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Research Article

Antioxidant Activities of the Medicinal Plants Used for Preparation of Fermentation Cakes of "Haanj", the Rice Based Alcoholic Beverage of Ahom Community People of Assam, India

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ABSTRACT

Eight plant species (*Selaginella sp*., *Ficus bhotanica*, *Lygodium microphyllum*, *Ipomoea cymosa*, *Melastoma malabathricum*, *Naravellia zeylanica*, *Glochidion arborescens* and *Rubus ellipticus*) used in folk medicines and also for the preparation of fermentation cakes of "Haanj", the rice based alcoholic beverages of Ahom community people of Assam, India, have been screened with respect to their antioxidant activity and total phenolic content. Hexane, ethyl acetate and methanol extracts of each plant material have been evaluated for the antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method and ABTS [2,2′- azinobis-(3-ethylbenzothaiaziline-6- sulfonate) [method. The Folin- Ciocalteu method was employed to determine total phenolic content expressing mgGAE/g. The methanol extract of *Melastoma malabathricum* was found to contain the highest TPC (9.598 ± 0.008 mg GAE/g). This variety was also found to have the highest scavenging activity against DPPH (IC_{50}value 37.266 ±0.056 µg/ml) and ABTS method (IC_{50} value 7.924 ± 0.016 µg/ml). The results obtained in the present study indicate that all plants can be a potential source of natural antioxidant.

Keywords: Plant extracts, Antioxidant activity, DPPH, ABTS, Total phenolic content.

INTRODUCTION

There has been an increasing interest in the therapeutic potential of medicinal plants as antioxidants[1]. Consequently, natural antioxidants from teas, wines, fruits, vegetables and spices are already exploited commercially either as antioxidant additives or as nutritional supplements[2]. As substances that have the capability to neutralize free radicals, antioxidants protect human body from free radicals which, are toxic by-products of natural cell metabolism and induce health problems including coronary heart disease, cancer, gastric problems, aging etc[3,4]. Antioxidants scavenge free radicals by hydrogen atom transfer or electron transfer to free radicals, thereby inhibit the radical induced chain reaction[5]. Fruits, vegetables and medicinal herbs are rich sources of natural antioxidant and hence can prevent diseases and lower health problems. Phenolic compounds, like flavonoids are responsible for antioxidant activity of plants[6,7]. Synthetic antioxidants have been reported to be restricted due to their health risks and toxicity[8]. As a consequence, in recent years, interest in natural antioxidant, especially of plant origin, has increased enormously[9]

North East India is one of the hotspot for biodiversity and medicinal plants in the world. This region is also famous for different tribes of human being and their diverse culture. The people of this area generally use a large number of plants found locally as folk medicine for treatment of various diseases[10, 11, 12] and for preparation of their traditional alcoholic beverages. Most of the tribes in this region use selected plants for making fermentation cakes for their traditional beverages and the plants are different in different tribes and also vary from place to place. Traditionally, Ahom community people of this region also use medicinal plants, found in this region, for treatment of various diseases and some of the plants are used for preparing fermentation cakes of "Haanj", the rice based alcoholic beverage. The number and variety of the plants used by the people differ from place to place within this region for making "Haanj". We selected the most commonly used eight plants only for study. The objective of the present study was to determine the antioxidant activity and total phenolic content of the different extracts of the selected eight medicinal plants which are also used for preparing fermentation cakes for preparing Haanj. This is a part of a programme of study on the effect of these plants on the various nutritional properties of the beverage.

MATERIALS AND METHODS

Plant materials and Chemicals

The plant materials were collected from their natural habitats from different places of Assam, North East India. In the map of India, Assam lies 89º42′ Eastern to 96º Eastern longitude and 24º8′ Northern to 28º2′ Northern latitude. *Selaginella sp*., *Ficus bhotanica*, *Lygodium microphyllum*, *Ipomoea cymosa* and *Melastoma malabathricum* were collected from Dibrugarh, *Naravellia*

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Table 1: % inhibition of eight plant extracts in different solvent by DPPH method

