AJHS
Asian Journal of Home Science
Volume **11** | Issue 1 | June, 2016 | 127-135

DOI: 10.15740/HAS/AJHS/11.1/127-135
e ISSN-0976-8351 ■ Visit us: *www.researchjournal.co.in*

Research Paper

Phytochemical content, antioxidant activity and reducing power of five ethnic medicinal plants of Manipur

■ OKRAM ABEMSANA DEVI, MAMONI DAS, ANANTA SAIKIA **AND** PRANATI DAS

Received: 02.03.2016; **Revised:** 27.04.2016; **Accepted:** 09.05.2016

■ **ABSTRACT :** Phytochemical content, antioxidant activity and reducing power of five ethnic medicinal plants of Manipur was analysed to study the antioxidant content commonly used by the traditional healers for the treatment of different inflammatory diseases and ailments. Antioxidant activity of methanolic extracts of five medicinal plants was investigated by using DPPH method which ranged from 47.82±0.041 per cent to 72.62±0.08 per cent inhibition. The phytochemical contents like total alkaloids, total flavonoids, total phenol, total carotenoids content ranged from 5.95±0.01 to 16.11±0.01 mg caffeine /100g on dry weight, 34.95±0.02 to 228.15±0.02 mg quercetin (QE)/100g on dry weight, 88.46±0.01 to 225.50±0.01 mg catechol equivalents (CE)/100g on dry weight, 0.81±0.005 to 3.80±0.005 mg/100g, respectively. Pearson correlation revealed a positive correlation between total phenol content, total flavonoids content, total carotenoids content and free radical scavenging activity (DPPH) of five medicinal plants extracts. However a negative correlation was found between total alkaloids content and free radical scavenging activity of medicinal plants extracts. The reducing power five medicinal plants extracts was statistically significant and positively correlated with DPPH free radical scavenging activity ($r = 0.651$; $p < 0.01$). The study revealed that the plants with higher antioxidant activity (DPPH) showed high absorbance. Higher absorbance indicates more reducing power.

■ **KEY WORDS:** Phytochemicals, Free radicals, Antioxidant, Reducing power, Medicinal plants

■ **HOW TO CITE THIS PAPER :** Devi, Okram Abemsana, Das, Mamoni, Saikia, Ananta and Das, Pranati (2016). Phytochemical content, antioxidant activity and reducing power of five ethnic medicinal plants of Manipur. *Asian J. Home Sci.*, **11** (1) : 127-135, **DOI: 10.15740/HAS/AJHS/11.1/127-135.**

See end of the paper for authors' affiliations

OKRAM ABEMSANA DEVI
Department of Food Science and Nutrition, College of Home Science, Assam Agricultural University, JORHAT (ASSAM) INDIA
Email : okramabemsana@gmail.com

Phytochemicals are the secondary metabolites produced by plants that are responsible for the smell, colour and flavour of fruits/vegetables/ plant foods. Phytochemicals present in the plants are reported to have antioxidants properties that will prevent the oxidative chain reaction initiated by the free radicals and counteract the damaging effects of reactive oxygen species (ROS) produced within the organism from molecular oxygen (Rahman, 2007). Free radicals are responsible for the aetiology of high number of chronic and degenerative diseases. There is a long history of medicinal usage of plants for the treatment of human disorders (Tanab *et al*., 2002). The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs

such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds (Dewick, 1996). According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80 per cent of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar and Muthuselvam, 2009). Medicinal plants contain phytochemicals or bioactive compounds such as phenol, carotenoids, tannins, alkaloids, terpenoids, steroids, flavonoids etc. which provide definite physiological action on the human body (Edcoga *et al.*, 2005). These compounds are synthesized by primary or rather secondary metabolism of living organisms. In the present study, quantitative phytochemical analysis were carried out in five medicinal plants namely *Cissus adnata* (Kongouyen), *Clerodendrum serratum* (Moirang khanam), *Polygonum barbatum* (Yelang), *Allium hookeri* (Napakpi) and *Allium odorum* (Nakuppi) of Manipur (Plate 1) to assess its antioxidant activity and reducing power.

■ RESEARCH METHODS

Collection of plant material :

The required fresh plants/plant parts were collected on the advice of the traditional healers, from various places of Thoubal district (24°37´N and 93°30´E), Manipur, India and also from the local market of Manipur. The samples were collected during the month of December and January and also June and July, in the year 2013-2014.

