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Phytochemical screening and *in vitro* antioxidant activity of ethanolic extract of *Homalomena aromatica* (Araceae) root

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ABSTRACT

Ethanolic extract of Homalomena aromatica root was selected to evaluate their in vitro antioxidant potential. Antioxidant potential of the plant part was estimated by different in vitro antioxidant assay procedures and their EC_{50} values were calculated. Screening of phytochemical constituents and high performance thin layer chromatography and high performance liquid chromatography analysis of the ethanolic extract of H. aromatica were done for identification of some functional compounds in the plant. The ethanolic extract of H. aromatica exhibited maximum total antioxidant activity at 9 µg/ml and 20 µg/ml for all the seven models of free radical scavenging activity. At a concentration of 9 and 20 µg/ml of the plant extract, superior free radical scavenging activity was recorded. The extract showed the presence of phenolics and flavonoids; the reducing power of the extract increased linearly with concentration. The results of the present study showed that the crude ethanolic extract of H. aromatica contains high amount of phenolics and flavonoids which is also confirmed by HPTLC and HPLC analysis. In conclusion, the plant might be an alternative to the synthetic Antioxidants available in the market.

Key words: Antioxidant activity, *Homalomena aromatica*, DPPH, FRAP, ABTS.

INTRODUCTION

In recent years, there is an increasing interest in the antioxidants. The main reason for this interest is the protection of cells, their organelles (especially membranes) and metabolic pathways against oxygen free radicals and their reactive derivatives (ROS) [1]. Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which react with other molecules by taking or giving electrons, involved in many pathological conditions [2] and various physiological processes such as ageing, cancer, diabetes and atherosclerosis to name a few. Several studies have demonstrated that plant(s) produce potent antioxidants [3]. The most common reactive oxygen species (ROS) include superoxide (0^{2-}) anion, hydrogen peroxide (H_20_2), peroxyl (ROO^-) radicals, and reactive hydroxyl (OH^-) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO) [4]. Under normal state of affairs, the ROS generated are detoxified by the antioxidants nearby in the body and there is symmetry between the ROS generated and the antioxidant present. However, due to ROS over production and/or derisory

antioxidant argument, this equilibrium is hindered favoring the ROS gain that culminates in oxidative hassle. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA [5]. Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the expediency of antioxidants in protection against these diseases is defensible. Epidemiological studies have brought into being that the intake of antioxidants such as vitamin C reduces the risk of coronary heart diseases and cancer [6]. Since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radical damage and extensive lysis, scavenging and diminishing the formation of oxygen-derived species are not 100% efficient, micro nutrients or antioxidants taken as supplements are particularly important in diminishing the cumulative oxidative damages. Among currently available drugs, synthetic drugs do have potential adverse reactions and which can be minimized to a greater extent through natural compounds. Still there are many natural drugs which are yet to be explored scientifically [7, 8].

Homalomena aromatica (Sprengel) Schott belongs to the family of Araceae and commonly known as "Sugandhmantri" (vernacular name). It is a rhizomatous perennial herb found in Assam, Chittagong hill of Bangladesh and Jampui in Tripura. It has also been reported from the foothills of Arunachal Pradesh, Nagaland, Mizoram and Manipur [9]. It is one of the common aromatic rhizomatous (semi-)aquatic herbs in North East (NE) India. Its geographical distribution is restricted to Guangxi and S. Yunnan region of China to Bangladesh through NE India, Laos and N. Myanmar to Vietnam through N. Thailand. *Homalomena aromatica* also provides significant protection against carbon tetrachloride induced hepatotoxicity in rats. Therefore in developing countries like India, it can be used as alternative to Silymarin in preventing as well as treatment of liver disorders as they are safe and cost effective [10]. In the entire NE India the aroma of rhizomes of *H. aromatica* is traditionally used for treating common cold of infants. Petiole of the plant is also used in curry as condiment for the pleasant scent by the people of this region. Presently, the commercial cultivation of this species is gaining popularity for its essential oil extracted from rhizomes [11]. Despite of its aroma and essential oil, the species has some other ethnobiological utility among the people of Assam. Its rhizome serves as good source of nutrition and is used for treating stomach problem, jaundice, stomach pain and diarrhea. In Chinese medicines also the aromatic rhizomes of all available species of Homalomena are used medicinally to treat injuries, fractures, joint and muscle pains, stomach pains, lumbago, intestinal parasites etc. and associated with liver and kidney meridians [12].

