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Evaluation of *in vitro* antioxidant activities of the methanol extracts of *Glinus oppositifolius* and *Trianthema decandra*

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SUMMARY

Glinus oppositifolius and *Trianthema decandra* belonging to the Ficoidaceae family were commonly used by tribal peoples for the treatment of liver disorders and cancer. The preliminary phytochemical screening of those plants showed the presence of flavonoids, terpenoids, tannins and saponins. The aim of this study was to evaluate the *in vitro* antioxidant activities of the methanol extracts of *Glinus oppositifolius* (MEGO) and *Trianthema decandra* (METD). The antioxidative capacities of MEGO and METD were determined by the following four complementary assay; DPPH radical scavenging assay, superoxide anion generation by xanthine-xanthine Oxidase assay, hydroxyl radical scavenging assay and Fe^{2+}–ascorbate induced by lipid peroxidation assay. The IC_{50} values of the both extracts were calculated from the inhibition curve. The IC_{50} MEGO and METD in DPPH, superoxide anion, hydroxyl radical scavenging and lipid peroxidation assay are 1.85, 7.31, 13.95, 22.82 and 2.21, 9.78, 14.87, 19.76 μg/ml respectively. Both the extracts exhibited a significant antioxidant effects.

Key words: Antioxidant; *Glinus oppositifolius*; *Trianthema decandra*

INTRODUCTION

In traditional medicinal system, the plant, *Trianthema decandra* belonging to the family, Ficoidaceae, is widely used in the treatments of asthma, hepatitis, cancer and suppression of menses. (Kirtikar and Basu, 1975). The plant, *Glinus oppositifolius* belonging to the same family, Ficoidaceae, is commonly used as stomachic, aperient, antiseptic, anticancer and suppressive agent of the lochia, bittter tonic for liver disorders. (Kirtikar and Basu, 1975; Chatterjee and Pakrashi, 1991). The preliminary phytochemical screening of those plants showed the presence of flavonoids, terpenoids, tannins and saponins. *Glinus oppositifolius* containing triterpenoids such as spergulagenin A, spergulatriol and spergulanic acid were reported earlier (Hariharan and Rangaswami, 1971; Kitagawa *et al*., 1976; Barua *et al*., 1980). Because of oxygen radical scavenging properties of different phytoconstituents derived from medicinal plants such as tannins, terpenoids, flavonoids, phenolic acids (Larson, 1988; Velioglu *et al*., 1998; Zheng *et al*., 2001; Cai *et al*., 2003) are used for the treatment of wide range of pharmacological properties. There has been growing interest in the investigation of the natural products from plants for the discovery of new antioxidants as well as an alternative route for the substitution of synthetic chemicals, side effects of which are always in question. The present study is aimed at assessing the *in vitro* antioxidative activities

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of the methanol extracts of *Glinus oppositifolius* and *Trianthema decandra.*

MATERIALS AND METHODS

Preparation of plant extracts

The whole plant material of *Glinus oppositifolius* was collected from Midnapore, West Bengal, during June-August when the plant was in full leaf and another plant, *Trianthema decandra* were collected from Kolli Hills Tamilnadu. The plant materials were taxonomically identified by the Botanical Survey of India, Shibpur, Howrah and the voucher specimen (GMC-1 and GMC-2) were retained in our laboratory for future reference. The collected plant material were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve number 40 and stored in an airtight container for further use. The air dried powered material of *Glinus oppositifolius* was defatted by extracting with petroleum ether in Soxhlet extraction apparatus. The defatted material was then extracted with methanol (80%). The solvent was completely removed under reduced pressure to obtain a dry mass and stored in vacuum dessicator. The yield of petroleum ether extract and methanol extracts were found to be 4.6% and 14.8% w/w respectively. The air dried power material of *Trianthema decandra* was also extracted with petroleum ether and methanol (80%) successively in Soxhlet apparatus. The solvent was also completely removed under reduced pressure and stored in vacuum dessicator. The yield of petroleum ether extract and methanol extracts were found to be 7.4 % and 13.8 % w/w respectively.

Reagents for estimation of biochemical markers and solvents were obtained from Sigma Chemical Co. and E-Merck, Mumbai respectively. The standard antioxidants were collected from Fine Chemicals. The colorimetric analysis was performed by using UV-Visible spectrophotometer, Merck. The rats used in Fe^{2+}-ascorbate induced by lipid peroxidation assay were supplied by Indian Institute of Chemical Biology (IICB), Kolkata.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of MEGO and METD were assessed using the DPPH free radical (Cuendet *et al*., 1997). 10 μl of the various concentrations of the extracts (from 1 μg/ml to 12 μg/ml in methanol) or standard was added to 5 ml of a 0.004% DPPH in methanol solution. DPPH in methanol solution was incubated at 37°C for 30 min. The degree of bleaching of the DPPH solution was measured at 517 nm. The corresponding blank reading was also taken. The formula used to calculate percentage of inhibition is as follows:

Percentage of inhibition = (Absorbance of control - Absorbance of test)/Absorbance of control

The antioxidant activity was expressed as IC_{50} (concentration in μg/ml is required to scavenge 50% DPPH free radical). The IC_{50} values of the extracts were calculated from the inhibition curve.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals. The attack of the hydroxyl radical on deoxyribose leads to the formation of TBARS, generated from the Fe^{3+}/ ascorbate/EDTA/H_2O_2 system (Kunchandy and Rao, 1990). The formed TBARS was measured at 532 nm.

