PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 9 (11): 1074-1077, 2010
ISSN 1680-5194

Evaluation of *in-vitro* Antioxidant Activities of Methanol Extracts of *Persea americana* and *Cnidosculus aconitifolius*

M.F. Asaolu[1], S.S. Asaolu[2], J.B. Fakunle[3], B.O. Emman-Okon[3], E.O. Ajayi[3] and R.A. Togun[4]
[1]Department of Biochemistry, University of Ado-Ekiti, P.M.B. 5363, Ado- Ekiti, Nigeria
[2]Department of Chemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria
[3]Department of Chemical Pathology, Obafemi Awolowo University, Ile-Ife, Nigeria
[4]Department of Heamatology and Immunology, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract: The present study was undertaken to investigate the antioxidant activities of *Persea americana* and *Cnidosculous aconitifolius*. The *in-vitro* antioxidant activity of the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* was evaluated using various experimental methods such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nitric oxide and reducing power radical- scavenging activity assay. Phytochemical screening as well as the amounts of total phenol and flavonoids were also determined. The present study revealed that both the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* possess significant antioxidant activities. However, *Persea americana* was found to have higher radical scavenging activity than *Cnidosculous aconitifolius* but the phenol content of *Cnidosculous aconitifolius* was higher than that of *Persea americana* whereas *Persea americana* was observed to possess more flavonoids than *Cnidosculous aconitifolius*.

Key words: Methanol extracts, radical-scavenging activity, antioxidant activities, phytochemical screening

INTRODUCTION

The development of a wide range of diseases such as malaria, diabetes, cardiovascular diseases and Parkison's diseases to mention few have been attributed to oxidative stress (Kremsner *et al*., 2000; Austin *et al*., 1994; Liao *et al*., 1997). Free radicals which are the products of oxidative stress (Utpal *et al*., 2008) are aggravated in these disease states leading to decrease in the antioxidant defense system.

Antioxidants have been found to play a major role in protecting the human body against damage induced by reactive free radicals (Halliwell and Gutteridge, 1990; Mates *et al*., 1999) by reacting with free radicals, chelating and also by acting as oxygen scavenger (Shahidi and Wanasundara, 1992; Buyukokuroglu *et al*., 2001).

Many studies have revealed that natural products and their derivatives possess efficient antioxidative characteristics (Rhee *et al*., 2009; Asaolu *et al*., 2010b). Besides, flavonoids and phenols, which are widely distributed in plants are known for their anticarcinogenic, antioxidant, inflammatory as well as free radical scavenging potentials (Miller, 1996; Frankel, 1994).

Worldwide, interest in natural products as antioxidants in reducing free radical induced tissue damage has led to a greater appreciation of the therapeutic potentials of plants. The use of natural antioxidants symbolizes safety in contrast to the synthetic products (Patel *et al*., 2010).

The presence of some phytochemicals and antioxidants has been reported in *Persea americana* and *Cnidosculous aconitifolius* which explained the hypotensive action of these plants in the treatment and management of cardiovascular diseases (Asaolu *et al*., 2010a, b, c). In this study, our purpose is to investigate and compare the *in vitro* antioxidative capacities of methanol extracts of *Persea americana* and *Cnidosculous aconitifolius*.

MATERIALS AND METHODS

Plant materials

Collection and identification of plant materials: Fresh leaves of *Persea americana* and *Cnidosculous aconitifolius* were collected from a farm in Ado-Ekiti, Ekiti State, Nigeria. Taxonomic identification of the plants was made in the Department of Plant Science, University of Ado-Ekiti, Nigeria.

Preparation of extracts: The leaves of *Persea americana* and *Cnidosculous aconitifolius* were washed with distilled water, air dried and ground into fine powder using a blender. The powdered leaves were defatted with n-hexane in soxhlet extractor and then extracted with methanol at room temperature. The extracts were concentrated using rotary evaporator.