Three new sesquiterpene alcohols, 1-β, 4-β, 7-∞- trihydroxyeudesmane, homalomenol A and homalomenol B were isolated from the roots of this plant and it has also anti-inflammatory, anti-gastric ulcer and anti-microbial activities, relaxing and calming effects [13]. In the present study, preliminary phytochemical testing showed the presence of high amount of flavonoids and phenolics content. This has prompted us to study the free radical scavenging activity of ethanolic extract of *H. aromatica* root, which may attribute to its diverse medicinal properties; moreover the plant has not been studied for its various medicinal properties except folklore claim. Literature survey revealed its work is mainly confirmed to its essential oil, but not the crude extract. Anti-ulcer and anti arthritic properties of the ethanolic root extract of the plant are also being investigated by us (Unpublished report) on the basis of its antioxidant property.

MATERIALS AND METHODS

Plant extract

Voucher specimens (IC Barua 4915 & 4922) are prepared by following the guidelines of Botanical Survey of India, poisoned with mercuric chloride and processed to deposit in the Central National Herbarium (CAL), Howrah, and the Kanjilal Herbarium (ASSAM), Shillong. The ethanolic extract of the root of *H. aromatica* (EHAR) were obtained in the month of January - February 2012 from Assam Agricultural University, (AAU), located in Jorhat, Assam, India.

Chemicals

Various chemicals *viz.* 2-Deoxy-D-ribose, butylated hydroxyanisole (BHA), 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) were obtained from Sigma Chemical. Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), EDTA, Ascorbic acid, Ammonium sulfate, polyvinyl polypyrrolidone (PVPP), Acetone, Ferrous chloride and Ferric chloride were purchased from Merck. All other reagents were of analytical grade. Solvents for HPLC, HPTLC analysis were obtained from Merck (HPLC grade).

Extract preparation
Two kg of dry plant materials was dipped in 6L ethanol in 10L round bottom flask and the mixture was mechanically stirred at room temperature for 7 days. Filtered off the solid residue and concentrated the filtrate under vacuum at 50^{o}C using rotary evaporator which gave 14.20 g of crude ethanol extract (EHAR) (Yield: 0.71%).

Estimation of half maximal effective concentration (EC_{50}) value
EC_{50} represents the amount of sample (µg extract/ml) necessary to scavenge free radicals by 50%. EC_{50} value is also the effective concentration at which the absorbance for reducing power is 0.5. Such EC_{50} value was calculated from the graph plotting inhibition percentage against extract concentration.

Preliminary Phytochemical Analysis
The extracts were subjected to preliminary phytochemical [14] testing of the rhizomes to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, saponins [15, 16, 17].

Determination of total *in-vitro* antioxidant activity
Determination of total phenolic content
Phenolic compounds are plant secondary metabolites produced either from phenylalanine or from its precursor shikimic acid [18]. The antioxidant potential of phenolic compounds has been shown in a number of *in-vitro* studies. They are capable of direct chain breaking antioxidant action by radical scavenging. In addition to having potential for independent antioxidant action, phenols have been suggested to spare essential antioxidants [19]. Total phenolic content is expressed at 765 nm using spectrophotometer (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300) at 0.1mg/g tannic acid equivalent using the following equation based on calibration curve, y= 0.1216 x where x=absorbance and y =tannic acid equivalent (mg/g) [20].

Determination of total flavonoid content
Aluminum chloride method was used for the determination of the total flavonoid[21] content of the extracts. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm and was recorded using spectrophotometer (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300). The concentrations of Total flavonoid content was calculated as quercetin mg/g using the following equation based on calibration curve, y=0.0255x where, x was absorbance and y, was the quercetin equivalent (mg/g).

Free radical scavenging activity
DPPH assay
The scavenging activity for DPPH radicals by EHAR was measured by the following method [22]. Assays were performed in 300 mL reaction mixtures, containing 200 mL of 0.1mM DPPH ethanol solution, 90 mL of 50mM Tris-HCl buffer (pH 7.4), and 10 mL of ethanol (as solvent blank) or test plant extracts and ascorbic acid were used as positive controls. After 30 min of incubation at room temperature, absorbance (540 nm) of the reaction mixtures was taken by UV spectrophotometer (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300). All determinations were performed in triplicate. The inhibitory effect of DPPH was calculated according to the following formula:

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Absorbance}_{control} - \text{Absorbance}_{sample})}{\text{Absorbance}_{control}} \times 100$$

EC_{50} was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition.

Superoxide radical scavenging activity
Superoxide anion scavenging activity of plant extracts was measured [23] with some modifications. The assay is based on the inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion. All the solutions are prepared in 100mM phosphate buffer (p.H 7.4). To 1ml of Nitroblue tetrazolium (NBT) (156mM) and 1ml of reduced Nicotinamide adenine dinucleotide(NADH) (468mM) 3 ml of plant extract (3-110 µg/ml) is mixed. The reaction was started by adding 100 µL) of phenazine methosulphonate (PMS) (60 µM) and the mixture was incubated at 25◦C for 5 minutes, measurements of absorbance was done at 560 nm (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300). The percentage inhibition was calculated.