Preparation of sample :

After collection the tender leaves or required plant parts were cleaned by removing the infested and diseased portion. The leaves were thoroughly washed under running water and finally in distilled water and shade dried till the leaves became very crisp. The dried plant material were then ground properly into fine powder in an electrical grinder and stored in an airtight container with identification labels. The ground plant species were stored in a refrigerator at 4°C. These powdered materials were used for further different chemical analysis.

Cissus adnata (Kongouyen)

Clerodendrum serratum (Moirang khanam)

Polygonum barbatum (Yelang)

Allium hookeri (Napakpi)

Allium odorum (Nakuppi)

Plate 1 : Selected medicinal plant samples

Determination of antioxidant activity :

Free radical scavenging assay :

Antioxidant activity was measured by using DPPH method according to Vani *et al*. (1997). Two grams of dried sample was extracted with 20 ml of methanol (99.5%). The extraction was done twice each for hours in shaking machine. The supernatant was filtered using whatman No. 1 filter paper after centrifuging the suspension at 10,000 rpm for 15 min, till analysis filtrated stored at -20°C. 100µl of aliquot of sample extract was taken in a test tube and added 2.9 ml of DPPH solution (0.005 mM solution of 2, 2 diphenyl-1-picryl-hydrazyl prepared in 99.5% methanol) after added this solution vortex vigorously. The test tube was incubated in dark for half an hour. The discoloration of DPPH was measured against blank at 517nm. Methanol was used as blank and DPPH methanolic solution was used as standard. Radical scavenging activity was calculated by the following formula :

$$\% \text{ inhibition} = \frac{(A_b - A_a)}{A_b} \times 100$$

where, A_b is the absorbance of blank

A_a is the absorbance of sample

Reducing power assay :

Reducing power of the sample was determined according to the method of Oyaizu (1986). 0.5 g of dried sample was extracted with 20 ml of methanol (99.5%). The extraction was done twice each for hours in shaking machine. The supernatant was filtered using whatman No. 1 filter paper. 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1 per cent potassium ferricyanide were added to 1 ml of methanolic sample extraction solution and mixed gently. The mixture was incubated at 50°C in a water bath for 20 minutes. Reaction was stopped by adding 2.5 ml of 10 per cent trichloroacetic acid (TCA) and the mixture were centrifuged at 4000 rpm for 10 minutes. From the top layer, 2.5 ml was transferred into the tubes containing 2.5 ml distilled water and 0.5 ml of 0.1 per cent ferric chloride ($FeCl_3.6H_2O$). The resulting solution was mixed well and after 5 minute the absorbance was measured at 700 nm against blanks. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard.

Total phenolic content :

Total phenol was assayed by the method proposed by Malick and Singh (1980). The homogenate was prepared with 0.5g of the powdered samples in 10-times volumes of 80 per cent ethanol in a pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The residue was re-extracted with 80 per cent ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water (5ml). Different aliquots (0.2 to 2.0ml) were pipetted out into test tubes. The volume in each tube was made up to 3.0ml with distilled water. To all the tubes, 0.5 ml of Folin-Ciocalteau reagent was added and mixed. After 3 minutes, 2.0ml of 20 per cent sodium carbonate solution was added to each tube. After mixing the tubes thoroughly, all the tubes were kept in a boiling water bath for exactly 1 minute, and allowed to cool. The absorbance was measured at 650 nm against a reagent blank. The concentration of the total phenols in the test sample was expressed as mg catechol/100g material.

Total flavonoids content :

The total flavonoids were determined according to Aiyegroro and Okoh (2010) with slight modification. Dried 0.5 g of sample powder was weighed and mixed with 5 ml of 80 per cent ethanol in 100 ml of conical flask and put on a shaker at 200 rpm for 24 hrs. After 24 hrs, the extracts were filtered through Whatman No. 42 filter paper. The volume of the filtrate was adjusted to 5 ml with 80 per cent ethanol. 0.5 ml of extracts of each sample was taken in a test tube followed by addition of 1.5 ml 95 per cent ethanol, 0.1 ml of 10 per cent aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation for 30 min at room temperature, the absorbance was measured at 415 nm in a spectrophotometer. Quercetin was used as standard to make the calibration curve.

Total alkaloids content :

The alkaloids present were estimated by using spectrophotometer (Shamsa *et al.,* 2008). The plant materials (5g) were ground and then extracted with methanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45^0 C to dryness. A part of plant residue

was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank and caffeine was used as standard. TAC was expressed in milligram caffeine equivalent (mg CE).