Analysis: The preliminary phytochemical screening of the methanol extracts of *Persea americana* and *Cnidosculous aconitifolius* was carried out using the

Corresponding Author: M.F. Asaolu, Department of Biochemistry, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria

method described by Sofowora (1986). Total phenols and flavonoids contents of the extracts were also determined (Ebrahimzadeh *et al*., 2009). Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of the extracts was performed as described by Batubara *et al*. (2009) while nitric oxide radical scavenging activity assay was carried out using the method of Green *et al*. (1982). The reducing power of the methanol extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* was evaluated according to the method of Okhawa *et al*. (1979).

Statistical analysis: Data were expressed as mean±SEM. The significant differences between *Persea americana* and *Cnidosculous aconitifolius* were assessed by Student's t test. A probability value of $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The results obtained from the phytochemical screening of the leaves of *Persea americana* and *Cnidosculous aconitifolius* showed the presence of saponins, tannins, flavonoids, alkaloids, phenols, anthraquinones, triterpenes and phlobatannins in these plants (Table 1). In DPPH radical-scavenging activity assay, the radical scavenging activity of the methanol extract of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* increases with increasing concentration (Table 3) but was less than that of BHA which served as the reference compound. This may account for the uses of DPPH as a substrate in evaluation of antioxidative activity of antioxidants (Duh and Yen, 1995). However, the scavenging effect was higher in *Persea americana* than in *Cnidosculous aconitifolius* (Tables 3 and 4). It can therefore be concluded that the extracts of the leaves of *Persea americana* and *Cnidosculous aconitifolius* exhibit a noticeable effect on scavenging free radicals.

Tables 5 and 6 presents the percentage inhibition of nitric oxide generation by methanol extracts of *Persea americana* and *Cnidosculous aconitifolius* respectively. The antioxidant activity of methanol extracts of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* was much weaker than that of quercetin which was used as a standard. Similar observation has been reported for other medicinal plants (Komal *et al*., 2010). The ability of the methanol extract of *Persea ameriana* to effectively scavenge free radical was higher compared with that of *Cnidosculous aconitifolius*.

The reducing power by methanol extract of *Persea americana* and *Cnidosculous aconitifolius* is as presented in Tables 7 and 8 respectively. The antioxidant activity of methanol extract was less than that of ascorbic acid.

Table 1: Phytochemical screening of methanol extracts of the leaves of *Cnidosculous aconitifolius* and *Persea americana*

Phytochemicals	*Cnidosculous aconitifolius*	*Persea americana*
Saponins	+++	++
Tannins	+	+
Flavonoids	++	++
Glycosides	ND	ND
Alkaloids	+++	+++
Phenols	++	+++
Anthraquinones	++	+++
Triterpenes	+	++
Steroids	ND	ND
Phlobatannins	+	++
Cardenolides	ND	ND

Table 2: % phenolic and flavonoid contents of methanol extracts of the leaves of *Cnidosculous aconitifolius* and *Persea americana*

Plant	Phenol (%)	Flavonoids (%)
Cnidosculous aconitifolius	1.80±0.43	0.35±0.11
Persea americana	1.22±0.52	0.58±0.09

Data are expressed as mean±SEM of triplicate tests

Table 3: Percentage DPPH radical scavenging activity of methanol extracts of the leaves of *Persea americana* and Butylated Hydroxyl Anisole (BHA)

Concentration (µg/ml)	% Inhibition by methanolic extract of *Persea americana*	% Inhibition by BHA
100	40.58±3.22	60.11±4.32
200	53.44±5.29	70.30±3.81
300	61.23±3.89	75.55±4.00
400	68.38±3.61	83.22±2.10
500	73.49±2.81	89.10±3.11
600	80.11±5.67	92.12±4.30

Data are expressed as mean±SEM of triplicate tests

Table 4: Percentage DPPH radical scavenging activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and Butylated Hydroxyl Anisole (BHA)

