52.
24. Moumita sanyal, Lalitharani. Pharmacognostic investigations of *Hemigraphis colorata* var. Paniculata. Society for Scientific Development in Agriculture and Technology,2014:9:836-839.
25. Gangadharan A, Nishath TK, Benny PJ. Evaluation of Phytochemical Analysis, Antioxidant and Anti-Elastase Activity of *Hemigraphis colorata*. International Journal of Pharmacognosy and Phytochemical Research, 2013:5(4):292-8.
26. Inayatullah S, Prenzler PD, Obied HK, Rehman AU, Mirza B. Bioprospecting traditional Pakistani medicinal plants for potent antioxidants. Food chemistry, 2012:132(1):222-9.
27. Ahmad I, Aqil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research,2007:162(3):264-75.
28. Montoro P, Braca A, Pizza C, De Tommasi N. Structure–antioxidant activity relationships of flavonoids isolated from different plant species. Food chemistry,2005:92(2):349-55.
29. Nithya TG, Jayanthi J, Ragunathan MG. Antioxidant activity, total phenol, flavonoid, alkaloid, tannin, and saponin contents of leaf extracts of *Salvinia molesta* DS Mitchell (1972). Asian Journal of Pharmaceutical and Clinical Research,2016:9(1):200-3.
30. Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature,1994:368(6473):753-6.
31. Antherden LM. Textbook of Pharmaceutical Chemistry. Oxford University Press, London,1969:8:813-814.
32. Mbagwu HO, Adeyemi OO. Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). Journal of Ethnopharmacology, 2008:116(1):16-20.
33. Bhattacharya A, Sood P, Citovsky V. The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. Molecular plant pathology,2010:11(5):705-19.
34. Grice HC. Safety evaluation of butylated hydroxyltoluene (BHT) in the liver, lung and gastrointestinal tract. Food and Chemical Toxicology, 1986:24:1127-30.
35. Tripathi R, Mohan H, Kamat JP. Modulation of oxidative damage by natural products. Food chemistry,2007:100(1):81-90.
36. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry,2005:53(10):4290-302.
37. Park MJ, Gwak KS, Yang I, Kim KW *et al*. Effect of citral, eugenol, nerolidol and α-terpineol on the ultrastructural changes of *Trichophyton mentagrophytes*. Fitoterapia,2009:80(5):290-6.
38. Patil SD, Sharma R, Srivastava S, Navani NK, Pathania R. Downregulation of yid C in *Escherichia coli* by antisense RNA expression results in sensitization to antibacterial essential oils eugenol and carvacrol. PLoS One,2013:8(3):57370.
39. Manrique Y, Gibis M, Schmidt H, Weiss J. Influence of application sequence and timing of eugenol and lauric arginate (LAE) on survival of spoilage organisms. Food Microbiology,2017:64:210-8.
40. Santiesteban-López A, Palou E, López-Malo A. Susceptibility of food-borne bacteria to binary combinations of antimicrobials at selected aw and pH. Journal of Applied Microbiology,2007:102(2):486-97.
41. Zhou L, Zheng H, Tang Y, Yu W, Gong Q. Eugenol inhibits quorum sensing at sub-inhibitory concentrations. Biotechnology letters,2013:35(4):631-7.
42. Gallo MB, Sarachine MJ. Biological activities of lupeol. International Journal of Biomedicine and Pharmaceutical Science,2009:3(1):46-66.
43. Nanadagopalan V, Gritto MJ, Doss A. GC-MS analysis of biomolecules on the leaves extract of *Sterculia urens* Roxb. Journal of Pharmacognosy and Phytochemistry,2015:3(6):193-6.