Total carotenoids content :

Total carotenoids content was determined according to Anaya (1999). 5 g of sample with 3 g of celite powder was ground with 50 ml cold acetone and filtered through whatman No. 4 filter paper. 40 ml of petroleum ether was taken in a 500 ml separating funnel and acetone extract was added in the funnel. Therefore the solution was washed 3-4 times with distilled water to discard the lower aqueous phase without discarding the upper phase. The upper phase was collected in 50 ml volumetric flask and 15g of anhydrous sodium sulphate was added to remove the residual water. The solution was again filtered and volume was made up with petroleum ether. The absorbance was record at 450nm in a spectrophotometer. The total carotenoids content was (µg/g) calculated as

$$= \frac{\text{Absorbance x volume (ml) x 10 x dilution factor}}{\text{Absorbance co - efficient (2592) x sample weight}}$$

Statistical analysis :

The results of all experiments performed were expressed as Mean ± SD of three determinations, the test of significance was applied wherever necessary and values obtained as $p<0.01$ were considered as statistically significant.

■ RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

Determination of radical scavenging activity (DPPH):

The primary characterization of scavenging ability of the plant extracts has been studied using a stable free radical DPPH. The results of radical scavenging activity of all the medicinal plants are shown in Fig. 1. There was a great variation of free radical scavenging activity (RSA) among the five medicinal plants of Manipur. The DPPH scavenging activity of five medicinal plants ranged from 47.82±0.041 per cent to 72.62±0.08 per cent inhibition, respectively. The highest DPPH scavenging activity was found in *Clerodendrum serratum* (72.62±0.08 %), followed by *Cissus adnata* (72.53±0.107), *Allium hookeri* (55.50±0.092), *Allium odorum* (51.72±0.011), *Polygonum barbatum* (47.82±0.041). An antioxidant inhibits the lipid peroxidation or other molecules providing protective against reactive oxygen species or by scavenging free radicals. DPPH method measures electron donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Determination of the free radical scavenging capacity or antioxidant potential of the test sample shows its effectiveness, prevention and repair mechanism against many health related disorders and diseases such as infections, diabetes, arthritis,

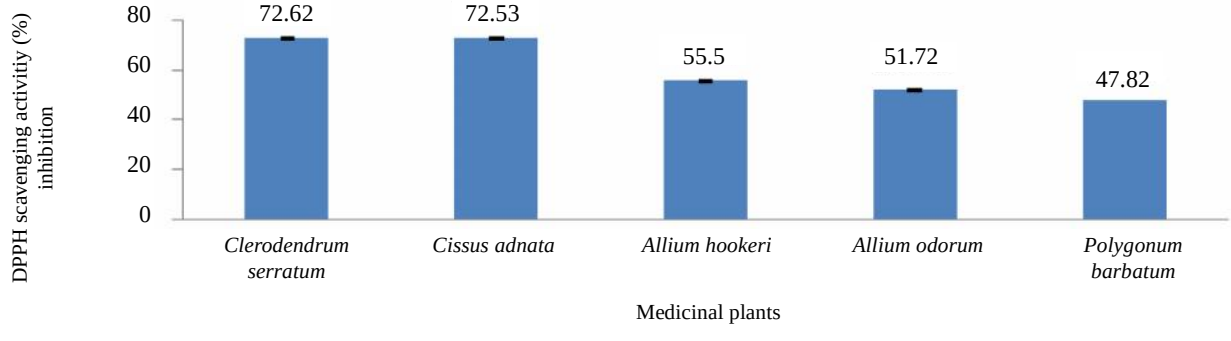

Fig. 1 : DPPH radical scavenging activity in 5 different ethnic medicinal plant extracts of Manipur

cardiovascular diseases, cancer, Alzheimer's diseases, AIDs etc. (Kumar and Abbas, 2012). The five medicinal plants of Manipur are rich in antioxidant contributed by the presence of many phytochemicals such as phenol, flavonoids, alkaloids, carotenoids etc. which may helps in preventing against free radicals

Reducing power of five medicinal plants :

The reducing power ability of the 5 medicinal plants of Manipur is given in Fig. 2.

