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Phytochemical analysis and antioxidant activities of *Homalomena aromatica* Schott.

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Abstract
Medicinal and Aromatic Plants play a significant role in economic, socio-cultural and ecological aspects of local communities all over the world. One such plant is *Homalomena aromatica* Schott. belonging to family Araceae. Its rhizome bears several medicinal properties like antidepressant, analgesic, antiseptic, anti-inflammatory, sedative, antispasmodic activities and treating joint pain. The rhizome even contains essential oil which has a high demand in perfumery and cosmetic industries. These medicinal and aromatic properties in the plant are due to the presence of certain phytochemicals and antioxidant property. The present study is carried out to ascertain the qualitative phytochemical constituents and antioxidant property of the plant. Antioxidant activity is evaluated by free radical scavenging activity of DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. Various significant bioactive compounds from the crude methanolic extract were identified and characterized by GC-MS analysis. The free radical scavenging activities were investigated based on the presence of phenols and flavonoids in the plant extract. Due to presence of various important bioactive compounds and antioxidant activity, the plant could be a significant source of modern synthetic drug production.

Keywords: Phytochemical, antioxidant, activities, *Homalomena aromatica*

Introduction
Medicinal and Aromatic Plants (MAPs) are groups of plants which are considered as valuable resources in nature for their special use as curative due to the presence of several phytochemicals. They produce a wide range of secondary metabolites and are well known sources of many novel drugs. These phytochemical compounds have significant physiological action on metabolism of the living systems. Such bioactive compounds include tannins, alkaloids, terpenoids, steroids, flavonoids, etc. [1]. As such, aromatic properties of plants are due to the presence of volatile substances which occur as essential oils, gum exudates, balsam and oleoresin in several parts of these plants such as root, rhizome, wood, bark, stem, foliage, flower and fruit. They act as raw materials for making perfumes, in cooking, pharmaceutical, liquor industries, etc. Extensive research on antioxidant activity of MAPs has gradually increased its potential benefits in disease prevention and health promotion. One such plant bearing numerous medicinal and aromatic properties is *Homalomena aromatic* Schott. The rhizome of the plant is traditionally used for treating common cold in infants and also in treating jaundice, diarrhea and asthma [2, 3]. It acts as aromatic stimulant which involve in high larvicidal activity against mosquito and also bears black-flies repellent properties [3, 4, 5]. Further, the petiole of this plant is used in curry as condiment for pleasant aroma by the people of Northeastern region of India. The extract of the whole plant is used in treating muscle weakness and rheumatism [6]. This herb is also used as blood purifier, cure for allergic eruption, treatment of boils in ear, in curing deafness, treatment for dandruff and healing of wounds in women after child birth [2].

The beneficial physiological and therapeutic effects of this plant results from the combinations of the phytochemicals present it. The information on the constituents of the plant clarifies the uses of the plants but only a small percentage have been investigated for their phytochemicals and only a fraction has undergone biological or pharmacological screening [7]. As more phytoconstituents are being identified and tested, traditional uses of the plants are being verified. Presence of many different phytochemicals also gives the hint for the presence of some antioxidant activity of the plant. Hence, the present study was aimed at screening phytochemicals and antioxidant properties of *H. aromatica* Schott.

Material and methods

Plant material

H. aromatic Schott., commonly known as '*Sugandh mantri'* (in Hindi) is a rhizomatous, perennial, terrestrial herb belonging to the family Araceae. It is a shade loving plant with short, erect, stout and tough stem growing upto an average height of 40-75cm (Fig. 1). The leaves are 18-34cm long and 14-23cm broad with long petioles and sheathing below and the leaf blades are ovate, often either cordate or sagittate in nature. The rhizome is covered with dark-brown leafy scales. The spa the is present with closely oppressed margins approximately of 7.5cm long, oblong, opening slightly above, scarcely differentiated into tube below. It is a monoecious plant with basal female flowers and male flowers at the *H. aromatic* top of the spadix. The flowers are mostly unisexual in nature and absent of perianth can be observed. The fruit are berry with few to many seeds. The seeds are small, ovoid and albuminous.