Name of the plants with code	DPPH inhibition (%) , (mean ± SD),		
	n-hexane extract	ethyl acetate extract	methanol extract
Ficus bhotanica (Fb)	30 ± 0.12	35.58 ± 0.13	76.72 ± 0.06
Glochidion arborescens (Ga)	26.40 ± 0.25	31.37 ± 0.16	35.30 ± 0.09
Ipomoea cymosa (Ic)	21.71 ± 0.09	26.55 ± 0.19	30.15 ± 0.18
Lygodium microphyllum (Lm)	37.86 ± 0.11	57.30 ± 0.16	60.74± 0.16
Melastoma malabathricum (Mm)	60.84 ± 0.09	84.54 ± 0.23	91.93 ± 0.10
Naravellia zeylanica (Nz)	33.02 ± 0.05	49.29 ± 0.12	83.37 ± 0.07
Rubus ellipticus (Re)	35.16 ± 0.12	51.95 ± 0.07	71.44 ± 0.08
Selaginella sp. (Ss)	16.12 ± 0.39	23.21 ± 0.17	28.05 ± 0.09

Table 2: IC_{50} values of eight plant extracts in different solvent by DPPH method

Name of the plants with code	IC_{50} value , (mean ± SD),(µg/ml)		
	n-hexane extract	ethyl acetate extract	methanol extract
Ficus bhotanica (Fb)	673.91 ± 1.23	545.50 ± 0.89	242.76 ± 0.13
Glochidion arborescens (Ga)	780.91 ± 3.80	654.74 ± 5.05	569.23 ± 1.16
Ipomoea cymosa (Ic)	1012.29 ± 6.33	806.39 ± 8.51	721.19 ± 3.07
Lygodium microphyllum (Lm)	541.45 ± 2.05	350.67 ± 0.91	327.30 ± 0.43
Melastoma malabathricum (Mm)	314.51 ± 0.55	160.98 ± 1.08	37.26 ± 0.05
Naravellia zeylanica (Nz)	626.41 ± 0.42	417.57 ± 0.89	230.07 ± 0.42
Rubus ellipticus (Re)	615.08 ± 1.76	404.80 ± 0.54	276.04 ± 0.06
Selaginella sp. (Ss)	1212.51 ± 37.25	885.59 ± 7.76	724.19 ± 3.75
Trolox (Tx, reference compound)	29.19 ± 0.024		

zeylanica was collected from Dhemaji, *Glochidion arborescens* was from Sivasagar and *Rubus ellipticus* from Tinsukia, Assam, India. The voucher specimens (*Selaginella sp.*: DCH-31, *F. Bhotanica*: DCH-32, *L. microphyllum*: DCH-33, *I. Cymosa*: DCH-34, *M. Malabathricum*: DCH-35, N. zeylanica: DCH-36, *G. arborescens*: DCH-37 and *R. Ellipticus*: DCH-38 were preserved in the Department of Chemistry, Dibrugarh University, Dibrugarh, Assam, India.

The chemicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS, gallic acid, trolox were purchased from Sigma. Folin-Ciocalteu reagent and methanol were obtained from Merck. Sodium carbonate, Potassium persulphate from Rankem and all other chemicals were of analytical grade.

Preparation of Crude plant extracts

The plant materials were washed with ethanol and then shade dried for 5 to 6 days and grinded the plant materials to make coarse powder. The dried powder was extracted with hexane, ethyl acetate and methanol respectively by using Soxhlet apparatus. The extracts were concentrated under reduced pressure with the help of a rotary vacuum evaporator (BUCHI). The residues were used to determine the radical scavenging activity.

Free radical scavenging activity: DPPH Assay

The scavenging activity of DPPH by the different plant extracts was determined by a slightly modified spectrophotometric method of Brand-Williams[13]. DPPH fresh solution in methanol was prepared daily, before UV measurement. For each measurement, 200µl of DPPH solution from this stock solution was made up to 3 ml by adding methanol to make a test solution. The absorbance of the test solution was recorded at 517nm. Antioxidant activity of the plant extracts were studied by adding 100 µl, 200 µl, 300 µl and 400 µl of the stock solution of the extract to a test solution of DPPH. The solution was shaken and then kept in the dark for 30 minutes at room temperature. The reduced absorbance was measured at 517nm and was compared with a control of methanol in a UV-Visible spectrophotometer (Hitachi). The percentage inhibition of the radicals due to antioxidant properties of the plant extract was calculated by using the equation (1):

$$\% \text{ inhibition} = [A_{control} - A_{sample}]/A_{control} \times 100 \text{.....} (1)$$

Where, $A_{control}$ = Absorption of DPPH solution without plant extract at (t=0)

A_{sample} = Absorption of DPPH solution in presence of plant extract at 30 min.