The reducing power of five medicinal plants was evaluated and the results were expressed as ascorbic acid equivalents. As observed from Fig. 2. *Polygonum barbatum* has minimum reducing power (0.61±0.003) and *Clerodendrum serratum* has maximum reducing power (2.19±0.05) amongst the five medicinal plant extracts. The results obtained were statistically significant and positively correlated with DPPH free radical scavenging activity ($r = 0.651$; $p < 0.01$). Reducing power is associated with antioxidant activity and serves as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Chanda *et al.*, 2009). Presence of reducers (antioxidants) causes the conversion of the Fe^{3+}/ ferricyanide complex used in this method to ferrous form. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of ferrous ions. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts (Soni and Sosa, 2013). The high value of the reducing ability of selected 5 medicinal plants indicates the high reducing power ability (Chung *et al.*, 2002). So, the high reducing power is indicative of the hydrogen donating ability of the active species present in the plant extract (Shimada *et al.*, 1992).

Phytochemical content :

Total alkaloids content :

The total alkaloids content of five medicinal plants

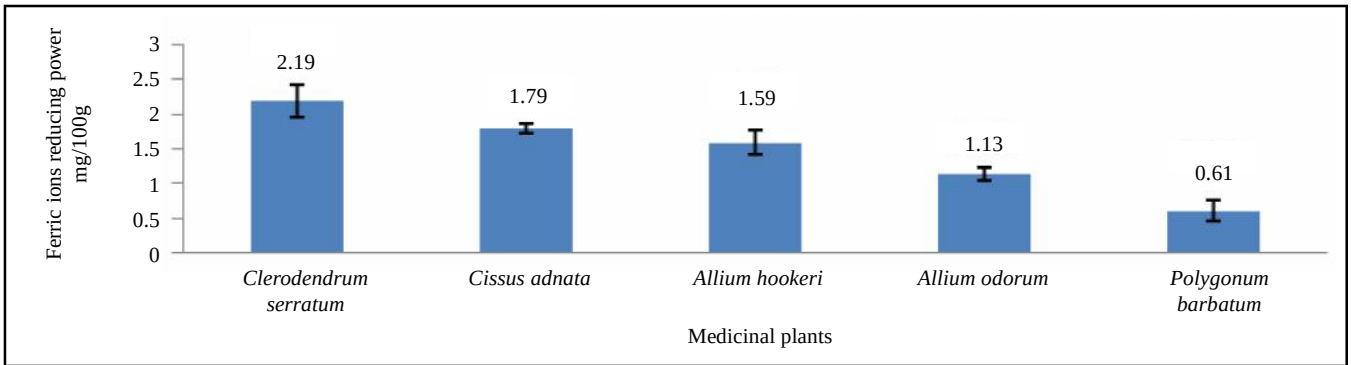

Fig. 2 : Reducing power in 5 different ethnic medicinal plant extracts of Manipur