Fig 1: *Homalomena aromatica* in its natural habitat

Collection of plant material

Mature plant material was collected from Lokhra, Guwahati (26.1106° N, 91.7466° E) and North Salmara, Bongaigoan (26.3797° N, 90.6133° E), Assam, India during the month of February, 2018. The collected specimens were identified consulting the voucher specimens available in GUBH, Department of Botany, Gauhati University, India.

Preparation of plant extracts

Freshly collected plant specimens were washed thoroughly under running tap water. Individual plants were cut into smaller pieces as three different parts i.e., leaves, petioles and rhizome and allowed to dry in shade. Finally, dried materials were ground into fine powder and stored in separate containers for further use.

The powdered samples were laid for extraction in a Soxhlet apparatus for 24 hour using two different solvents viz., methanol and water. 20g each leaf and petiole powder together (total 40g) was mixed thoroughly and packed into the thimble and extraction was carried out using 400ml methanol as solvent making a ratio of 1:10 (40g : 400ml). The temperature was set to 64.7 ºC and the extract was collected after 24 hrs. Similarly, the extraction was also carried out for water (at 100 ºC). Further, extracts of rhizome for both methanol (64.7 ºC) and water (100 ºC) were also prepared. The extracts were dried using a lyophilizer and weight of extracts was calculated before and after solidification of the extracts. The condensed extracts were stored in airtight container at 4°C until and were used for the various phytochemical screening [7, 8].

Preliminary phytochemical screening

The methanolic and aqueous extracts of *H. aromatic* Schott. were laid for qualitative phytochemical screening for identification of various classes of active phytochemical constituents follows alkaloids, tannins, saponins, cardiac glycosides, sterols, flavonoids, phenolic compounds, quinones, proteins, fixed oils, starch, coumarins, gum and mucilages, phlobatannins, and terpenoids [9 - 15].

DPPH antioxidant scavenging

The free radical scavenging activity was calculated by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method [16]. DPPH solution of 0.1 mM was prepared freshly in methanol and kept away from light for 30 min and then the initial absorbance was measured at 517 nm using UV-Vis Spectrophotometer. Final concentration of standard ascorbic acid and plant extracts were made at different concentrations (0.25µg, 0.25µg, 0.75µg and 1.0 µg) were taken and the volumes were adjusted to 1ml with methanol for methanolic sample and water for water sample. The final volume to be assayed was filled by adding 1ml of methanolic DPPH solution to 1ml of different concentrations of plant extract in an eppendorf tube of 2ml. Three separate tests were performed for the sample. The tubes were allowed to incubate in dark for 30min at 27ºC. Both methanol and water was used as blank for methanolic and water samples and the experiment was expressed as the inhibition percentage (%) of free radical by the sample and was calculated using the formula follows:

$$\text{Radical Scavenging (\%)} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Where, Abs control is the absorbance of DPPH + methanol
Abs sample is the absorbance of DPPH radical + sample (i.e., extract or standard).

GC-MS analysis

GC-MS was used for identification of few major bioactive compounds from the crude methanolic extract of *H. aromatic*. GC-MS was carried out on a GC Clarus 680 & MS Clarus 600C Perkin Elmer system composed of a gas chromatograph connected to a mass spectrometer (GC-MS) instrument. The instrument type was PE AutoSystem GC with built-in autosampler. The column length was 60m and diameter was 250µm with vacuum compensation. The total run time was 56 minute and the sampling rate was 1.5625pts/s. An injection volume of 1µl was employed (a split ratio of 10:1) and helium gas was used as a carrier gas at a constant flow rate of 1ml/ min. For the Oven program the initial and maximum temperature was 60ºC and 350ºC. The initial hold and equilibration time was maintained for 3 min and 2 min respectively. The oven temperature was programmed from the initial temperature of 60ºC with an increase of 6°C/min to 200°C for 3 min hold, and then 6°C/min to 300°C for 10 min hold.

Calculation of the Yield value for each extract

The total yield value of contents after extraction process was estimated by standard method[17].

$$\text{Yield value} = \frac{\text{Extracts obtained}}{\text{Total amount of crude drug}} \times 100$$

Results and Discussion

Qualitative phytochemical analysis

Phytochemical studies carried out for plant extracts of *H. aromatica* using methanol and water as solvents showed different reactivity due to their variations in solvent polarity and different results were obtained which indicated the presence or absence of different phytochemical constituents. Methanolic rhizome extract of *H. aromatic* showed the presence of alkaloids, tannins, saponins, cardiac glycosides, phenolic compounds, phlobatannins, and terpenoids whereas flavonoids, quinones, proteins, fixed oils, starch, sterols and gums and mucilages were not detected [17].