ABTS radical cation scavenging activity

Radical scavenging activities of the plant extracts were also measured by ABTS radical cation scavenging method[14, 15, 16]. Briefly, a stock solution of ABTS radical cation was prepared by dissolving equal amount of ABTS solution (7 mM, 25 mL in deionised water) with potassium persulphate ($K_2S_2O_8$) (140 mM, 440 µL). The mixture was left to stand in the dark at room temperature for 15-16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the previous solution and diluting it in methanol to obtain the absorbance 0.700 ± 0.02 at 734 nm (ABTS working solution should be replaced every five days at least because the free radical degrades easily). The plant extracts (0.1ml) at different concentrations were mixed with the ABTS working solution (2.9 mL) and the reaction mixture was allowed to stand at 30ºC for 6 minutes, then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm, at which point the antioxidants present in the extracts began to inhibit the radical, producing a reduction in absorbance, with a quantitative relationship between the

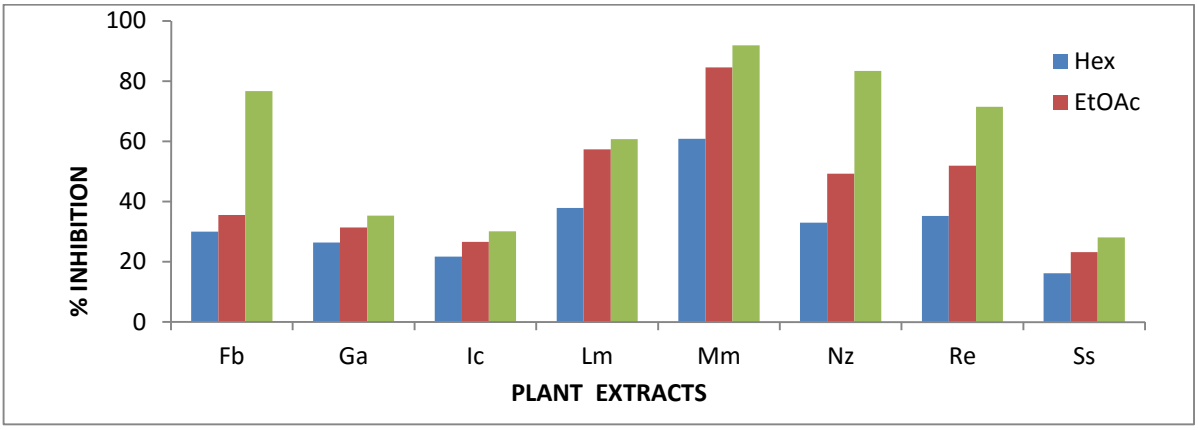

Figure 1: % inhibition of eight plant extracts in different solvent by DPPH method

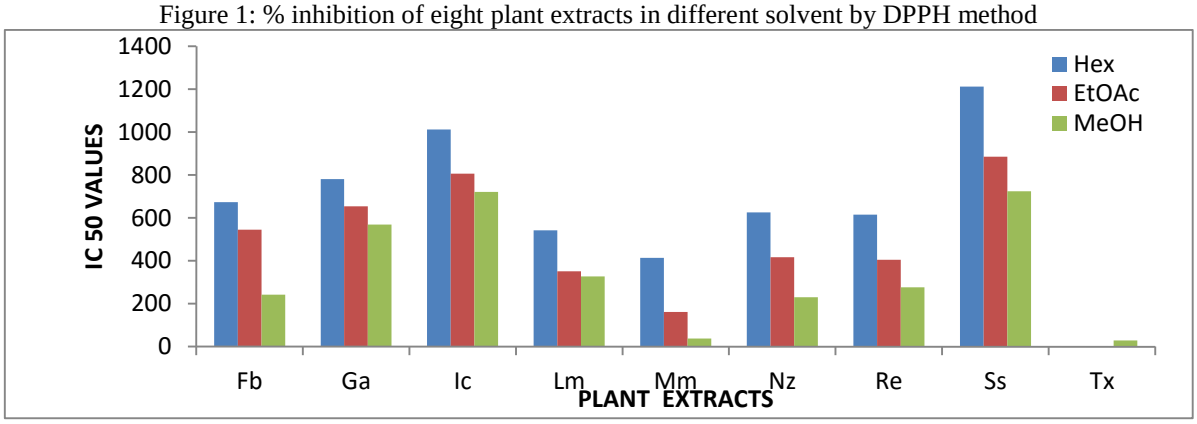

Figure 2: IC_{50} values of eight plant extracts in different solvents by DPPH method

