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In-vitro antioxidant activity of *Clerodendron inerme* (l.) *Ga*ertn leaves

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ABSTRACT

The antioxidant properties of methanolic and petroleum ether extract of *Clerodendron inerme*, that probably involve free radical mechanisms, were evaluated by the methods, namely the DPPH (1,1-diphenyl-2-picryl hydrazyl) assay, hydroxyl scavenging assay and the lipid peroxidation assay. The *Clerodendron inerme* methanol extract (CIME) was most active in the DPPH test with an IC_{50} value of 19.20 ± 0.27 μg/mL. The hydroxyl radical scavenging activity of the pet ether extract showed maximum activity 69.28 ± 2.1% at 100 μg/mL. In the lipid peroxidation assay the methanolic extract showed the highest effectiveness with inhibition values of 36.38 ± 1.3 % at 100 μg/mL. The present study suggests that the tested extract of the plant have moderate to potent antioxidant activity.

Keywords: *Clerodendron inerme* (L.) Gaertn.; Antioxidants; DPPH assay; lipid peroxidation assay.

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INTRODUCTION

In living systems, free radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually pro-duced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxi-dase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics.In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can result inincreased radical activity and damage. Free radi-calsor oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes [1-4].

Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties [5-12].

Clerodendron inerme (L.) Gaertn (Family- Verbenaceae) is a straggling much branched shrub 0.9 - 2.1 m. long, sometimes scandent [13,14] are found in the wastelands, hedges and banks of river, sea in various tropical parts of India [14,15]. It is sufficiently found in the Coastal South Orissa of India. The plant is well known under vernaculars as 'Glory Bower genus, Garden Quinine' in English [16-17] 'Cholora' in Oriya, and 'Lanjai, Sangkupi' in Hindi and 'Kundali, Samudrayuthika, Vanajai, Vanayuthika' in Sanskrit. The powdered leaves are useful in fresh juice of leaves used as alternative in scrofula and venereal diseases [14]; poultice in buboes [13, 15]; vermifuge, used as bitter tonic and febge in malaria especially of children. A bath prepared with them is used in mania [13]. Considering the importance of this area, antioxidant potential of *Clerodendron inerme* was evaluated. These plant extracts are used in Asian countries for various diseases where free radicals are thought to be involved. Three methods namely free radical scavenging activity by DPPH test, hydroxyl radical scavenging activity and lipid peroxidation by thiobarbituric acid (TBA) assay were employed.

MATERIALS AND METHODS

Plant material

Clerodendron inerme leaves were collected during the month of August 2007 from the rural belt of Mohuda hills in Ganjam District, Orissa, India, identified and authenticated by Prof. S. K. Dash, HOD, PG Department of Bioscience, College of Pharmaceutical Sciences, Mohuda; comparing with the voucher specimen (CI-I) present in the herbarium, has been kept in the laboratory for future references. The collected plants were washed and air-dried under the shade, cut into small pieces, powdered by a mechanical grinder and passed through 40-mesh sieve and stored in a closed vessel for future use.

Preparation of extract

The dried, powdered leaves of *Clerodendron inerme* (250 g) were extracted successively with 1200 mL of petroleum ether (60–80°C) and 1200 mL of methanol in soxhlet apparatus. A dark green coloured petroleum ether extract was obtained. The same powdered leaves (marc), after proper drying, were extracted with methanol (18 h) to produce a greenish brown semisolid mass. The extractions were carried out until the solvents became colourless. These extracts were again dried and concentrated by evaporating the solvent completely under vacuum at the range of boiling points of solvent (Methanol at 64°C and petroleum ether at 35°C) using rotatory evaporator. The methanol extract yield 15.7% w/w and petroleum ether extract yield 3.0% w/w with respectively. The chemical constituents of the extract was identified by qualitative analysis and confirmed by the thin layer chromatography. The dried extract was stored at 4°C. These extract used to test the free radical scavenging activity, hydroxyl scavenging activity and lipid peroxidation activity. All extractive solvents are of analytical grade reagents (AR) and purchased from S.D. Fine Chemicals, Mumbai, India.

DPPH free radical scavenging activity

The free radical scavenging activity of the extracts was determined using DPPH. The DPPH assay measures hydrogen atom (or one electron) donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is a purple colored stable free radical; it becomes reduced to the yellow colored diphenyl picryl hydrazine. A methanol and petroleum ether DPPH-solution (0.15%) was mixed with serial dilutions (1 to 50 μg/mL) of extracts and shaken vigorously. The tubes were allowed to stand at 27^0C for 15 min. The change in absorbance of samples was measured at 517 nm using a UV spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage [18].