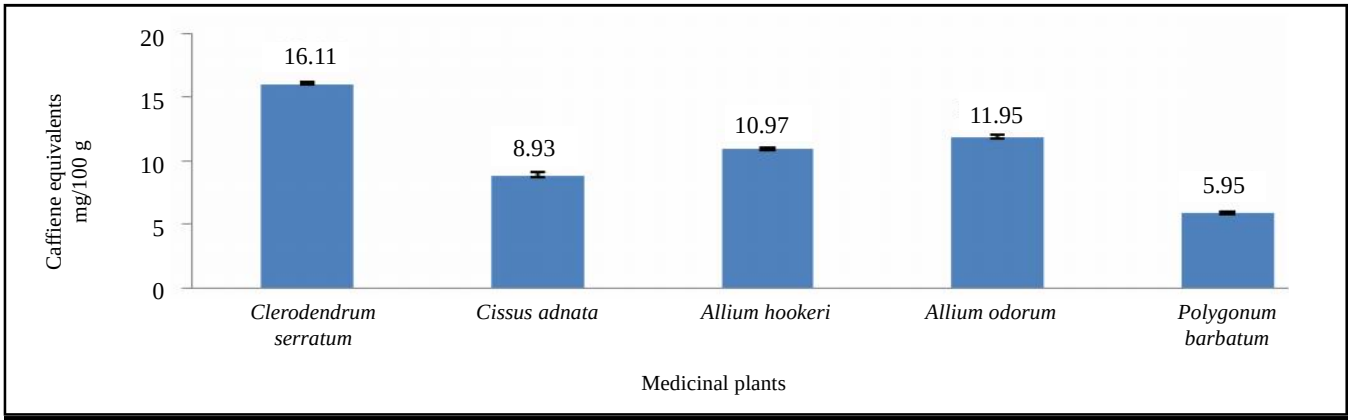

Fig. 3 : Total alkaloids content in 5 ethnic medicinal plant extracts of Manipur

was evaluated and the results were expressed as caffeine equivalents. Total alkaloids content of five medicinal plants showed significant variation, ranging from 5.95±0.01 to 16.11±0.01 mg caffeine /100g dry weight. The highest alkaloids content was found in *Clerodendrum serratum* (16.11±0.01) and lowest content in *Polygonum barbatum* (5.95±0.01). Antioxidant activity of alkaloids is that they have an important role in preventing various diseases by increasing superoxide dismutase activity, decreasing superoxide anion and malondialdehyde (MDA) formation and bind with catalyzing metal ions (transition metals like iron and cupper ions), which can reduce the concentration of metal ions in lipid peroxidation (Schroeter *et al.*, 2002). Alkaloids with hydroxyl substitution and a partially desaturated pyridine ring are most active which can scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) by rapid donation of a hydrogen atom to radicals (Pietta, 2000). The antioxidant activity of the 5 medicinal plants also contributed by alkaloids not only phenols and flavonoids content by rapid donation of a hydrogen atom to radicals which are responsible for the treatment of various diseases including diabetics, cancer, cardiac dysfunction, respiratory disorders etc. and as local anaesthesia and in relief of pain (Huang *et al.*, 1996).

Total flavonoids content :

The total flavonoids content of five medicinal plants was evaluated and the results were expressed as quercetin equivalents. From the Fig. 4 it was observed that total flavonoids content of five medicinal plants showed significant variation, ranging from 34.95±0.02 to 228.15±0.02 mg Quercetin (QE)/100g of dry weight. The highest flavonoids content was found in *Cissus adnata* (228.15±0.02 mg/100g) and lowest content in *Allium hookeri* (34.95±0.02). The antioxidant property of flavonoids is that they are potent free radical scavengers due to presence of double bond in hydroxyl position in their molecule. Flavonoids interferes oxidation of lipids and other molecules by rapid donation of hydrogen atoms to reactive oxygen free radicals (Schroeter *et al.*, 2002). High flavonoids content of 5 medicinal plants may also contribute the antioxidant activity of medicinal plants by rapid donation of hydrogen atom to the free radicals which helps in preventing against free radicals induced degenerative diseases.

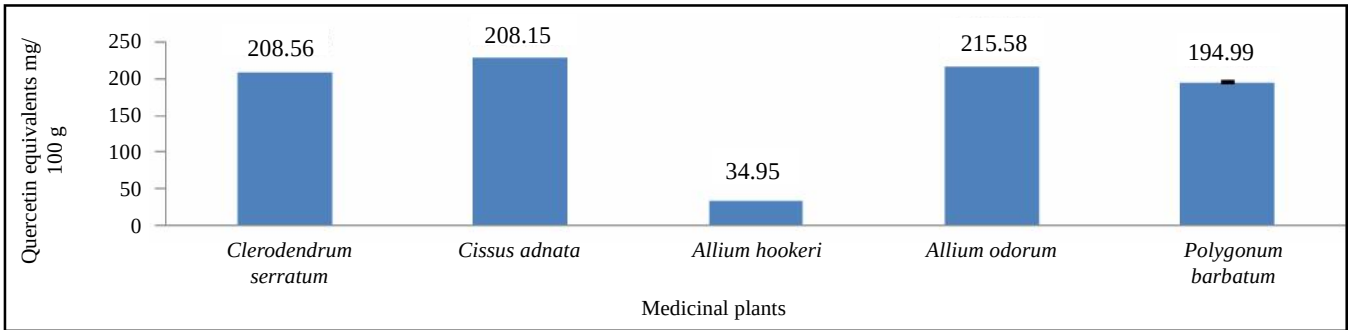

Fig. 4 : Total flavonoids content in 5 different ethnic medicinal plant extracts of Manipur