Nitric Oxide (NO) Radical scavenging activity
Sample of various concentration were used to determine their effect on the NO radical scavenging activity [24] using sodium nitroprusside generating NO system compared with their parent compound. The Griess reagent (1% sulfanilamide 2% O-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride) is added to sample which stoichiometrically reacts to form a chromophore whose absorbance was measured at 546nm using UV spectrometry (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300). Ascorbic acid was used as standard.

Hydroxyl radical scavenging activity
The scavenging activity for hydroxyl radical was measured by studying the competition between deoxy ribose and test extract for the hydroxyl radical generated by Fenton's reaction [25]. Reaction mixture of the plant extract of different concentration reacting with 2-Deoxy –D- ribose (28 mM), EDTA (1.04 mM), $FeCl_3$ (0.2 mM) and ascorbic acid (1mM) and were incubated at 37◦C for 1 hour. The damage imposed on deoxyribose due to the free radical was determined colorimetrically by measuring the Thiobarbituric acid reactive substances (TBARS).

Ferric Reducing Antioxidant Power (FRAP) Assay [23]
Various concentrations of the sample and standard solutions (0.15ml each) were taken, 25 ml of acetate buffer (pH. 3.6) and 2.5 ml of 2 Mm ferric chloride and 2.5ml of TPTZ were mixed separately and allowed to incubate at 40º C for 1 hr and then various concentration (3-110 mg/ml) of EHAR and 0.15ml each was allowed to react with 2.85 of FRAP solution for 30 minutes in the dark. Reading of the colored product was taken at 593nm (Multi GoScan, Thermo Fischer Scientific, Model No: 1119300). All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Free radical-scavenging ability by ABTS radical
The free radical-scavenging activity was determined by ABTS radical cation decolorization assay [26]. ABTS was dissolved in water to a 7 µM concentration. ABTS radical cation ($ABTS^{-+}$) was produced by reacting ABTS stock solution with 2.45 µM potassium per sulfate (final concentration) and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion, the samples containing the ABTS, solution were diluted with redistilled water to an absorbance of 0.700±0.02 at 734 nm (Thermo Fischer Scientific, Model No: 1119300) and equilibrated at 30º C. Reagent blank reading was taken. After addition of 3.0 ml of diluted ABTS solution, the absorbance reading was taken exactly 6 min after initial mixing. The results were corrected for dilution and expressed in l µM trolox per 100 g dry weight (dw). All determinations were performed in triplicate.

Reductive ability assay
Reducing power assay [27] method is based on the principle that substances which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of the ethanolic extract and standards increases with the increase in amount of sample and standard concentrations.

Analysis of extracts by HPTLC
A densitometer HPTLC of EHAR was performed for the characteristic fingerprinting profile. The standard quercetin and gallic acid (Sigma) were prepared in methanol at 1 mg/ml and 40mg/ml concentration respectively. The solutions were centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. The sample (10µl) was loaded as 8 mm band length in the 10 × 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5, (Switzerland), The sample loaded plates were kept in TLC twin trough developing chamber (after saturation with solvent vapour) and loaded on the TLC plate with the solvent system Toluene: Ethyle acetate: Formic acid (4.5:3:0.2) and chloroform: ethyl acetate: formic acid (7.5: 6: 0.3) as mobile phase. Finally the plate was kept in scanner stage and scanning was done in 254 nm.

Analysis of extracts by HPLC-DAD
The extracts were analyzed using a HPLC system Dionex (UHPLC 3000) equipped with Binary Gradient Pump, column heater and degasser online, photodiode array detector (Dionex, UHPLC 3000) and chromalon Software. Separation was achieved using a reversed phase column C18 (4.6×250mm 4 µm), PROD, ACCLAIM at temperature of 25°C. DAD detection was employed at the wavelength range between 210 and 500 nm. Samples were dissolved in the corresponding solvent of the extract at 10 mg/mL. The volume of sample injected was 20 µL using an L-7200 autosampler. The mobile phase was a mixture of Methanol:Acetonitrile:Water (60:20:20 v/v) (A) and 0.1% O-

phosphoric acid:Acetonitrile (400:600 v/v) (B) and the flow rate was 1 mL/min. The elution system was in isocratic mode.

Statistical Analysis

Triplicate analyses were performed on a number of samples, established according to the total plant samples investigated and element analysed. For an assessment of the analytical relevance of the results, standard procedures of statistical calculation were used. All numeric data were expressed as mean ± standard deviation (SD). For statistical analysis, the commercially available software package graph pad prism (Windows) was used. Mean values did not differ significantly.