Reaction mixture (1 ml) containing deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), H_2O_2 (1 mM), phosphate buffer (20 mM, pH 7.4) and various concentrations of methanol extract of *Glinus oppositifolius* and *Trianthema decandra* were incubated at 37°C for one hour. After the incubation period TBARS thus formed was measured at 532 nm (Ohkawa *et al*., 1979). The percentage inhibition was calculated by comparing the results of the various concentrations of the tested extract with that of the control group. The IC_{50}

values of the extracts were calculated from the inhibition curve.

Superoxide anion generation by xanthine-xanthine oxidase assay

Superoxide anion scavenging property was measured by using xanthine–xanthine oxidase system (Chang *et al.*, 1994). Reaction mixture (0.9 ml) containing 50 mM potassium phosphate buffer (pH 7.8), xanthine (0.05 mM) and NBT (0.6 mM) and various concentrations of the methanol extract of *Glinus oppositifolius* and *Trianthema decandra* in ethanol was incubated for 10 min. The reaction was initiated by the addition of 0.1 ml of xanthine oxidase (25 mU/ml in phosphate buffer). The mixture was allowed to stand at room temperature for 10 minutes and the absorbance of formazan chromophore thus formed was measured against a blank solution at 570 nm. The IC_{50} values of the extracts were calculated from the inhibition curve.

Fe^{2+}–ascorbate induced lipid peroxidation

Lipid peroxidation induced by Fe^{2+}–ascorbate system in rat liver homogenate was estimated by the method of Ohkawa *et al*. (1979). Reaction mixture (0.5 ml) containing 0.1 ml of 25% w/v rat liver homogenate in Tris–HCl buffer (20 mM, pH 7.0); 0.1 ml of KCl (30 mM); 0.1ml of $FeSO_4 \cdot 6H_2O$ (0.16 mM); 0.1 ml of ascorbic acid (0.06 mM) and 0.1 ml of various concentrations of the methanol extract of *Glinus oppositifolius* and *Trianthema decandra* were incubated at 37°C for one hour (Bishayee and Balasubhramanian, 1971). After the incubation period, the lipid peroxide formation was measured by using the method described elsewhere (Ohkawa *et al*., 1979). The IC_{50} values of the extracts were calculated from the inhibition curve.

RESULTS AND DISCUSSION

The antioxidative capacities of the methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* were evaluated by the following four complementary assay; DPPH radical scavenging assay, superoxide ($O_2^{\cdot}$) anion generation by xanthine-xanthine oxidase assay, hydroxyl ($OH^{\cdot}$) radical scavenging assay and Fe^{2+}–ascorbate induced by lipid peroxidation assay. The different concentrations of *Glinus oppositifolius* (MEGO), *Trianthema decandra* (METD) and standard drug providing 50% inhibition in the free radicals generation (DPPH, superoxide and hydroxyl) and lipid peroxidation *in vitro* were summarized in Tables 1, 2, 3 and 4.

MEGO and METD exhibited the significant DPPH radical scavenging property with IC_{50} of 1.85 and 2.20 μg/ml respectively. The DPPH assay method has been generally utilized to perform the free

Table 1. Effect of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on DPPH assay

Tested materials	Concentration (μg /ml)	Percentage of inhibition	IC_{50} (μg /ml)
MEGO	1.5	45.82 ± 1.56	1.85
	3.0	62.28 ± 1.84	
	6.0	80.68 ± 1.67	
	12.0	90.25 ± 2.23	
METD	1.5	42.38 ± 1.32	2.21
	3.0	57.26 ± 1.54	
	6.0	79.82 ± 1.94	
	12.0	88.23 ± 1.08	
L-ascorbic acid	1.5	47.25 ± 1.25	1.72
	3.0	64.82 ± 1.75	
	6.0	83.45 ± 1.65	
	12.0	96.82 ± 1.86	

All values represent mean ± S.E.M.

