J. Assam Sc. Soc. Vol. 57. No. 1 & 2 **ISSN 0587-1921**
December 2016; Pp. 82-89

IN VITRO ANTIOXIDANT ACTIVITY AND PHYTO-CHEMICAL CONSTITUENT OF LEAF EXTRACT OF *GNETUM GNEMON*

***Chandana Choudhury Barua, Mondira Bora** and **Prakash Haloi**
Department of Pharmacology and Toxicology
College of Veterinary Science, Khanapara, Guwahati - 781022
****Corresponding author:*** chanacin@gmail.com

ABSTRACT

Different extracts from the leaves of *Gnetum gnemon* viz ethanol, hydroethanol and aqueous were evaluated for phyto-chemical constituents and in vitro antioxidant activity by DPPH, reductive ability assay and superoxide anion scavenging activity. Qualitative screening of the plant extract revealed the presence of different phyto constituent's viz. steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins. Quantitative analysis for the three extracts were done for total phenolics and total flavonoids. Among the three extracts, maximum phenolic content was found in ethanolic extract (0.148±0.025mg tannic acid/g dm) and maximum flavonoids content was found in aqueous extract (0.048±0.008 mg quercetin/g dm). The result reveals that the plant exhibit potent antioxidant property which might be used as a natural source of antioxidant.

Key words: *Gnetum gnemon*, Antioxidant activity, Phenolics, Flavonoids

INTRODUCTION

In the present era, plants have got wide variety of applications in the field of medicine field due to their easy availability and being ecofriendly (Fredrick et al., 2013). According to WHO (World Health Organisation) more than 80% of the world's population relies on

traditional medicine for their primary health care needs. The medicinal values of the plants are attributed to bioactive phytochemical constituents present within it. These bioactive phytochemical constituents in medicinal plant include alkaloids, flavonoids, phenolic compounds, tannins, anthracene derivatives and essential oils (Krishnaiah et al., 2009). Such phytochemicals are believed to exert potent beneficial action that supports and promote human health. Since they are natural products, they are generally considered as safe and free from side effect unlike synthetic drug. This has stimulated search for natural products from various plants with high antioxidant potential (Okamura et al., 1993). Literature survey revealed that many medicinal plants have antioxidant activity that are effective against several diseases such as cancer, atherosclerosis, cerebral cardiovascular diseases, diabetes, hypertension, and Alzheimer's disease (Liu et al., 2003; Devasagayam et al., 2004). Phytoconstituents like phenolics and flavonoids are known to have antioxidant activities. Antioxidants interfere with the oxidative processes by scavenging free radicals and by acting as electron donors (Gulcin et al., 2005). There is an increasing interest nowadays in searching for compounds with antioxidant activity among various indigenous herbs, spices and medicinal plants.

Gnetum gnemon L. is a native plant of Indonesia. It is a rare gymnosperm species which is a perennial slender shrub attaining a height of 3 to 4 feet. In NE India they are popularly known by Karbi name "Hanthu" or Assamese name "Letera" in Assam. The common names of *Gnetum gnemon* are bago, melinjo, benjilo, bagoe, maninjau, voe, khalet peedae, phak,miang kaniang, liang, gam cay and bet (Cradiz et al., 2001). In Nagaland and parts of Manipur of North Eastern India, the green leaves and twigs are in great demand. The leaves are consumed as vegetables. It is also boiled and taken as soup. Traditionally, such soup is given to woman after child birth and the popular belief is that it has a tonic effect and helps to regain health. The seeds are roasted and eaten as delicacy and sold in local market. As per old record, it is available in moist deciduous forests of Nagaland, parts of Manipur and Upper Assam. The plant is used in folkloric medicine to treat convulsions and for arthritis, bronchitis and asthma (Iliya et al., 2002). The stilbenoids obtained from Ggnemon derivatives is responsible for various antioxidant and antimicrobial activity (Iliya et. al., 2002) The crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various components present in the whole extract (Hamberger et al., 1991).The present study was undertaken to evaluate the in vitro antioxidant property along with the phytochemical constituents of *Gnetum gnemon*.

MATERIALS AND METHODS

Collection of the plant and preparation of herbarium

Leaves of Gnetum gnemon were collected during the month of July-August, 2011 from Karbi Anglong. The plant specimens were identified by Dr. Iswar C. Barua, taxonomist, AAU,

Jorhat, Assam and voucher specimens (CAL 0000027000) were deposited in the herbarium in the Dept.of Natural Products Chemistry, NEIST, Jorhat and AAU, Khanapara.

Preparation of the various extract

About 500 g finely cut leaves were homogenised with sufficient quantity of ethanol in a conical flask for about 16 hours. Then the mixture was filtered and the filtrate were collected. The plant materials were further extracted this way three/four times. The filtrate obtained were pooled and distilled under reduced pressure at 50^0C. The final drying was done in a lyophilizer. The lyophilzed extract was scraped off and transferred to a tarred glass container. This is the stock extract of the plant. In the same way hydroethanol and aqueous extract were prepared to get stock extracts.