RESULTS AND DISCUSSION

In the present status, there is a strong need for an effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases like cancer, cardiovascular diseases etc [28]. They are also involved in autoimmune disorders like rheumatoid arthritis, cancer etc. Therefore, research for the determination of the natural antioxidants source is significant.

The total Phenolic and flavonoid content

Phenolic compounds are known as powerful chain breaking antioxidants because of their scavenging ability due to their hydroxyl groups [29, 30]. The phenolic compounds may contribute directly to antioxidative action [31]. The aluminium chloride colorimetric method uses wavelength scan of the complexes of the sample and standard with aluminum chloride showed that the complexes formed by flavonoids (quercetin) with C-3 or C-5 hydroxyl group [32] revealing total flavonoids content in the extract. Flavonoids are the most ubiquitous groups of plant secondary metabolites. This class of compounds has good antioxidant potential and their effects on human nutrition and health are considerable. Phenols are the major plant compounds with potential antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. It was known that plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities [33,34].Total phenol and total flavonoid content of the ethanol extract was significantly higher in EHAR, i.e. 9.00±0.001 (mg tannic acid/g of dry plant material) $\times 10^{-3}$ and 7.00±0.001(mg quercetin/g of dry plant material) $\times$ 10^{-3} respectively. This was further confirmed by DNA finger printing using HPTLC and HPLC method.

***In vitro* free radical-scavenging activity**

In the present study, seven different methods were used successfully for the evaluation of the antioxidant potential of the extract viz. DPPH˙ radical-scavenging assay, Superoxide radical scavenging activity, Nitric Oxide Radical scavenging activity, Reducing ability on FRAP, hydroxyl radical scavenging activity and ABTS radical scavenging activity. Several publications have reported the relationship between a high phenolic content and antioxidant activity [36, 37] and this correlation was confirmed in this study. Moreover, different methods used in this study also indicate its diverse antioxidant properties. The percentage of total antioxidant activity of ethanolic extract of EHAR was estimated and the results are presented in Fig. 1.

Table 1. Ferric ion reducing antioxidant power of standard ascorbic acid and ABTS radical scavenging activity with EHAR at different concentrations (µg/ml)

Concentration	Reductive Inhibition (EHAR)	Reductive Inhibition (Ascorbic acid)	Concentration	ABTS radical scavenging activity of (EHAR)
0.1	164.40 ± 0.46	176.60 ± 3.33	1	5.79 ± 3.88
0.5	174.20 ± 0.15	181.00 ± 0.56	2	6.29 ± 2.11
1	177.20 ± 0.15	186.00 ± 0.57	3	11.43 ± 2.25
2	241.20 ± 5.90	189.00 ± 0.57	4.5	12.30 ± 1.20
3	247.50 ± 4.78	190.30 ± 0.33	9	14.81 ± 3.32
9	322.30 ±0.15	403.30 ±1.66	20	12.6 ± 3.33

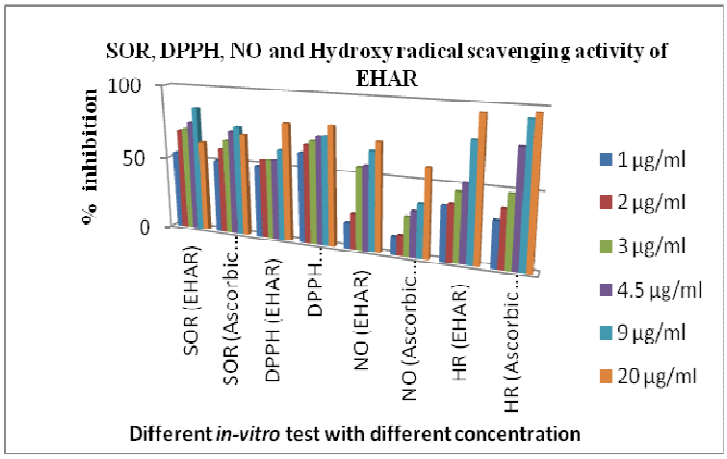

Fig. 1. SOR, DPPH, NO and Hydroxy radical scavenging activity of EHAR and standard ascorbic acid at different concentrations (µg/ml)

Table 2: Reductive ability activity of EHAR and standard ascorbic acid at different concentrations (µg/ml)

Concentration (µg/ml)	Reductive ability inhibition (EHAR)	Reductive ability inhibition (ascorbic acid)
100	0.187 ± 0.64	0.34 ± 0.40
200	0.147 ± 0.01	0.90 ± 0.71
300	0.160 ± 0.20	1.25 ± 1.02
400	0.165 ± 0.02	2.05 ± 0.55
500	0.214 ±0.03	2.45 ± 0.18
600	0.461 ± 0.17	2.51 ± 0.12

Free radical scavenging activity (DPPH Assay)

The radical scavenging activity of EHAR extract was maximum with 79.9±2.91% scavenging activity for 20 µg/ml extract (Fig 1). The EC_{50} value was 1.30 µg/ml. These results indicated that extract exhibited the ability to quench the DPPH radical at 20 µg/ml.