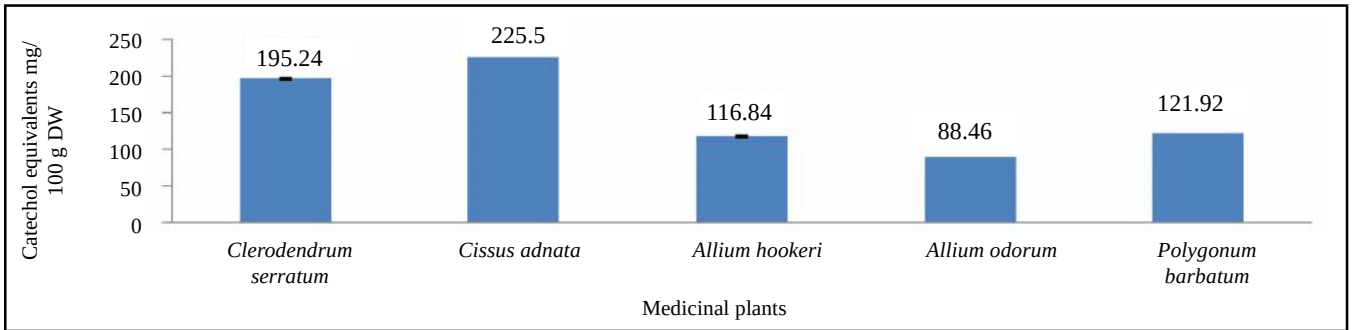

Fig. 5 : Total phenol content in 5 different ethnic medicinal plant extracts of Manipur

Total phenol content :

The total phenol content of five medicinal plants was evaluated and the results were expressed as catechol equivalents. Total phenol content of five medicinal plants showed significant variation, ranging from 88.46±0.01 to 225.50±0.01 mg catechol equivalents (CE)/100g of dry weight. The highest phenol content was found in *Cissus adnata* (225.50±0.01) and lowest in *Allium odorum* (88.46±0.01). The antioxidant activity of phenolic compounds is related to the acid moiety and the number and the relative positions of hydroxyl groups (in the 2- and 4- positions or in the 3-, 4-, 5-positions confer the greatest antioxidant activity) on the aromatic ring structure (Seifu, 2012). Polyphenol protects from oxidative damage by donating hydrogen or electron to the free radicals and plays a beneficial role in reducing the risk of coronary heart disease, diabetes, arthritis and cancer (Willcox and Bodeker, 2004). The antioxidant activity of 5 medicinal plants may be contributed by the presence of phenol which inhibits the oxidative mechanisms that are responsible for many health related disorders and diseases. So, the higher phenol contents in the 5 medicinal plants contribute more antioxidant activity which is therapeutically helpful in preventing many degenerative diseases.

Total carotenoids content :

The total carotenoids content of five medicinal plants showed slight variation, ranging from 0.81±0.005 to 3.80±0.005 mg/100g, respectively. It was observed that the highest carotenoids content was found in *Allium odorum* (3.80±0.005 mg/100g) and lowest in *Allium hookerii* (0.81±0.005). The antioxidant property of carotenoids is that they are very efficient physical and chemical quenchers of singlet oxygen ($^1O_2$), as well as potent scavengers of other reactive oxygen species (ROS). The presence of carotenoids in the medicinal plant extracts playa a protective role in a number of ROS-mediated disorders such as cardiovascular diseases, several types of cancer or neurological, as well as photosensitive or eye-related disorders (Fiedor *et al*., 2005). The presence of carotenoids in 5 medicinal plants also leads to the antioxidant property of the medicinal plants.

Correlation between phytochemical content and free radical scavenging activity :

Pearson correlation showed that there was a significant positive correlation between total phenol content and antioxidant activity of 5 medicinal plant extract as determined by DPPH radical scavenging assay ($r=0.565$; $p<0.01$) of 5 medicinal plants extract. Phenolic compounds contribute a greater antioxidant property mainly due to their redox properties and are capable of neutralising lipid free radicals and preventing decomposition of hydro-peroxides into free radicals and

Table 1 : Pearson correlation co-efficient between DPPH (%) and different phytochemical contents of five medicinal plants

Variable	Correlation with DPPH in five medicinal plants
Total phenol (mg/100g)	0.565**
Total flavonoids (mg/100g)	0.587**
Total alkaloids (mg/100g)	-0.309^{NS}
Total carotenoids (mg/100g)	0.505**