Phytochemical analysis of leaf extract

Preliminary qualitative screening of the leaf extract was carried out for the presence of different phytoconstituents viz. steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the method of Harborne (1991).

The qualitative tests revealed that phenolics and flavonoids were the most prominent compounds and subsequently quantitative estimates were done for them. For this the extract was prepared from the stock at a concentration of 0.1 mg/ml. Ethanol was used as solvent. Total phenolics were estimated as per the protocol of Chang et. al (2001) which involve modified folin ciocalteu method. After colour development absorbance was taken in a spectrophotometer (Chemito, UV 2100) at 650 nm. Ethanol was used as blank and from the absorbance values total phenolics were estimated from the standard curve prepared with tannic acid as standard. The values were expressed as mg/g tannic acid equivalent. Flavonoids were estimated from the same extract as per the method of Ordonez et. al (2006). After colour development the absorbance values were taken in a spectrophotometer at 415 nm. From the absorbance values quantification was done using the standard curve prepared with quercetin. The values were expressed as mg/g quercetin equivalent.

In vitro antioxidant activity

In vitro antioxidant activities of the leaf extract were estimated using three different methods to compare the results and to facilitate a logical conclusion.

(DPPH) 2, 2-Diphenyl-1-picryl Hydrazyl scavenging activity

The free radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl Hydrazyl scavenging (DPPH Sigma-Aldrich) were determined as per the protocol of Ayoola et al., (2006). Vitamin C was used as antioxidant standard at different concentrations. Extracts of different volumes were taken and final volume was made upto 2 ml by adding required amount of solvent. To this 2 ml of DPPH (0.1 mM in methanol) were added and homogenised. The mixture was incubated at room temperature in dark for 30 minutes after which reduction in colour was measured in a spectrophotometer at 517 nm. From the absorbance values radical scavenging activity (RSA) was calculated as below.

$$RSA\% = \frac{A_o - A_s}{A_o} \times 100$$

Where - A_o = Negative control (unbleached DPPH)
A_s = Absorbance for individual sample

Reductive ability

Reducing power was assayed as per the protocol of Jayaprakash et. al (2001). One ml of different concentrations of the plant extract (in the range of 100 to 600 µg/ml) were mixed with 2.5 ml potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50^0C for 20 minutes and absorbance was measured at 700 nm (Multiskan, Thermo Scientific). For quantification a standard curve was prepared using a gradient solution of gallic acid. From the curve reductive power was worked out and expressed as GAE mg/ g.dm.

Superoxide anion scavenging activity : Superoxide anion scavenging activity of plant extract was measured according to the method of Robak *et al.,* (1988) with some modifications. All the solutions were prepared in 100mM phosphate buffer (pH 7.5). One ml of nitro blue tetrazolium chloride (NBT) (156 µM), 1 ml of NADH (468 µM) and 3 ml of plant extracts were mixed. The reaction was started by adding 100 µl of phenazine methosulphate (PMS, 60 µM) and the mixture was then incubated at 25° C for 5 minutes. Thereafter absorbance was taken in a spectrophotometer at 560 nm (Multiskan, Thermo Fisher Scientific).

Three replications were made for each sample and the mean and standard error of mean (±SEm) were computed. Statistical analysis was performed by one-way analysis of variance (ANOVA) among different treatment groups followed by Tukey-Kramer test (Graph Pad Prism 5.0). $P < 0.05$ was considered to indicate statistical significance.

RESULT AND DISCUSSION

Qualitative screening of Phyto constituents

Extracts prepared with different solvents exhibited differences in the phytochemical profile. The phytochemical screening of ethanolic extract of *G. gnemon* shows the presence of diterpenes, phenolics, glycosides, triterpenes. The hydroethanolic extract of *G. gnemon* showed the presence of diterpenes, phenolics, alkaloids, triterpenes and the aqueous extract showed the presence of phenolics,triterpenes, tannins, glycosides and alkaloids. The findings indicate that different phytochemicals have different solubility in various solvents.