The methanolic leaf and petiole extract showed the presence of alkaloids, tannins, saponins, cardiac glycosides, phenolic compounds, coumarins and fixed oils whereas the extract was devoid of flavonoids, sterols, proteins, quinones, starch, gums and mucilages, terpenoids, and phlobatannins.

The water extract of rhizome showed the presence of alkaloids, tannins, saponins, flavonoids, phenolic compounds, quinones, fixed oils, coumarins, gums and mucilages and terpenoids whereas starch, cardiac glycosides, sterols, phlobatannins, and proteins were absent.

Water extract of leaf and petiole showed the presence alkaloids, tannins, saponins, flavonoids, phenolic compounds, fixed oils, coumarins, gums and mucilages, and terpenoids whereas extract was devoid of cardiac glycosides, sterols, quinones, phlobatannins, proteins, and starch.

Phytochemical screening was carried out to detect the phytochemicals in the methanol and aqueous extracts of leaf and petiole mixture and rhizome. The methanolic and aqueous extract were used for qualitative phytochemical screening to detect the presence of alkaloids, tannins, saponins, cardiac glycosides, sterols, flavonoids, phenolic compounds, quinones, proteins, fixed oils, starch, coumarins, gum and mucilages, phlobatannins, terpenoids which were performed accepting the standard procedures (Table 1) [18-20].

Table 1: Preliminary phytochemical screening of extracts of *H. aromatic* ('+' for presence, '-'for absence)

Sl. No.	Phytochemical test	Reagents used (Test performed)	Inference for presence of phytochemicals	Results			
				Methanolic extract (Rhizome)	Water extract (Rhizome)	Methanolic extract (Leaf/petiole)	Water extract (Leaf/petiole)
1	Alkaloids test	Mayer's reagent	Appearance of white precipitate	-	-	-	-
		Wagner reagent	Appearance of reddish brown precipitate	+	+	+	+
2	Tannins test	$FeCl_3$ test	Formation of yellow brown precipitate.	+	+	+	+
3	Saponins test	Froth test	Formation of stable foam.	+	+	+	+
4	Cardiac Glycosides test	Salkowski test	Reddish brown color indicates the presence of a steroidal ring	+	-	-	-
		Kellar-kiliani test	Formation of brown ring at the interphase.	+	-	+	-
5	Sterols test	Salkowski test	Appearance of red color at the lower layer.	-	-	-	-
6	Flavonoids test	Lead Acetate test	Formation of reddish brown precipitate.	-	+	-	+
7	Phenolic compounds test	Lead Acetate test	Formation of bulky white precipitate.	+	+	+	+
8	Quinones test	Alcoholic KOH test	Appearance of red to blue color.	-	+	-	-
9	Proteins test	Biuret test	Appearance of pink colour in ethanoic layer.	-	-	-	-
10	Fixed oils test	$CuSO_4$ test	Appearance of blue coloration.	-	+	+	+
11	Starch test	Iodine test	Color changes to blue / violet color.	-	-	-	-
12	Coumarins test	NaOH test	Appearance of Yellow coloration.	+	+	+	+
13	Gums and Mucilage test	Ethanol test	Formation of White or cloudy precipitate.	-	+	-	+
14	Phlobatannins test	HCl test	Deposition of a red precipitate.	+	-	-	-
15	Terpenoids test	Salkowski's Test	Appearance of reddish brown color.	+	+	-	+

DPPH radical scavenging activity

In the DPPH (1, 1-diphenyl-2-picryl hydrazine) radical scavenging assay, antioxidants react with DPPH, and convert it to yellow coloured DPPH. The degree of discoloration indicates the radical scavenging activity. In this test, *H. aromatica* extract exhibited a considerable antioxidant activity but not more than the standard ascorbic acid. The antioxidant activity of ascorbic acid is highest with 60.35% DPPH scavenging at 1mg/ml concentration and IC50 value of 18.01µg/ml.