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method reported by Klein and co-workers. Various concentrations (5, 10, 20, 40, 60, 80 and 100 μg/mL in methanol and petroleum ether) of extracts were taken in different test tubes and evaporated to dryness. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80^0C-90^0C for 15 min. The reaction was terminated by the addition of 1mL of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and made up to 1L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank [19].

Lipid peroxidation by thiobarbituric acid (TBA) assay

TBA reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm. Normal albino rats of the Wistar strain were used for the preparation of liver homogenate. The perfuged liver was isolated and 10% w/v homogenate was prepared with 0.15 M KCl. The homogenate was centrifuged at 1000 g for 15 min, and clear cell free supernatant obtained was used for the study of *in vitro* lipid peroxidation. Different concentrations (5, 10, 20, 40, 60, 80 and 100 μg/mL in methanol and petroleum ether) of extracts were taken in test tubes and evaporated to dryness. One milliliter of 0.15 M KCl and 0.5 mL of rat liver homogenate were added to the test tubes. Peroxidation was initiated by 100 μl of 0.2 mM ferric chloride. After incubation at 37^0C for 30 min, the reaction was terminated by adding 2 mL of ice-cold HCl (0.25 M) containing 15% trichloroacetic acid (TCA), 0.38% TBA, and 0.5% butylated hydroxytoluene (BHT). The reaction mixtures were heated at 80^0C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatant was measured at 532 nm. An identical experiment was performed to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any extract. The percentage inhibition was calculated [20].

Statistical analysis

All the values are expressed in mean ± SEM and one-way ANOVA using Dunnets test was performed.

RESULTS AND DISCUSSION

Three *in-vitro* tests, the DPPH radical scavenging action, hydroxyl scavenging and the lipid peroxidation assay for antioxidant activity were used to assess the antioxidant properties of methanolic and petroleum ether extracts of *Clerodendron inerme.* Together all the three tests provide a better assessment of antioxidant properties.

Table 1: DPPH radical scavenging assay

Plant extract	IC_{50} value ± SEM (n = 3)
Clerodendron inerme methanolic extract	19.20 ± 0.27*
Clerodendron inerme petroleum ether extract	28.42 ± 0.43

*P<0.05, Significance vs standard (n = 3).

Free radical scavenging potential of MeOH extract at different concentrations was tested by the DPPH method. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to 1, 1-diphenyl-2-picrylhydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. Results showed that the methanolic extract of *Clerodendron inerme* was most active in the DPPH test with an IC_{50} value of 19.20 ± 0.27 μg/mL followed by petroleum ether extract with IC_{50} value of 28.42 ± 0.43 μg/mL (Table1).

Table 2: Hydroxyl radical scavenging activity

Plant name	% Hydroxyl scavenging activity ± SEM						
	5 μg/mL	10 μg/mL	20 μg/mL	40 μg/mL	60 μg/mL	80 μg/mL	100 μg/mL
Clerodendron inerme methanolic extract	2.01 ± 0.1	3.42 ± 0.2	7.81 ± 0.6	11.24 ± 1.4	16.24 ± 1.6	21.21 ± 1.5	30.86 ± 2.3
Clerodendron inerme petroleum ether extract	9.11 ± 2.3	21.42 ± 0.2	39.75 ± 1.4	42.24 ± 1.6	57.11 ± 2.6*	62.42 ± 2.0*	69.28 ± 2.1*
Vitamin C	11.24 ± 1.0	20.42 ± 1.0	31.71 ± 2.4	39.24 ± 1.6	61.25 ± 2.2	68.12 ± 1.4	80.24 ± 1.2

*P<0.05, Significance vs standard (n = 3).

Table 3: Lipid peroxidation assay

Plant name	Lipid peroxidation % Inhibition ± SEM (n = 3)						
	5 μg/mL	10 μg/mL	20 μg/mL	40 μg/mL	60 μg/mL	80 μg/mL	100 μg/mL
Clerodendron inerme methanolic extract	2.28 ± 1.0	10.48 ± 1.6	18.68 ± 1.6	22.61 ± 1.6	28.11 ± 2.3	31.24 ± 2.5*	36.38 ± 1.3*
Clerodendron inerme petroleum ether extract	4.28 ± 1.1	7.72 ± 1.8	12.80 ± 1.2	19.62 ± 1.4	24.26 ± 2.1	30.44 ± 2.4*	39.14 ± 2.1*
Vitamin C	4.21 ± 2.1	13.22 ± 1.4	18.26 ± 0.2	21.83 ± 1.2	30.24 ± 2.6	36.68 ± 1.4	43.26 ± 0.3

*P<0.05, significance vs standard (n = 3).

Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells [21]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids [22]. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radicals formed by the oxidation react with DMSO to yield formaldehyde, which provides a convenient method for their detection by treatment with Nash regent.

The hydroxyl radical scavenging activity of the pet ether extract showed maximum activity 69.28 ± 2.1% at 100 μg/mL (Table 2). In the lipid peroxidation assay the methanolic extract showed the highest effectiveness with inhibition values of 36.38 ± 1.3 % at 100 μg/mL(Table 3). The activity of the extracts is attributed to their hydrogen-donating ability [23]. It is known that free radical cause auto-oxidation of unsaturated lipids in food [24]. On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid [22].

CONCLUSION

The data obtained reveal that the activity of methanolic extract in DPPH and lipid peroxidation assay *Clerodendron inerme* extract is more potent than petroleum ether extract of *Clerodendron inerme.* In hydroxyl

radical assay petroleum ether extract of *Clerodendron inermeis* is more potent than methanolic extract of *Clerodendron inerme.*

However, the present results suggest that the tested extract of the plant have moderate to potent antioxidant activity. Therefore the further investigations are necessary for encompassing the *in-vivo* behavior of the studied plant extracts.

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REFERENCES

[1] Saha MN, Alam MA, Aktar R, Jahangir R. Bangladesh Journal Pharmacology 2008; 3: 90-96.

[2] Polterait O. Current Org. Chem. 1997; 1: 415-440.

[3] Prior RL. American Journal of Clinical Nutrition 2003; 78: 570S-578S.

[4] Lee SE, Hwang HJ, Ha JS. Life Sci 2003; 73: 167-179.

[5] Makari HK, Haraprasad N, Patil HS, Ravikumar. The Internet J. Aesthetic and Antiaging Medicine 2008; 1: 1-10.

[6] Augustin S, Claudine M, Christine M, Christian R. Crit Rev Food Sciences 2005; 45: 287-306.

[7] Aviram M. Free Rad Res 2000; 33: S85-S87.

[8] Polterait O. Current Org Chem 1997; 1: 415-440.

[9] Prior RL. American Journal of Clinical Nutrition 2003; 78: 570S-578S.

[10] Trease GE and Evans WC. Pharmacognosy: A Physicians's Guide to Herbal Medicine. Bailliere, Tindall. London. 1989; 13: 886-893.

[11] Lu Y and Foo Y. Food Chem 2000; 75: 197-202.

[12] Koleva II, Beek TV, HLinssen JP, Groot AD and Evstatieva LN. Phytochemical Analysis 2002; 13: 8-17.

[13] Kirtikar KR, Basu BP. Indian Medicinal Plants, 1998; 3: 515-779.

[14] Chatterjee A, Pakrashi SC. The Treatise on Indian medicinal plants, 1995; 4: 222-223.

[15] Chopra IC, Nayar SL, Chopra RN. Glossary of Indian medicinal plants, 1956; 1: 71.

[16] Shrivastava N, Patel T. Medicinal and Aromatic Plant Science and Biotechnology 2007; 142-149.

[17] PLANTS 2001, National Plant Data Center, Baton Rouge, LA. Available: http://plants.usda.gov.

[18] Viturro C, Molina A, Schmeda-Hischmann G. Phytother Res 1999; 13: 422-24.

[19] Thabrew MI, Hughes RD, McFarlane IG. Antioxidant activity of *Osbeckia aspera*. Phytother Res 1998; 12: 288-90.

[20] Placer ZA, Cushman LL, Johnsom BC. Anal Biochem 1996; 16: 359-64.

[21] Shimada KK, Kujjikawa KY. J Agri Food Chem 1992; 40: 945-48.

[22] Jain PK, Veerasamy R, Sharma S, Agrawal RK. Turk J Biol 2008; 32, 197-202.

[23] Sherwin ER. J Am Oil Chem Soc 1978; 55: 809-14.

[24] Haslam E. J Natr Prod 1996; 59: 205-15.