Determination of total phenolics and total flavonoids

In the present study, among the three extracts (ethanol, hydroethanol and aqueous), maximum phenolic content was found in ethanolic extract (0.148 ± 0.025 mg/g dm.) and maximum

Table 1 : *Invitro* antioxidant activity of various extract of *G. gnemon* by DPPH assay

Plants extracts of *G. gnemon* Solvent	**Extract µg/ml**					**IC_{50} (µg/ml)**
	0.05	**0.10**	**0.50**	**1.0**	**2.0**	
	Percent inhibition					
Ethanol	51.61 ±4.00	56.00 ±1.000	68.83 ±4.000	72.88 ±1.000	79.28 ±5.000	5.73
Hydroethanol	52.60 ±2.000	65.3 ±1.000	70.93 ±4.000	79.50 ±2.000	82.10 ±3.000	5.17
Aqueous	61.18 ±2.000	72.54 ±4.000	78.00 ±2.500	80.86 ±2.000	84.20 ±1.000	4.36
Vitamin- C	72.17 ± 1.45	76.18 ±0.48	80.08 ±0.24	84.75 ± 0.24	92.80 ± 0.60	1.56

Table 2 : Reductive ability of various extracts of *G.gnemon.*

Solvent	**Reductive ability GAE µg/g (concentrations in µg/ml)**				
Extracts	100	200	300	400	500
Ethanolic	0.806 ±0.004	0.901 ±0.001	0.966 ±0.010	1.115 ±0.002	1.177 ±0.015
Hydroethanol	0.757 ±0.006	0.822 ±0.008	0.861 ±0.009	0.955 ±0.013	1.065 ±0.017
Aqueous	0.639 ±0.010	0.741 ±0.009	0.811 ±0.004	0.860 ±0.007	0.911 ±0.005
Vitamin- C	0.835 ±0.63	1.190 ±0.41	1.513 ±0.60	1.718 ±0.90	2.011 ±0.54

Table 3 : Superoxide anion scavenging activity of various extracts of *G.gnemon*

Solvent	***Superoxide anion scavenging activity* Per cent inhibition**						**IC_{50} (µg/ml)**
Extracts	10	20	40	60	80	100	
Ethanol	55.49 ±0.445	59.63 ±0.409	63.55 ±0.478	72.00 ±0.204	74.35 ±0.204	83.05 ±0.803	3.44
Hydro-ethanol	56.01 ±0.503	57.78 ±0.459	62.08 ±0.792	71.90 ±0.644	75.96 ±0.174	81.92 ±0.416	3.68
Aqueous	55.23 ±0.692	64.15 ±0.465	72.16 ±0.580	76.15 ±0.597	80.11 ±0.520	88.26 ±0.921	3.18
Vitamin-C	60.00 ±0.41	67.16 ±0.43	76.60 ±0.40	80.11 ±0.62	86.44 ±0.52	95.97 ±0.91	2.18

flavonoid was found in aqueous extract (0.048 ± 0.008 mg/g dm.) This indicate that phenolic compound of *G. gnetum* has high solubility in ethanol while flavonoids have maximum solubility in water. The principal antioxidant constituent of plants based products are phenolics and flavonoids. Plant extracts rich in phenolic acids exhibit strong antioxidant and antiradical activity in vitro (Mary et al., 2003). Parshad et al., (1998 and Chang et al., 2007) have reported that phenolics and flavonoids are scavengers of reactive oxygen species (ROS) and are viewed as promising therapeutic drugs for free radical pathogenesis. They are rich in polyphenolic compounds, has a potent antioxidant property to scavenge free radicals that cause oxidative damage to lipids, proteins, and nucleic acids (Shui and Leong, 2004).

Antioxidant Activity

For all the extracts there were gradual increase in RSA with increasing concentration of extract in the range of 0.05 μg/ml to 2.0 μg/ml. Among the extracts best antioxidant activity based on DPPH reduction was observed in case of aqueous extract. In case of 0.05 μg/ml, RSA was 61.18 Beyond this there was dose dependant increase in antioxidant activity and for 2.0 μg/ml RSA% was 84.20% The IC-50 value for aqueous extract was 4.36 μg/ml. The corresponding value for vitamin -C was 72.17 / to 92.8/ with IC-50 value of 1.56 μg/ml. The antioxidant activity based on reducing power assay was comparable to that of DPPH reduction assay. Reductive ability was assayed with varying concentration of extract in the range of 100 to 500 μg/ml with an increment of 100 μg/ml. For reductive ability best observation were recorded for ethanolic extract. For 100 μg/ml, reductive ability was 0.806 GAE mg/g.dm. There was a linear dose dependant increase in reductive ability and for 500 μg/ml, the reductive ability was highest with 1777GAE mg/g.dm. However, hydroethanolic extract and aqueous extracts also exhibited good degree of reductive ability. By comparison vitamin -C exhibited reductive ability in the range of 0.835 GAE mg/g.dm to 2.011 GAE mg/g.dm. For 100 and 500 μg/ml respectively Superoxide anion scavenging activity was assayed with extracts in increasing concentration from 10 to 100 μg/ml. For all the extracts there were linear dose dependant increase in antioxidant activity. Best observation was recorded for aqueous extract. For lowest dose of 10 μg/ml antioxidant activity was 55.23 % which increased progressively with increase in concentration of extract. For highest dose of 100 μg/ml it was 88.26%. By comparison for the standard vitamin-C, antioxidant activity varied from 66.00 to 95.97% for 10 and 100 μg/ml respectively. The trend was further reflected in the IC-50 values. A number of workers working with different vegetables, fruits and other plants have reported antioxidant activities of varied degree Okamura et. al 1993, (Liu et. al 2003, Ayoola et. al 2006). Such antioxidant activities are mainly attributed to phytochemicals like phenolics, flavonoids etc. Robak and Gryglewsky, 1988 (Gulcin et. al. 2005). There are reports that G.netum gnemon is used in folklore medicine to treat convulsions and for arthritis, bronchitis and asthma (Iliya et. al. 2002). The stilbenoids obtained from *G. gnemon* are known to be responsible for various