** Correlation is significant at the 0.01 level (2-tailed).
NS=Non-significant

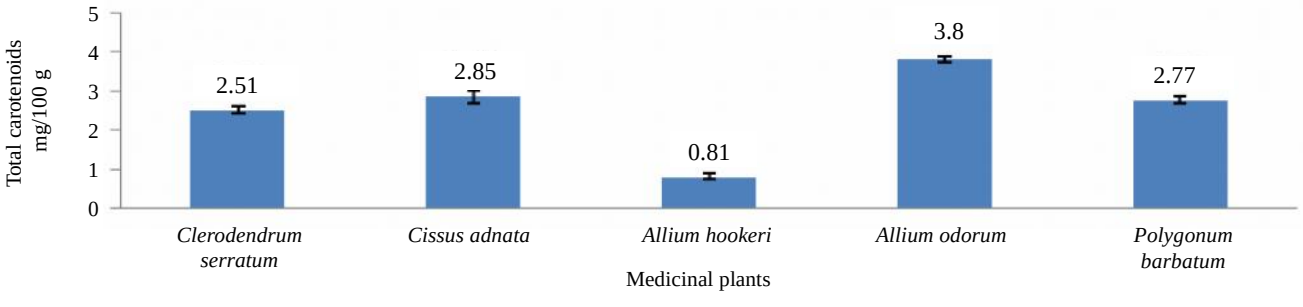

Fig. 6 : Total carotenoids content in 5 different ethnic medicinal plant extracts of Manipur

also act as highly effective free radical scavengers which are mainly due to their redox properties and can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Li *et al.*, 2006).

Positive correlation was also observed between total flavonoids content and antioxidant activity by DPPH radical scavenging assay ($r=0.587$; $p<0.01$) of 5 medicinal plants extract. Free radical quenching activity of flavonoids depends on the specific substitution pattern of free hydroxyl group on their structure specially B ring with 3', 4' dihydroxy groups and 3-OH group at C ring are essential features for strong antioxidant activity (Amic *et al.*, 2003).

There was no correlation was found between DPPH radical scavenging activity and total alkaloids contents of 5 medicinal plants extract. Many research studies found negative correlation between the total alkaloids content and DPPH free radical scavenging activity. Sheik and Chandrashekar (2014) revealed that the negative correlation observed between the total alkaloids and the DPPH radical scavenging activity could be due to the presence of some of the active phenolic compounds in plant extract contributing towards scavenging of free radicals. The presence of aromatic –OH group in alkaloids are responsible for their antioxidant efficiency, similarly to phenolic antioxidants, via chain-breaking mechanism by donation of hydrogen (Lucia *et al.*, 2004).

Pearson correlation also showed that there was a significant positive correlation between total carotenoids content and antioxidant activity by DPPH radical scavenging assay ($r=0.505$; $p<0.01$) of 5 medicinal plants extract. The free radical scavenging activity and also stated that antioxidant activity of carotenoids depend on the number of double bonds and capacity to quench radicals by hydrogen atom transfer or by accepting electrons from radicals.

Conclusion :

From the present study it can be concluded that five ethnic medicinal plants of Manipur which are used by the traditional healers/maibas/maibis are rich in flavonoids, carotenoids, alkaloids, phenols, and have higher antioxidant capacity and potential to reduce and scavenge ROS which are produced due to oxidative stress. Therefore it may be helpful in the prevention and treatment of oxidative stress induced inflammatory diseases like diabetes, cardiovascular diseases, cancer, arthritis, gout, neurodegenerative diseases, respiratory tract infections and skin disorders etc. Along with the free radical scavenging activity, these plants also have high reducing power which provides the potentiality or ability of these medicinal plants to reduce and scavenge free radicals. The study also provided baseline data for future studies geared towards the therapeutic benefits of common medicinal plants of Manipur.

Acknowledgement :

The authors are thankful to Mr. N. Janaki Singh, Mr. P. Kumar Singh, Mr. O. Iboyaima Singh and Mr. L. Nabakishor Singh for their help in the collection of plant materials and identification of specimens in making this study possible. The authors are also thankful to the Assam Agricultural University, Jorhat for providing necessary support and for helping in analysing the samples.

Authors' affiliations:
MAMONI DAS, ANANTA SAIKIA AND PRANATI DAS, Department of Food Science and Nutrition, College of Home Science, Assam Agricultural University, JORHAT (ASSAM) INDIA

■ REFERENCES

Aiyegroro, O.A. and Okoh, A.I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. *BMC compl. And Alt. Med*., **10**:21.

Amic, D., Amic, D.B. and Trinajstic, N. (2003). Structure related scavenging activity relationships of flavanoids. *Croat. Chem*., **76**: 55-61.

Anaya, A.L. (1999). Allopathy as a tool in the management of biotic resources in agroecosystems, *Crit. Rev. Plant Sci.*,**18**: 697-739.