Table 3: % inhibition of eight plant extracts in different solvent by ABTS radical cation scavenging method

Name of the plants with code	% inhibition of ABTS radical cation, (mean ± SD)		
	n-hexane extract	ethyl acetate extract	methanol extract
Ficus bhotanica (Fb)	15.35 ± 0.03	99.72 ± 0.24	98.65 ± 0.49
Glochidion arborescens (Ga)	9.58 ± 0.06	20.91 ± 0.11	22.86 ± 0.05
Ipomoea cymosa (Ic)	18.05 ± 0.11	41.79 ± 0.21	82.70 ± 0.01
Lygodium microphyllum (Lm)	16.27 ± 0.04	37.83 ± 0.04	39.92 ± 0.04
Melastoma malabathricum (Mm)	6.41 ± 0.06	41.81 ± 0.06	99.73 ± 0.05
Naravellia zeylanica (Nz)	9.92 ± 0.08	20.06 ± 0.06	26.21 ± 0.16
Rubus ellipticus (Re)	16.84 ± 0.12	66.6 ± 0.12	98.68 ± 0.11
Selaginella sp. (Ss)	12.90 ± 0.05	13.65 ± 0.05	38.29 ± 0.18

reduction and the concentration of antioxidants present in the studied sample. The radical scavenging activity was given as ABTS radical scavenging effect that was calculated by equation (2):

% inhibition = $[(A_{control}-A_{sample})/A_0] \times 100$..... (2)

Where, $A_{control}$ = Absorption of ABTS solution without plant extract at (t=0)

A_{sample} = Absorption of ABTS solution in presence of plant extract at 6 min.

Determination of total phenolic content (TPC)

The amount of total phenolics in the plant extracts were determined by the Folin-Ciocalteu reagent (FCR) method[17, 18]. To 1 mL of a dilute extract of each plant, 1ml of 10% dilute FCR, 2ml of aq. Na_2CO_3 (7.5%) and 2ml of distilled H_2O were added and the resulting mixture was allowed to stand for 30 minutes, and the total phenolics were determined using an UV-Visible spectrophotometer at 760nm. The average of triplicate measurements was used to calculate the phenolic content as mg gallic acid equivalents (GAE)/g dry weight of the plant extract. Gallic acid was used for the preparation of standard curve. Standard curve of Gallic acid was shown in fig. 5.

Statistical analysis

All analysis was performed in triplicate. The data were recorded as means ± standard deviations (SD). Correlation coefficients (R) were calculated using MS Excel Software.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

Free radical scavenging activity of the n-hexane, ethyl acetate and methanol extracts of all the eight plants were evaluated by DPPH method. The results of percentage

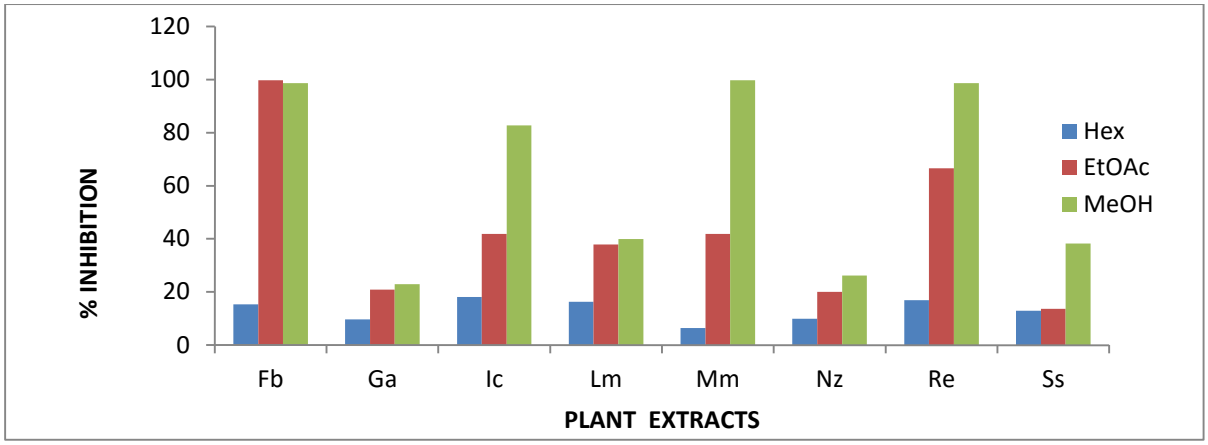

Figure 3: % inhibition of eight plant extracts in different solvent by ABTS method