antioxidant and antimicrobial activity (Iliya et. al 2002). In traditional medicine system it is the crude extract which is administered. However, the crude extracts of plants are pharmacologically more active than the isolated active principles due to the synergistic effects of various components present in the whole extract (Hamberger et. al 1991). The findings of the present study with support from literature lend support to the traditional knowledge prevalent in N.E India about the medicinal values of *G.netum gnemon*.

ACKNOWLEDGEMENT

The Authors are grateful to the Department of Biotechnology (DBT), New Delhi, Govt. of India for financial help and Director of Research, AAU, Khanapara for providing necessary facilities.

REFERENCE

1. Ayoola G A., Sofidiya T., Odukoya O. and Coker H.A.B. (2006) Phytochemical screening and free radical scavenging activity of some Nigerian Medicinal plants. *Journal of Pharmaceutical Science and Pharmaceutical Practice*, 8:133-136
2. Chang H., Huang G., Agrawal D.C., Kuo C., Wu C. and Tsay H. (2007) Antioxidant activities and polyphenol contents of six folk medicinal ferns used as "Gusuibu". *Botanical Studies*, 48:397-406
3. Cradiz R.T., Florido H.B. and Bago (2001) *Gnetum gnemon* In *Research Information Series on Ecosystems*,13(2):16
4. Devasagayam T.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S.S. and Lele R.D. (2004) Free radicals and antioxidants in human health: current status and future prospects. *Journal Association Physician India*, 52: 794-804
5. Fredrick C. A., Onyekaba T.U., Charity C.E., Chibueze I. and Valentine C.E. (2013) Preliminary phytochemical investigations and evaluation of antimicrobial activity of nhexane extract of the leaves of *Synclisia scabrida* family menispermaceae. *Research Journal Pharmaceutical Science*, 2(3):1-5
6. Gulcin I., Alici HA. and Cesur M. (2005) Determination of *in-vitro* antioxidant and radical scavenging activities of protocol. *Pharmacology Bulletin*, 53:281-285
7. Hamberger M. and Hastettman K. (1991) Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry, 30:3864-3874
8. Harborne J. B. (1991) Phytochemical Methods. *A Guide to Modern Techniques of Plant Analysis*, Chapman and Hall, London, 176-201
9. Iliya I., Ali Z., Tanaka T., Iinuma. M., Furusawa M., Nakaya K.I., Murata J. and Darnaedi D.(2002) Four new stilbene oligomers in the root of *Gnetum gnemon*. Helvetica Chimica Acta, 85(8): 253
10. Jayaprakash G.K., Singh R.P. and Sakariah K.K. (2001) Antioxidant activity of grape seed extracts on peroxidation models. *Journal of Agricultural Food Chemistry*, 55:1018-1022
11. Krishnaiah D., Devi T., Bono A. and Sarbtly R. (2009) Studies on phytochemical constituents of six Malaysian Medicinal plants. *Journal of Medicinal Plant Resource*, 3(2): 67-72

12. Liu R.H. (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American Journal of Clinical Nutrition*, 78(3): 517 -520
13. Mary N.K., Achuthan C.R., Babu B.H. and Padikkala J. (2003) *In vitro* antioxidant and antithrombotic activity of Hemidesmus indicus (L) R. Br. *Journal of Ethnopharmacology*, 87 : 187-191
14. Okamura H., Mimura A.,Yakou Y., Niwano M. and Takahara Y. (1993) Antioxidant activity of tannins and flavonoids in eucalyptus rostrata.*Phytochemistry*,33:557-561
15. Ordonez A.A.L. Gomez J.G., Vattuone M.A.and Isla M.I. (2006) Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry*, 97:452–458
16. Parshad R., Sanford KK., Price FM., Steele VE., Tarone RE., Kelloff GJ.and Boone CW.(1998) Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells. Anticancer Research,18:3263-3266
17. Robak J. and Gryglewski RJ. (1988) Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, 37: 837–841
18. Shui G.H. and Leong L.P. (2004) Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *Journal of Chromatography*, 67-75