Antioxidant property analysis

It was observed that the methanolic and water extract of *H. aromatica* in both rhizome and leaf & petiole have demonstrated dose dependent increase in the DPPH radical scavenging activity. Ascorbic acid (Standard) has shown IC50 at 18.01µg/ml concentration obtained by equation (y = 1.686x + 53.74) whereas four different extract has shown variations in DPPH radical scavenging activity. The methanolic extract of rhizome has shown IC50 at 33.31µg/ml concentration obtained by equation (y = 2.905x + 48.39) whereas the methanolic extract of Leaf & Petiole has shown IC50 at 34.38µg/ml concentration obtained by equation (y = 2.969x + 46.25), the water extract of rhizome has shown IC50 at 42.83µg/ml concentration obtained by equation (y = 4.897x + 35.075), while the Water extract of Leaf & Petiole has shown IC50 at 44.41µg/ml concentration obtained by equation (y = 5.381x + 30.075) (Table 2-3; Fig. 2-3).

Table 2: Analysis of DPPH radical scavenging

Working Concentration (µg/ml)	Scavenging (%)				
	Ascorbic acid	Methanol extract (Rhizome)	Methanol extract (Leaf/Petiole)	Water extract (Rhizome)	Water extract (Leaf/Petiole)
0.25	55.18±0.6	51.09±0.3	49.40±0.4	40.17±0.7	36.34±0.9
0.50	57.47±0.2	54.43±0.2	51.89±0.5	44.18±0.5	40.36±1.0
0.75	58.82±0.3	57.26±0.3	55.21±0.1	50.55±0.4	44.52±0.8
1.0	60.35±0.7	59.83±0.5	58.19±0.2	54.37±0.05	52.89±0.5

Table 3: Concentration of extract at DPPH radical scavenging activity 50% (IC50)

Sample extract	DPPH IC50 (µg/ml)
Ascorbic acid	18.01
Methanolic extract (Rhizome)	33.31
Methanolic extract (Leaf/petiole)	34.38
Water extract (Rhizome)	42.83
Water extract (Leaf/petiole)	44.41

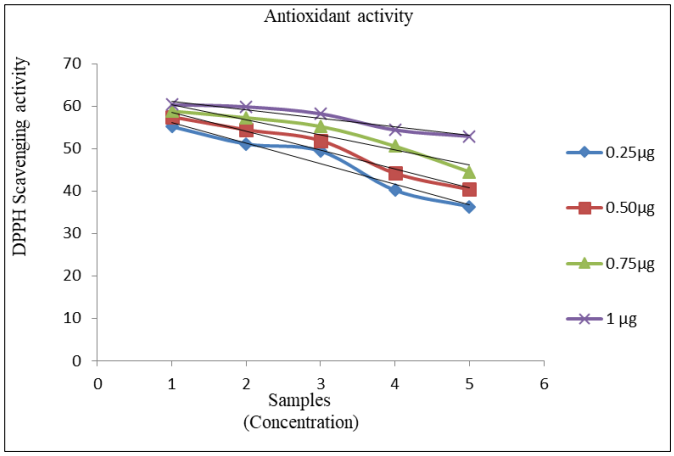

Fig 2: DPPH radical scavenging activity (%) versus *H. aromatic* concentration (µg/mL).

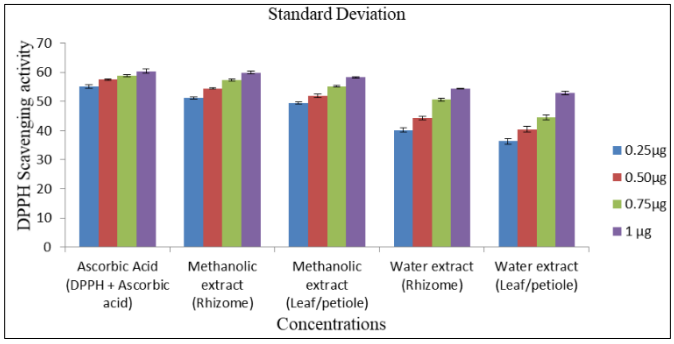

Fig 3: Standard deviation for antioxidant activity assay

GC-MS analysis

GC-MS is the best techniques to detect the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. GC-MS chromatogram analysis of the methanolic extract of *H. aromatic* showed several peaks which indicated the presence of numerous phytochemical compounds. The peak is measured from the base to the tip of the peak [21]. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. Among them some of the important bioactive phytoconstituents and their activity have been identified in methanolic rhizome and leaf and petiole extracts following the active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage (Table 4).