Arunkumar, S. and Muthuselvam (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World J. Agril. Sc.*, **5** (5):572-576.

Chanda, S., Dave, R., Kaneria, M. and Nagani, K. (2009). Seaweeds: a novel, untapped source of drugs from sea to combat infectious diseases. *Curr. Res. Technol. Edn. Top. Appl. Microb. Biotechnol.*, pp. 473-480.

Chung, K.T., Lu, Z. and Chou, M.W. (2002). Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem. Toxicol.*, **36**: 1053-1060.

Dewick, P.M. (1996). Tumor inhibition from plants : Tease and Evans. In : *Plants With Antiprotozoal Activity*. 14th edn. Phillipson, J.D. and Wright, C.W. (eds.). Tease and Evans, Pharmacognosy, WB Saunders Company, London., pp. 6-12.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). *Phytochemical constituents of some Nigerian medicinal plants. African J. Biotechnol.*, **4**(7): 685-688.

Fiedor, J., Fiedor, L., Haessner, R. and Scheer, H. (2005). Cyclic endoperoxides of β-carotene, potential pro-oxidants, as products of chemical quenching of singlet oxygen. *Biochimica et Biophysica Acta.,* **1709**: 1-4.

Huang, S. W., Frankel, E. N., Schwarz, K., Aeschbach, R. and German, J.B. (1996). Antioxidant activity of carnosic acid and methylcarnosate in bulk oils and oil-in-water emulsions. *J. Agric. Food Chem.,***44**: 2951-2956.

Kumar, K. and Abbas, S.G. (2012). Ethnomedicinal composition depends on floristic composition: A case studied in Sal forests of Jharkhand. *Internat. J. Pharmacy & Life Sci.*, **3** (5) : 1710-1719.

Li, B.B., Smith, B. and Hossain, M.M. (2006). Extraction of phenolics from citrus peels: I. Solvent extraction method. *Separ. Purif. Technol.,* **48**: 182-188.

Lucia, R., Magdalena, M., Daniela, K. and Milan, S. (2004). Antiradical and antioxidant activities of alkaloids from Mahonia aquifolium. Structural aspects, *Bioorg. Med. Chem*., **12** (17): 4709-4715.

Malik, C.P. and Singh, M.B. (1980). In: *Plant Enzymology and histo Enzymology.* Kalyani Publishers New Delhi, p. 286.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.,* **7** : 307-15.

Pietta, P.G. (2000). Flavonoids as antioxidants. *J. Nat. Products.,* **63**: 1035–1042.

Rahman, K. (2007). Studies on free radicals, antioxidants, and cofactors, *Clin Interv Aging.,* **2**: 219-236.

Schroeter, H., Boyd, C., Spencer, J.P.E., Williams, R.J., Cadenas, E. and Rice-Evans, C. (2002). MAPK signaling in neurodegeneration: Influences of flavonoids and of nitric oxide. *Neurobiol. Aging.,* **23** : 861-880.

Seifu, T. (2012). Ethnobotanical and Ethnopharmaceutical Studies on Medicinal Plants of Chifra District, Afar Region, North Eastern Ethiopia. M.Sc. Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Shamsa, F., Hamidreza, M., Rouhollah, G. and Mohammadreza, V. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J. Pharm. Sci*., **32**: 17-20.

Sheik, S. and Chandrashekar, K.R. (2014). Antimicrobial and antioxidant activities of *Kingiodendron pinnatum* (DC.) Harms and *Humboldtia brunonis* Wallich: endemic plants of the Western Ghats of India. *J. Natn. Sci. Foundation Sri Lanka.,* **42**(4): 307-313.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. *J. Agril. Food Chem*., **40**: 945-948.

Soni, A. and Sosa, S. (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *J. Pharm. Phytochem.,* **2** (4): 22-29.

Tanab, H., Yoshida, M. and Tomita, N. (2002). Comparison of the antioxidant activities of 22 commonly used herbs and spices on the lipid oxidation of pork meat. *J. Animal Sci*., **73**: 389-393.

Vani, T., Rajani, M., Sarkar, S. and Shishoo, C.J. (1997). Antioxidant properties of the ayurvedic formulation, Triphala and its constituents. *Inter. J. Pharmac.,* **35**: 313-317.

Willcox, M.L. and Bodeker, G. (2004). Traditional herbal medicines for malaria. *Clin. Rev. British Med. J.,* **329**: 1156–1159.