Superoxide radical scavenging activity

Superoxide anion is also very harmful to cellular components [23]. Robak and Glyglewski (1998) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. As shown in Fig. 1, the superoxide radical scavenging activities of the plant extract increased markedly at a concentration of 9 µg/ml was 85.3 ± 2.02 of the plant extract. The EC_{50} value was 0.94 µg/ml. The results suggest that the plant extract is a more potent scavenger of superoxide radical.

Nitric Oxide (NO) Radical scavenging activity

It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas, chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis etc. The toxicity of NO increases greatly when it reacts with superoxide radical, forming the highly reactive peroxynitrite anion ($ONOO^-$) [38, 39]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study showed that the extract has more potent nitric oxide scavenging at a concentration of 20 µg/ml, was found to be 72.6±0.88 of the plant extract (Fig 1), the EC_{50} values was 2.9 µg/ml. which is greater than the standard ascorbic acid.

Hydroxyl radical scavenging activity

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins. In the present study, the hydroxyl radical-scavenging effect of the extract, in a concentration of 9 µg /ml, was found to be 95.2±1.22%

(Fig 1). The EC_{50} value was found to be 1.70 µg/ml. Hence, the extract can be considered as a good scavenger of hydroxyl radicals.

Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant potential of EHAR was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the extract and ascorbate at various concentrations (0.1, 0.5, 1, 2, 3, 9 µg/ml) were examined and the values are presented above in Table 2. The maximum reducing ability at 20 µg/ml for plant extract and ascorbate was 322.3±0.15% and 403.3±1.66% respectively.

Free radical-scavenging ability by ABTS radical

ABTS is a blue chromophore produced by the reaction between ABTS and potassium per sulfate. Addition of the plant extract to this pre-formed radical cation reduced it to ABTS in a concentration-dependent manner. The results were compared with trolox standard curve (R^2= 0.9586) and the activity was found to be increased in from 5.79% to 14.81% at a concentration of 1-9 µg/ml. Therefore, the result also demonstrates that the extract is a potent antioxidant (Table 3). The ethanolic extract of EHAR exhibited a maximum total antioxidant activity at 9 µg/ml and 20 µg/ml for all the seven models of free radical scavenging activity. Hence in this concentration range, the plant extract possessed higher free radical scavenging activity.

Reductive ability assay

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of extract. Greater absorbance at 700 nm indicated greater reducing power. Table 4 represents the reductive capabilities of the ethanolic extract of EHAR. In the concentration range, all the extracts demonstrated reducing power that increased linearly with concentration at 100, 200, 300, 400, 500, 600 µg/ml. The reducing power of the extract might be due to their hydrogen-donating ability. Possibly, the plant contains high amounts of reductone, which could react with radicals to stabilize and terminate radical chain reactions.

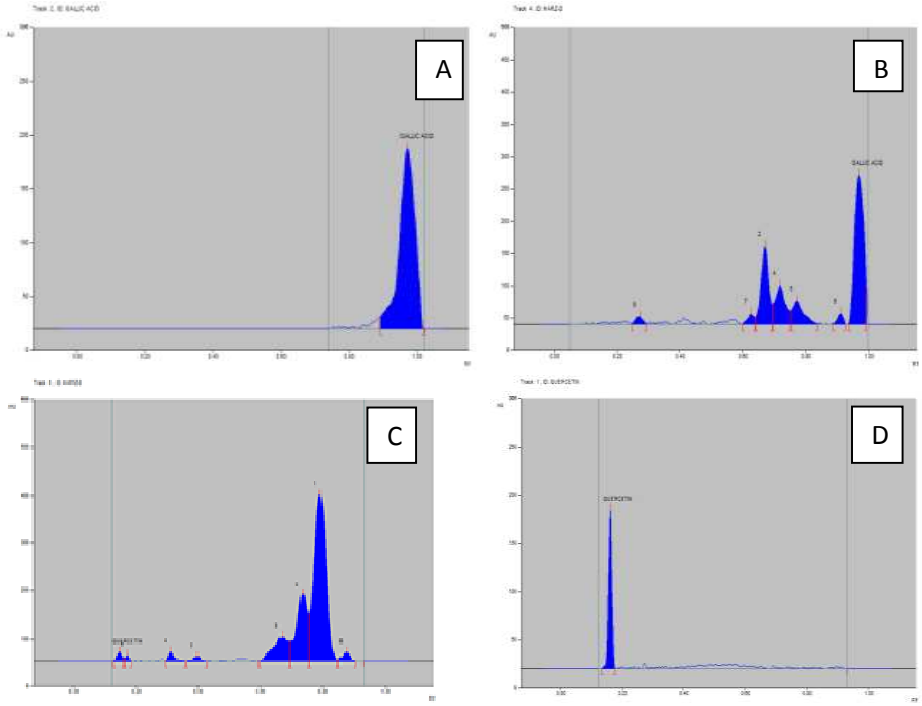

Fig 2. HPTLC and digital scanning phytochemical finger prints for ethanol extract of EHAR showing the presence of Quercetin (A) and Gallic acid (C), Standard Quercetin (B) and standard Gallic acid (D) taken at 254 nm wavelength