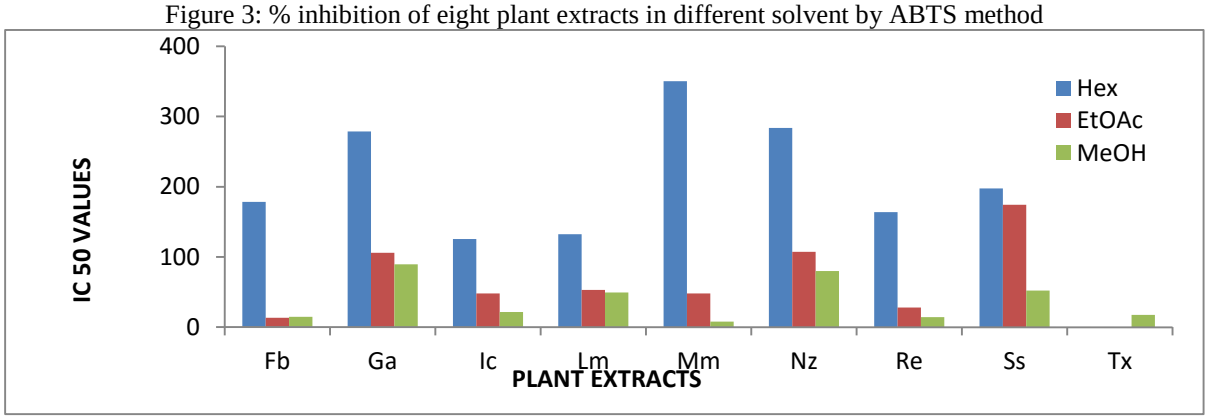

Figure 4: IC_{50} values of eight plant extracts in different solvents by ABTS method