Table 4: Activity of important phytocomponents identified in methanolic rhizome and leaf and petiole extract of *H. aromatica* by GC-MS analysis. (RT = retention time, MW = molecular weight)

No.	RT (min)	Name of the compound	Molecular formula	MW (g/mol)	Peak Area %	Uses
1	42.277	Oleic Acid	C18H34O2	282	2.221	The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient.
2	41.016	Undecenoic/ Undecylenic Acid	C12H20O2	184	2.965	Undecylenic acid is an active ingredient in medications for skin infections, and to relieve itching, burning, and irritation associated with skin problems. For example, it is used against fungal skin infections, such as athlete's foot, ringworm, *Tineacruris*, or other generalized infections by *Candida albicans.*
3	41.218	Dichloroaceti c acid, tridec-2-ynyl ester	C15H24O2Cl2	307	47.792	Dichloroacetic acid (DCA) are used for cosmetic treatments (such as chemical peels and tattoo removal) and as topical medication for the chemoablation of warts, including genital warts. It can kill normal cells as well. Salts of DCA are used as drugs since they inhibit the enzyme pyruvate dehydrogenase kinase. Early reports of its activity against brain cancer cells led patients to treat themselves with DCA, which is commercially available in non-pharmaceutical grade.
4	50.515	Caryophyllen e	C15H24	204	35.539	Caryophyllene or (−)-β-caryophyllene, is a natural bicyclic sesquiterpene that is a constituent of many essential oils, especially clove oil.
5	50.420	Epiandrostero ne	C19H30O2	290	12.448	Epiandrosterone is a steroid hormone, well-known in the fitness world as being a great pro-hormone. Of course, it is a precursor to the popular steroid stanolone and this means that it can convert to DHT (dihydrotestosterone) in the body and help to loss the fat in the body.
6	37.421	Palmitic Acid or Hexadecanoic Acid	C16H32O2	256	2.779	Palmitic acid has many functions in cosmetics, from detergent cleansing agent to emollient. In moisturizers, palmitic acid is a very good emollient.
7	41.016	9,12-Octadecadien oic acid (Linoleic Acid)	C18H32O2	280	2.965	Linoleic acid is used in making quick-drying oils, which are useful in oil paints and varnishes. These applications exploit the easy reaction of the linoleic acid with oxygen in air, which leads to crosslinking and formation of a stable film called linoxyn. Linoleic acid has become increasingly popular in the beauty products industry because of its beneficial properties on the skin. Research points to linoleic acid's anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties when applied topically on the skin. Linoleic acid lipid radicals can be used to show the antioxidant effect of polyphenols and natural phenols.
8	41.022	4-Decenoic	C10H18O2	170	0.973	It is used as flavouring agent in different food items.

		Acid				
9	37.421	Methyl Laurate (Methyl Dodecanoate)	C13H26O2	214	2.779	Methyl dodecanoate can be used in a wide range of applications including the manufacturing of lubricants, metalworking fluids, solvents, oiling agents and many others.
10	39.367	2-Octanol	C8H18O	130	1.041	2-Octanol is mainly used as: flavor agents, low-volatility solvent; resins (paints, coatings, adhesives, inks, etc.), agrochemicals, mineral extraction, defoaming agent such as pulp & paper, oil, gas, cement, coatings, coal, etc. It can also be used as a chemical intermediate for production of various other chemicals which includes surfactants, cosmetic, plasticizers, pesticides, lubricants, fragrances.