HPTLC analysis

When comparison was made with standard flavonoid quercetin and phenolic content, gallic acid peaks shows match with the standards. Standard quercetin (1mg/ml) and gallic acid (10mg/ml) showed single peak in HPTLC

chromatogram (Fig 2). The mobile phase consists of Toluene:Ethyle acetate:Formic acid (4.5:3:0.2) and Chloroform:Ethyl acetate:Formic acid (7.5:6:0.3) gave Rf values of 0.16 and 0.97 for quercetin and gallic acid respectively. In HPTLC profile, eight peaks were visible in the Fig-8.A and seven peaks were visible in the Fig 2 and Table 5. In Fig 2 A, Peak 1 shows R_f of 0.16 with area of 327.9 were calculated, in Fig 2 C, Peak 7 shows R_f of 0.97 with area of 5045.8 were calculated. From this data, the content of quercetin and gallic acid which were present in EHAR, showed to be 1.90% and 50.01% respectively. Other peaks in the HPTLC chromatograms indicated the presence of other chemical constituents. The data are shown in Table 3 (A, B, C, D).

Table 3. Chromatographic profile of ethanol extract of EHAR at mobile phase Toluene:Ethyle acetate:Formic acid (4.5:3:0.2) (A) and at mobile phase chloroform:ethyl acetate:formic acid (7.5:6:0.3) (C), Standard Quercetin (B) and Gallic acid (D)

A

Peak	R_f value (min)	Peak area (AU)	Area %	Assigned substances
1	0.16	327.9	1.90	Quercetin
2	0.17	171.8	1.00	P6
3	0.32	405.9	2.35	P4
4	0.40	278.2	1.61	P7
5	0.67	2016.4	11.69	P3
6	0.74	3640.1	21.11	P2
7	0.79	9934.8	57.61	P1
8	0.88	470.1	2.73	P5

B

Peak	R_f value (min)	Peak area (AU)	Area %	Assigned substances
P1	0.16	1313.8	100	Quercetin

C

Peak	R_f value (min)	Peak area (AU)	Area %	Assigned substances
P1	0.97	2170.4	100	Gallic acid

D

Peak	R_f value (min)	Peak area (AU)	Area %	Assigned substances
1	0.27	195.4	1.94	9
2	0.63	231.5	2.29	8
3	0.67	2130.0	21.11	7
4	0.72	1373.6	13.61	2
5	0.77	904.1	8.96	4
6	0.91	209.4	2.08	5
7	0.97	5045.8	50.01	Gallic acid

HPLC analysis

Reversed-phase column C18 and mobile phase used by high-performance liquid chromatography diode-array detection (HPLC DAD) were appropriate to the characterization of the ethanolic extract. Fig. 3 shows chromatograms at 254 nm of these extract, which contain flavonoids and phenolic acid derivatives in different proportions. Chromatographic analysis showed the presence of phenolic acid (gallic acid) and flavonoid (quercetin) in the ethanolic extract of EHAR in Fig. 3 (A) and (B) respectively. Gallic acid showed the RT at 2.85 while quercetin showed its RT at 3.31.