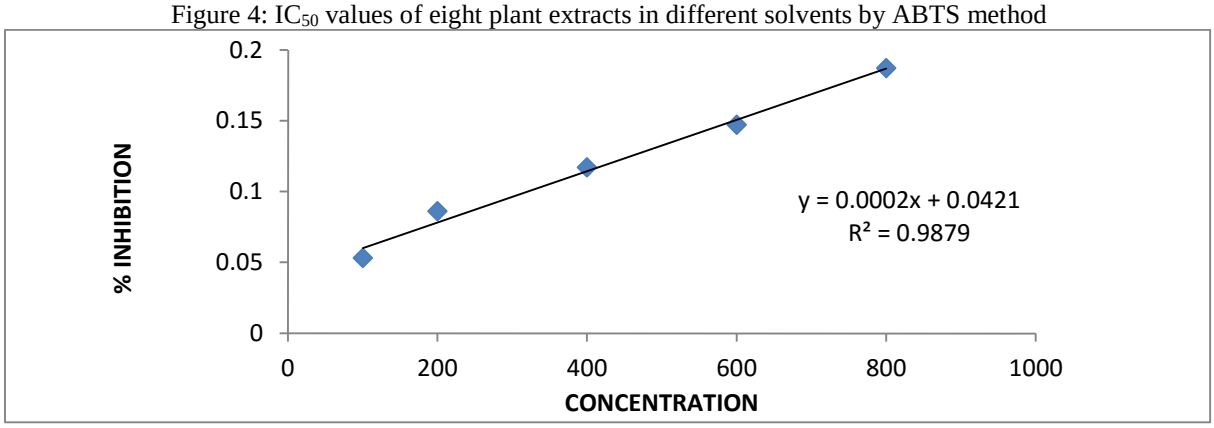

Figure 5: Standard curve of Gallic acid, % inhibition vs. concentration

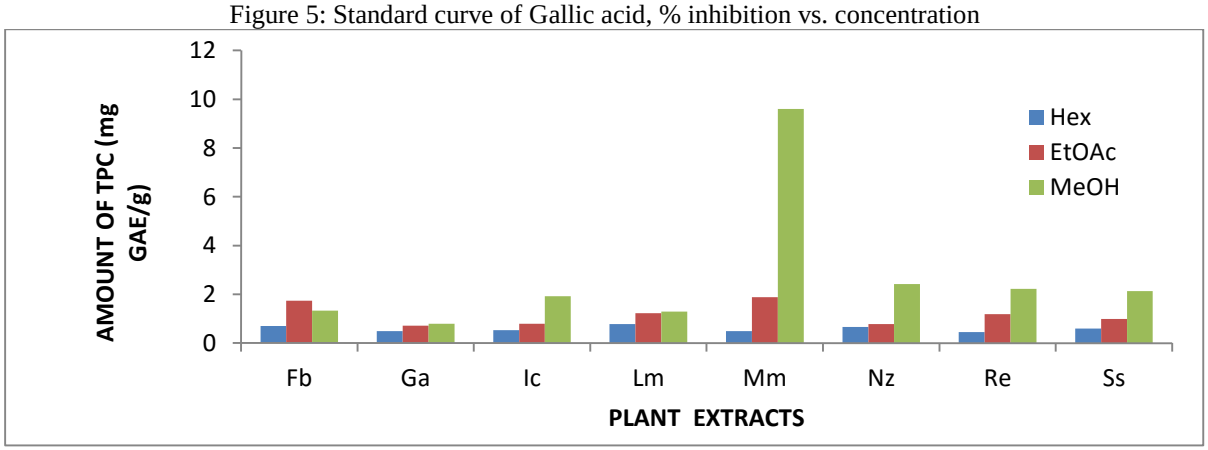

Figure 6: Amount of TPC (mg GAE/g) of eight plant extracts in different solvents

inhibition are shown in table 1 and fig. 1. IC_{50} values were calculated from plotted graphs of scavenging activity against the concentrations of samples. The IC_{50} values were reported in table 2 and Fig. 2. The methanol extracts of all the eight plants exhibited the highest DPPH radical scavenging activity at a concentration of 400µg dry extract/ml of solvent. The ethyl acetate and n- hexane extracts were considerably less effective scavengers than the methanol extracts, with the ethyl acetate extracts being more active than n- hexane extracts. IC_{50} value was also calculated. IC_{50} value is the concentration of the sample required to scavenge 50 per cent of the free radical present in the system. IC_{50} value is inversely related to the percentage inhibition of the plant extracts. The highest percentage inhibition was found in methanol extract of *Melastoma malabathricum* having percentage inhibition (91.93 ± 0.10) and IC_{50} value (37.26 ± 0.05µg/ml) and the lowest value of percentage inhibition was exhibited by the hexane extract of *Selaginella sp* having percentage inhibition (16.12±0.39) and IC_{50} value (1212.51 ± 37.26 µg/ml). Trolox was used as the reference compound and IC_{50} value was found 29.19 ± 0.024 µg/ml. The IC_{50} value of Trolox was found to be low in comparison to all plant extracts but comparing to methanol extract of *Melastoma malabathricum* IC_{50} value have minimal difference with high antioxidant activity.

ABTS radical cation scavenging activity

This method was based on measurement using ABTS radical cation. ABTS radical cation assay was applicable for both lyophilic and hydrophilic antioxidants. The percentage inhibition and IC_{50} value of the eight plant extracts in three solvents (hexane, Ethyl acetate and methanol) are shown in table 3 and table 4 and graphical representations are presented in fig. 3 and fig. 4 respectively. The methanol extracts of all the eight plants except *Ficus bhotanica* plant exhibited the highest ABTS radical cation scavenging activity at a concentration of 40µg dry extract/ml of solvent. In case of *Ficus bhotanica*, ethyl acetate extract exhibited highest percentage inhibition (99.72 ± 0.24) compared to the methanol and hexane extract. According to table 3 and 4, the highest % inhibition was found in methanol extract of *Melastoma malabathricum* having percentage inhibition (99.73 ± 0.05) and IC_{50} value (7.92 ± 0.01 µg/ml). The lowest percentage inhibition was found in hexane extract of *Melastoma malabathricum* having percentage inhibition (6.41 ± 0.06) and IC_{50} value (350.51 ± 2.17 µg/ml). All the other plant extracts also demonstrated the significant antioxidant activities against ABTS radical cation. Trolox was used as reference compound and IC_{50} value was found to be 17.47 ± 0.01 µg/ml. It was observed that the ethyl acetate extract of *Ficus bhotanica*, methanol extracts of *Ficus bhotanica*, *Rubus ellipticus* and *Melastoma malabathricum* have lower value of IC_{50} than that of Trolox which indicates that these extracts have higher antioxidant activity than Trolox.