Concentration (µg/ml)	% Inhibition by methanolic extract of *Cnidosculous aconitifolius*	% Inhibition by BHA
100	30.33±2.11	40.23±1.90
200	41.34±3.22	50.11±2.14
300	52.46±2.81	60.22±1.14
400	59.45±2.51	71.34±4.88
500	61.67±5.98	79.23±2.14
600	72.78±6.54	83.14±4.23

Data are expressed as mean±SEM of triplicate tests

Generally, the results of the present study indicate that the methanol extracts of the leaves of both *Persea americana* and *Cnidosculous aconitifolius* showed strong antioxidant activity. This might be as a result of the presence of some phytochemicals most importantly flavonoids and phenols which are well known

Table 5: Percentage Nitric oxide radical scavenging activity of methanol extracts of the leaves of *Persea americana* and curcumin

Concentration (µg/ml)	% Inhibition by methanolic extract of *Persea americana*	% Inhibition by curcumin
40	20.89±6.82	44.33±1.89
80	38.11±4.18	55.49±2.67
100	58.41±2.18	63.34±3.62
120	65.33±1.21	71.38±2.10
140	70.44±3.42	80.14±3.90
160	75.31±4.11	85.77±4.20

Data are expressed as mean±SEM of triplicate tests

Table 6: Percentage Nitric oxide radical scavenging activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and curcumin

Concentration (µg/ml)	% Inhibition by methanolic extract of *Cnidosculous aconitifolius*	% Inhibition by curcumin
40	18.49±2.18	40.21±2.33
80	33.64±3.11	51.31±3.84
100	52.14±4.31	60.41±2.00
120	61.22±2.17	68.81±1.95
140	68.21±1.48	75.22±4.80
160	73.14±2.33	82.19±3.18

Data are expressed as mean±SEM of triplicate tests

Table 7: Percentage antioxidant activity of methanol extracts of the leaves of *Persea americana* and ascorbic acid in reducing power method

Concentration (µg/ml)	Reducing power of methanol extract of *Persea americana*	Reducing power of methanol extract of Ascorbic acid
50	42.17±2.14	51.92±2.18
100	51.34±2.64	59.22±3.27
150	65.22±5.36	70.18±2.14
200	72.31±4.11	72.48±4.22
250	83.47±3.18	90.50±3.81
300	90.12±2.41	96.00±4.81

Data are expressed as mean±SEM of triplicate tests

Table 8: Percentage antioxidant activity of methanol extracts of the leaves of *Cnidosculous aconitifolius* and Ascorbic acid in Reducing Power method

Concentration (µg/ml)	Reducing power of methanol extract of *Cnidosculous aconitifolius*	Reducing power of methanol extract of Ascorbic acid
50	31.21±2.81	43.16±4.18
100	43.11±1.89	47.38±3.01
150	55.22±4.10	65.14±4.08
200	70.05±3.21	67.98±3.21
250	75.14±2.38	85.84±5.24
300	85.22±3.18	90.49±4.21

Data are expressed as mean±SEM of triplicate tests

antioxidants. Phenols in plants are known for their scavenging ability as a result of the presence of hydroxyl groups in them. In some plants, a significant association has been reported between phenols and antioxidant activity (Gulcin *et al*., 2002) as phenols help in stabilizing lipid peroxidation (Yen *et al*., 2005). However, antioxidative activity of methanol extract of the leaves of *Persea americana* was much more effective than that of *Cnidosculous aconitifolius* but less effective than that of ascorbic acid, quercetin and BHA. This may be due to the presence of some chemicals in the methanol extract of the leaves of *Persea americana* which play a significant role in the antioxidant capacity. This needs to be investigated.

REFERENCES

Asaolu, M.F., S.S. Asaolu, I.G. Adanlawo, B.T. Aluko, Y.R. Alli-Smith, Y. Ibitoye and A.M. Abidakun, 2010a. Comparative chemical compositions of the leaves of some selected antihypertensive medicinal plants in Nigeria. Der Pharm. Chem., 2: 11-15.

Asaolu, M.F., S.S. Asaolu and I.G. Adanlawo, 2010b. Evaluation of phytochemicals and antioxidants of four botanicals with antihypertensive properties. Int. J. Pharm. Bio. Sci., 1: 1-7.