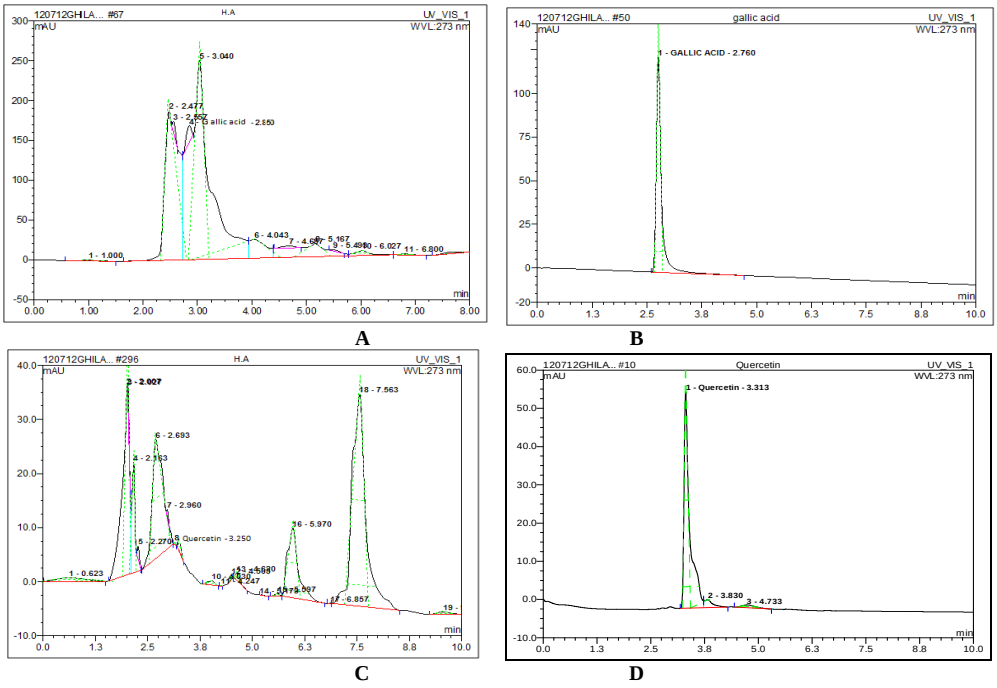

Fig 3. Chromatograms of the extracts of EHAR and standards by HPLC-DAD method at 254 nm. (A) Extract showing phenolic acid; (B)Standard gallic acid; (C) Extract showing flavonoid; (D) Standard quercetin

H. aromatica rhizomes are rich source of essential oils, was isolated and subjected to gas chromatography-mass spectrum (GC-MS) analysis [38]. Fifty-five chemical constituents were reported from *H. aromatica* rhizomes of which T-murolol (5.32%), viridiflorol (3.69%), α-selinene (2.19%), M-cymene (2.19%) and γ-Muurolene (1.81%) were identified and reported for the first time. Other major components were identified as linalool (62.5%), terpene-4-ol (7.08%), δ-cadinene (5.57%), α-cadinol (3.71%) and spatulenol (1.81%)[38]. It's essential oil showed high antimicrobial activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum fulvum*, *Microsporum gypseum*, *Trichosporon beigelii* and *Candida albicans* [39]. All these activities are attributed to its phytochemical and antioxidant properties. Polyphenols and flavonoids are the plant derived secondary metabolites, which significantly confer protection against development and progression of many chronic pathological diseases including cancer, diabetes, cardio-vascular problems and aging via their antioxidant activity [40] since flavonoid, phenolic compounds are the major contributors to the antioxidant activities of plants [41]. EHAR showed the presence of flavonoid and phenolics according to the HPTLC and HPLC data shown in Fig. 2 and Fig. 3.

CONCLUSION

In conclusion, the results of the present study showed that the crude ethanolic extract of EHAR contains high amount of phenolics and flavonoids which is also shown by HPTLC and HPLC analysis. A positive and significant correlation existed between antioxidant activity and total phenolics. Subsequently, it showed a good antioxidant activity and might be an alternate to synthetic antioxidants available in the market. The highest antioxidant activity was observed at a concentration of 9 µg/ml and 20 µg/ml which is significant for the entire free radical scavenging assays, thus this concentration range might be the optimal dose for *in vitro* study. The present study suggests that the ethanolic extract of root of *H. aromatica* might be a potential source of natural antioxidant. Hence, its beneficial effect on animal and human health may be derived from its antioxidant properties to afford protection against various diseases as mentioned above. We have found significant anti inflammatory and anti ulcer activity in the ethanol extract of EHAR in another study which is due its phytoconstituents flavonoid as detected in our present study. It's *in vivo* antioxidant and anti ulcer activity has been investigated with ethanol extract. Based on these studies we are now aiming to isolate its active principle and further isolation of pure compound.