Total phenolic content (TPC)

It is well known that phenolic compounds are potential antioxidants and free radical-scavengers; hence, there should be a close correlation between the content of phenolic compounds and antioxidant activity. In the present study, the total phenolic content of eight plants of various solvent extracts was investigated. The results were given in Table 5 and fig.5. The total phenolic content varied in the different extracts and ranged from 0.451 ± 0.003 to 0.780 ± 0.008 mg GAE/ g, 0.708 ± 0.003 to 1.888 ± 0.005 mg GAE/g and 0.798 ± 0.003 to 9.598 ± 0.008 mg GAE/g for hexane, ethyl acetate and methanol extract respectively. The extract with the highest total phenolic content was methanol extract of *Melastoma malabathricum* (9.598 ± 0.008 mg GAE/g). The extract with lowest total phenolic content was found in hexane extract of *Rubus ellipticus* (0.451 ± 0.003 mg GAE/g). The highest values were obtained for methanol extracts of all eight plants. A good correlation was observed between radical scavenging activity and the total phenolic content of the eight investigated plants.

CONCLUSION

All the extracts showed good percentage inhibition. But the methanol extracts of the all the eight plants exhibited higher radical scavenging activity in DPPH and ABTS method than other extracts except in one case, where the ethyl acetate extract of *Ficus bhotanica* showed a little higher scavenging activity against ABTS method. The methanol extracts were also found to have high TPC. The result of the present study provides evidence that all of these plants can be considered as valuable sources of antioxidant component of human diet. The antioxidant activity of all these investigated plants used for the preparation of fermentation cake may have contributions on the antioxidant activity of rice based alcoholic

Table 4: IC_{50} values of eight plant extracts in different solvent by ABTS radical cation scavenging method

Name of the plants with code	IC_{50} value, (mean ± SD),(µg/ml)		
	n-hexane extract	ethyl acetate extract	methanol extract
Ficus bhotanica (Fb)	178.54 ± 3.26	13.26 ± 0.02	14.76 ± 0.08
Glochidion arborescens (Ga)	278.79 ± 2.12	105.63 ± 0.44	89.44 ± 0.47
Ipomoea cymosa (Ic)	125.62 ± 0.93	47.98 ± 0.28	21.45 ± 0.01
Lygodium microphyllum (Lm)	132.38 ± 1.17	53.02 ± 0.05	49.32 ± 0.10
Melastoma malabathricum (Mm)	350.51 ± 2.17	47.86 ± 1.06	7.92 ± 0.01
Naravellia zeylanica (Nz)	283.62 ± 2.19	107.19 ± 0.51	79.67 ± 0.13
Rubus ellipticus (Re)	163.89 ± 1.32	27.96 ± 0.49	14.15 ± 0.02
Selaginella sp. (Ss)	197.51 ± 0.97	174.33 ± 0.47	52.09 ± 0.51
Trolox (Tx, reference compound)	17.47 ± 0.01		

Table 5: Total phenolic contents (TPC) of eight plant extracts (GAE: mg/g of extract)

Name of the plants with code	Total phenolic content, (mean ± SD) (mg/g)		
	n-hexane extract	ethyl acetate extract	methanol extract
Ficus bhotanica (Fb)	0.706 ± 0.003	1.733 ± 0.003	1.335 ± 0.005
Glochidion arborescens (Ga)	0.488 ± 0.005	0.708 ± 0.003	0.798 ± 0.003
Ipomoea cymosa (Ic)	0.533 ± 0.003	0.796 ± 0.003	1.926 ± 0.003
Lygodium microphyllum (Lm)	0.780 ± 0.008	1.221 ± 0.003	1.293 ± 0.003
Melastoma malabathricum (Mm)	0.496 ± 0.003	1.888 ± 0.005	9.598 ± 0.008
Naravellia zeylanica (Nz)	0.656 ± 0.003	0.785 ± 0.005	2.421 ± 0.003
Rubus ellipticus (Re)	0.451 ± 0.003	1.190 ± 0.005	2.228 ± 0.003
Selaginella sp. (Ss)	0.596 ± 0.003	0.988 ± 0.003	2.131± 0.003

beverages, "Haanj", prepared by Ahom community people of Assam, India. Further studies regarding the antioxidant activities of "Haanj" is currently under progress.

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