Structures of some important compounds are as follows

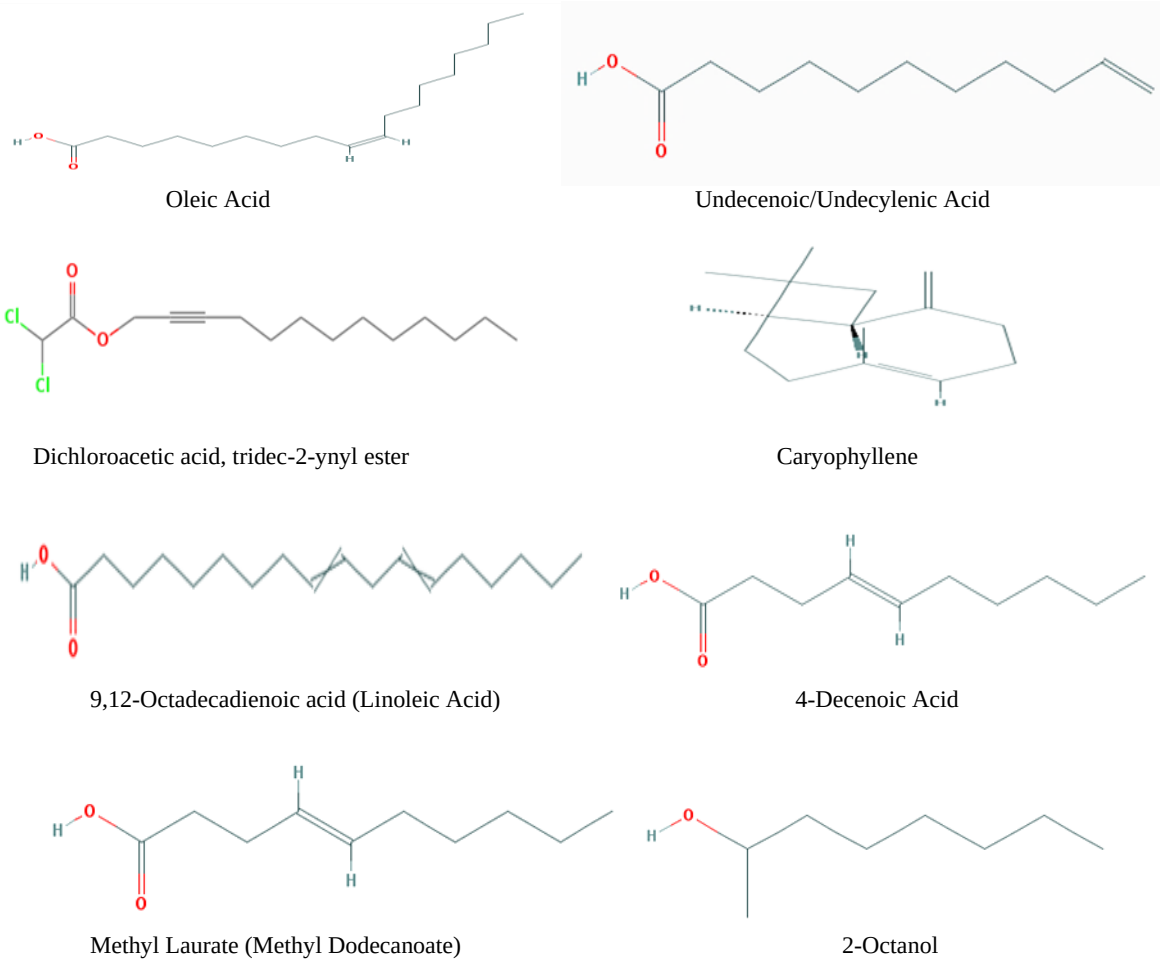

Fig 4: Structures of some important compounds identified in methanolic rhizome and leaf & petiole extract of *H. aromatica* by GC-MS

Table 5: Yield value of the GC-MS analysis

Plant parts used	Rhizome		Leaf and petiole	
Solvent used	Methanol	Water	Methanol	Water
Weight before solidification	128.81g	207.17g	67.16g	226.26g
Weight after solidification	12.32g	2.91g	5.12g	9.41g
Yield value	30.80%	7.27%	12.8%	23.52%