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REFERENCES

[1] AK Tiwari. *Current Science*, **2001**, 81, 1179–1187.
[2] DL Madhavi; SS Deshpande; DK Salunkhe, *Food antioxidants: Technological, Toxicological. Health perspectives*, Marcel Dekker, New York, **1996**, pp. 267-359.
[3] S Khlifi; YE Hachimi; A Khalil; N Es-Safi; A Belahyan; R Tellal; E Abbouyi. *Indian Journal of Pharmacology*, **2006**, 38, 276-280.
[4] DA Joyce. *Advance Drug Reaction Bulleti*, **1987**, 127, 476–479.
[5] JL Farber. *Environmental Health Perspective*, **1994**, 102, 17-24.
[6] R Marchioli; C Schweiger; G Levantesi; L Tavazzi; F Valagussa. *Epidemiological and clinical trial data Lipid*, **2001**, 36, 53-63.
[7] T Devaki; K Shivashangari**.** *Journal of Natural Remedies*, **2005**, 5, 102-107.
[8] Z Hamzah; C Peter; M Mashhor. *Gardens' Bulletin Singapore*, **2012**, 64, 523–527.
[9] B Dutta; M Lahkar. *International Journal of Pharmacy and Pharmaceutical Science*, **2013**, 5, 975-1491.
[10] KR Kirtikar, BD Basu. *Indian Medicinal Plants*, **2004**, 4, 2619-2620.
[11] DB Deb. The Tripura state, Today & Tomorrows Print Publication. New Delhi. **1983**; 12: 399-400.
[12] TV Sung; B Steffan; W Steglich; G Klebe; G Adam. *Homalomena aromatica*. *Phytochemistry*, **1992**, 31, 3515-3520.
[13] AJ Das; R Kumar; M Athar; DS Rawat. *International Research Journal of Pharmacy*, **2013**, 4, 146-149.
[14] JB Harborne. Phytochemical Screening – Guide to modern techniques of plant analysis, 2nd ed., Chapman and Hall, New York, **1991**, pp. 653.
[15] KR Khandelwal. Practical Pharmacognosy, 2nd ed., Nirali Prakashan**,** Pune, India, **2009**, pp 149-156.
[16] CK Kokate. Practical Pharmacognosy, 4th ed., New Gyan Offset Printers, Delhi, India, **2000,** 107-109.
[17] A Kumar; R Ilavarasan; D Jayachandran; P Aravindhan. *Pakistan Journal of Nutrition*, **2009**, 8, 83-85.
[18] A Nurmi. Antioxidant studies on selected *Lamiaceae* herbs *in vitro* and in humans, Yliopistopaino University press, Helsinki, Finland, **2008**, 10, pp. 952-978.
[19] K Wolve; X Wu; R Spitz. *Journal of Natural Cancer Institute*, **2003**, 95, 1211-1218.
[20] E Ordon; J Gomez; M Vattione; M Isla. *Food Chemistry*, **2006**, 97, 452-458.
[21] A Cotelle; JL Bernier; JP Catteau; J Pommery; JC Wallet; EM Gaydou. *Free Radical Biology and Medicine*, **1996**, 20, 35.
[22] R Robert; P Nicoletta. *Free Radical Biology and Medicine*, **1999**, 26, 1231–1237.
[23] N Sreejayan; N Rao. *Journal of Pharmacy and Pharmacology*, **1997**, 49, 105.
[24] I Benzie; J Strain. *Analytical Biochemistry*, **1996**, 239, 70-76.
[25] E Kunchandy. *International Journal of Pharmacognosy*, 1990, 58, 237.
[26] J Robak; R Gryglewki. *Biochemical Pharmacology*, **1998**, 37, 837.
[27] PS Policegoudra; S Goswami. *Journal de mycologie médicale*, 2012, 22, 83-87.
[28] L Aruoma. *Journal of American Oil and Chemical Society*, **1998**, 75, 199–212.
[29] F Shahidi. *Critical Review of Food Science Nutrition*, **1992**, 32, 67–103.
[30] T Hatano; R Edamatsu; M Hiramatsu; A Mori; Y Fujita; A Yasuhara. *Chemical Pharmaceutical Bulletin*, **1989**, 37, 2016–2021.
[31] PD Duh; YY Tu; GC Yen. *LMT- Food Sci. Tech, Lebnesmittel-Wissenschaft und Technologie*, **1999**, 32, 269–277.
[32] C Chang; M Yang; H Wen. *Food and Drug Analysis*, **2002**, 10, 178-182.
[33] YS Velioglu; G Mazza; L Gao; BD Oomah. *Journal of Agricultural Food Chemistry*, **1998**, 46, 4113-4117.
[34] I Ismail; M Hassan. *African Journal of Biotechnology*, **2011**, 10, 601-609.
[35] MC Gutteridge. *Biochemical Journal*, **1984**, 224, 761−767.
[36] DA Joyce. *Adverse Drug Reaction Bulletin*, **1987**, 127, 476–479.
[37] S Anandjiwala; MS Bagul; M Arabia; M Rajani. *Indian Journal of Pharmaceutical Science*, **2008**, 70, 31-35.
[38] K Jayprakash; R Singh. *Journal of Agriculutral Food Chemistry*, **2001**, 55, 1018.
[39] BP Kanti; SR Ibrahim. *Oxidative Medical Cell Longevity*, **2009**, 2, 270–278.

[40] MA Abdulla, FH Bayaty, LT Younis. *Journal of Medicinal Plants Research*, **2010**, 4, 1253–1259.
[41] RJ Oliveira; GR Souza. *Journal of Young Pharmacists*, **2013**, 5, 102-105.