Asaolu, M.F., S.S. Asaolu, A.O. Oyeyemi and B.T. Aluko, 2010c. Hypolipemic effects of methanolic extracts of *Persea americana* seeds in hypercholestrolemic rats. J. Med. Med. Sci., 1(4).

Austin, M.A., J.E. Hokanson and J.D. Brunzell, 1994. Oxidative stress and ageing. Curr. Opin. Lipid., 5: 395-403.

Batubara, I., T. Mitsunaga and Ohashi, 2009. Screening anti-acne potency of Indonesian medicinal plants: Antibacterial, lipase inhibition and antioxidant activities. J. Wood Sci., 55: 230-235.

Buyukokuroglu, M.E., I. Gulcin, M. Okatay and O.I. Kufrevioglu, 2001. *In-vitro* antioxidant properties of dantrolene sodium. Pharmacol. Res., 44: 491-494.

Duh, P.D. and G. Yen, 1995. Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light. Food Chem., 54: 127-131.

Ebrahimzadeh, M.A., S. Eshanifar and B. Eslami, 2009. *Sambucus ebulus elburensis* fruits: A good source for antioxidants. Phcog. Mag., 4: 447-450.

Frankel, J.L., 1994. Nutritional benefits of flavonoids. Paper presented at International conference on food factors: Chemistry and Cancer Prevention. Hamamatsu, Japan; Abstract C6-2.

Green, L.C., D.A. Wanger, J. Glogowski, P.L. Skipper, J.K. Wishnok and S.P. Tannenbaum, 1982. Antioxidant properties. Biochem., 126: 131-138.

Gulcin, I., M.E. Buyulcokuroglu, M. Oktay and O.I. Kufrevioglu, 2002. On the *in vitro* antioxidative properties of melatonin. J. Pineal. Res., 33:167-171.

Halliwell, B. and J.M.C. Gutteridge, 1990. Role of free radicals and catalytic metal ions in human disease: An overview. Method Enzymol., 186: 1-85.

Komal, M., K. Parimal and S.S. Khadabadi, 2010. *In vitro* antioxidant activity of methanolic extract of *Erythrina indica*. Der Pharm. Lettr., 2: 16-21.

Kremsner, P.G., B. Greve, B. Lell, D. Luckner and D. Schmid, 2000. Malaria anaemia in African children associated with high oxygen radical production. Lancet, 355: 40-41.

Liao, L., R.M. Starzyk and D.N. Granger, 1997. Arterioscler. Thromb. Vasc. Biol., 17: 437-444.

Mates, J.M., C. Perez-Gomez and I. Nunez de Castro, 1999. Antioxidant enzymes and human diseases. Clin. Chem., 32: 595-603.

Miller, A.L., 1996 Antioxidant flavonoids: Structure, function and clinical usage. Alt. Med. Rev., 1: 103.

Okhawa, H., W. Ohishi and K. Yagi, 1979. Analytical Biochemistry, 95: 351.

Patel, R.V., P.R. Patel and S.S. Kajal, 2010. Antioxidant activity of some selected medicinal plants in Western Region of India.

Rhee, M.H., H.J. Park and J.Y. Cho, 2009. Salicornia herbacea: Botanical, chemical and pharmacological review of halophyte marsh plant. J. Med. Plants Res., 3: 548-555.

Shahidi, F. and P.D. Wanasundara, 1992. Phenolic antioxidants. Crit. Rev. Food. Sci. Nutr., 32: 67-103.

Sofowora, A., 1986. The state of medicinal plant research in Nigeria. Ibadan University Press, pp: 113-160.

Utpal, B., A. Sahu, S. Ali, L. Kasoju, A. Singh and H. Sharanabasava, 2008. Oxidative stress and free radical changes. Food Res. Int., 41: 1-15.

Yen, W.J., L.W. Chang and P.D. Duh, 2005. Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. Food Sci. and Tech., 38: 193-200.