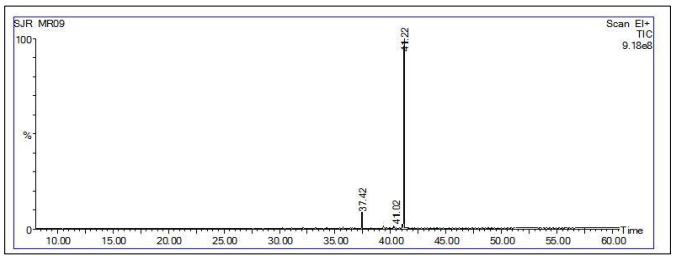

Fig 5: GC-MS chromatogram of methanolic rhizome extract of *H. aromatica*

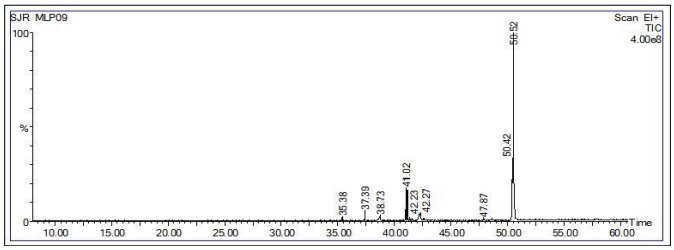

Fig 6: GC-MS chromatogram of methanolic leaf/petiole extract of *H. aromatica*

The plant kingdom has proved to be an important source material for therapeutic uses, such as treatment of various diseases and thus they are known to serve as an important source for most of the world's pharmaceuticals. In present time, the use of traditional medicinal plants and herbal drugs

are generally increasing. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances includes tannins, alkaloids, terpenoids, steroids and flavonoids [22, 23]. Following this important perspective, the investigations were carried out on phytochemical and antioxidant activity analyses of the plant *Homalomena aromatica* Schott. In the present investigation, the phytochemical analysis of methanol and water extracts of rhizome and leaf/petiole extracts of *H. aromatica* showed the presence of various groups of secondary metabolites such as alkaloids, tannins, saponins, cardiac glycosides, flavonoids, phenolic compounds, quinones, fixed oils, coumarins, gums and mucilages, phlobatannins, and terpenoids which are potential source of diverse range of medicines or important bioactive compound for human benefits. By GC-MS analysis, several compounds were detected but some of the significant individual compounds were identified which posses medicinal and other economic values. Some of the identified major biochemical compounds through GCMS analysis are Oleic acid, Undecenoic acid, Dichloroacetic acid, 2-Octanol, Caryophyllene, Methyl Laurate (Methyl Dodecanoate), 4-Decenoic acid, 9, 12-Octadecadienoic acid (Linoleic acid), Palmitic acid or Hexadecanoic acid, Epiandrosterone. Phenolic compounds plays significant role in cancer prevention and treatment. Most of the phenolic compounds are derived from the medicinal herbs and dietary plants which mostly include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects). Presence of phenolic compounds and its derivatives at an optimum level suggest the analysis of antioxidant assay from the crude methanol and water extract of *H. aromatica.* The methanolic and water extracts of *H. aromatica* were analyzed for their possible antioxidant activities using DPPH (1, 1-diphenyl-2-picryl hydrazine) radical scavenging assay, taking ascorbic acid as standard at various different concentration. In this assay, *H. aromatica* extract exhibited a considerable amount of antioxidant activity but not more than the standard ascorbic acid. The methanolic extract (rhizome) has showed IC50 value at 33.31µg/ml concentration which possess higher inhibition property than the methanolic extract (leaf and petiole) showed IC50 value at 34.38µg/ml, Water extract (Rhizome) showed IC50 value at 42.83µg/ml, and Water extract (leaf and petiole) showed IC50 value at 44.41µg/ml. While the IC50 value of standard ascorbic acid was found to be 18.01µg/ml concentration. The present antioxidant analysis showed that this plant possess moderate antioxidant activity and can be considered as a good source of natural antioxidant and should be further analyzed for their hidden chemical and biological effects [24-26].

Conclusion

Medicinal and aromatic plants represent a treasure repository of structurally diverse bioactive molecules. Most of the best plant medicines are the amalgamation of their constituents. The beneficial physiological and therapeutic effects of plant materials typically result from the combinations of these secondary phytochemicals or the metabolites present in the plant. The information on the constituents of the plant clarifies the uses of the plants but only a small percentage have been investigated for their phytochemicals and only a fraction has undergone biological or pharmacological screening. As more phytoconstituents are being identified, characterized and tested. As a result of which the traditional ethnomedicinal uses of the plants are being verified. Through this study it could be concluded that the phytochemical and antioxidant property analyses of *Homalomena aromatica* Schott. would provide significant information for further identification and characterization of many unrevealed active phytochemical constituents which may be responsible for the various medicinal, aromatic properties and antioxidant activity of the plant.

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