Table 2. Effect of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on Superoxide anion generation by Xanthine-Xanthine oxidase assay

Tested materials	Concentration (μg /ml)	Percentage of inhibition	IC_{50} (μg /ml)
MEGO	3.0	25.39 ± 1.48	7.31
	6.0	42.82 ± 2.02	
	12.0	70.45 ± 1.98	
	24.0	90.28 ± 1.96	
METD	3.0	20.55 ± 1.62	9.78
	6.0	31.15 ± 1.58	
	12.0	57.85 ± 1.12	
	24.0	80.38 ± 1.32	
Curcumin	3.0	22.35 ± 1.54	8.82
	6.0	35.45 ± 1.76	
	12.0	62.82 ± 1.85	
	24.0	85.68 ± 2.15	

All values represent mean ± S.E.M.

Table 3. Effect of methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on Hydroxyl radical scavenging activity

Tested materials	Concentration (μg /ml)	Percentage of inhibition	IC_{50} (μg /ml)
MEGO	6.0	27.35 ± 1.35	13.95
	12.0	43.27 ± 1.75	
	18.0	65.45 ± 2.65	
	24.0	85.82 ± 1.37	
METD	6.0	25.62 ± 1.01	14.87
	12.0	40.39 ± 2.17	
	18.0	60.85 ± 1.98	
	24.0	80.20 ± 1.63	
Curcumin	6.0	30.25 ± 1.78	13.12
	12.0	45.68 ± 2.53	
	18.0	72.39 ± 1.89	
	24.0	90.56 ± 1.67	

All values represent mean ± S.E.M.

radical scavenging effects of natural antioxidants (Blois, 1995). The hydrogen atoms or electron donation capabilities of corresponding extracts were measured from the bleaching of purple coloured methanol solution of DPPH. In this method, MEGO and METD showed significant antioxidant property with IC_{50} values of the nearly same range as that of standard antioxidant, L-ascorbic acid.

Another important method to evaluate antioxidative activity is Superoxide anion generation by Xanthine-Xanthine oxidase assay. Xanthine oxidase catalyzes the oxidation of Xanthine to uric acid in presence of molecular oxygen to yield $O_2^{\cdot}$ radical and H_2O_2 (Mayumi *et al*., 1993; Vina *et al*., 2000). Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion, perhydroxy radical and hydroxyl radical. Oxidative stress plays an important role in the pathogenesis of various diseases such as atheroclerosis,alcoholic liver cirrhosis,and cancer etc. The prevention of $O_2^{\cdot}$ radical generation is beneficial for the treatment of those diseases. The significant antioxidative activity with IC_{50} values 7.31 and 9.78 μg/ml respectively was shown by MEGO and METD. In Superoxide radical inhibition, it was found that MEGO was to be more effective and METD was less effective than curcumin, a

Table 4. Effect of the methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* on Fe^{+2} ascorbate induced lipid peroxidation

Tested materials	Concentration (μg /ml)	Percentage of inhibition	IC_{50} (μg /ml)
MEGO	2.5	5.42 ± 1.05	22.82
	5.0	15.29 ± 1.58	
	10	28.82 ± 2.05	
	20	47.28 ± 1.75	
	40	60.65 ± 1.23	
	80	85.56 ± 1.45	
	160	93.25 ± 1.62	
METD	2.5	5.22 ± 1.78	19.76
	5.0	13.92 ± 1.25	
	10	29.35 ± 0.98	
	20	51.12 ± 1.45	
	40	75.35 ± 1.39	
	80	83.25 ± 1.89	
	160	90.35 ± 1.48	
Quercitin	2.5	6.82 ± 1.24	15.95
	5.0	15.38 ± 1.44	
	10	35.29 ± 1.87	
	20	56.68 ± 1.94	
	40	78.25 ± 2.01	
	80	88.66 ± 1.65	
	160	95.28 ± 1.56	

All values represent mean ± S.E.M.

well-known antioxidant.

The hydroxyl radical is the most reactive oxygen radical (Nakken *et al*., 1965; Michaels *et al*., 1973), which is formed *via* Fenton's reaction in the living systems (Repine *et al*., 1981). In the oxidative metabolism, the detrimental byproduct, hydroxyl radical, causes the molecular damage of nerve in the living organism. These radicals have major direct or indirect role in several pathological conditions such as brain ischemia, parkinson's disease, hepatitis and carcinogenesis. In this assay method, MEGO and METD significantly scavenged the hydroxyl radical generated from Fe^{+3}-ascorbate-EDTA-H_2O_2 system (Fenton's reaction). The hydroxyl scavenging property of MEGO and METD were shown to be less effective than the standard antioxidant, curcumin. The IC_{50} values of MEGO, METD and curcumin were 13.95, 14.87 and 13.12 μg/ml respectively.

Poly unsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low density lipoproteins. The PUFAs allow for fluidity of cellular membranes. A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. Lipid peroxidation could cause cancer promoting mutations or cell death. The propagation cycle of lipid peroxidation is broken by either enzymatic inactivation of ROS or non enzymatic reactions due to the intervention of free radical scavengers and antioxidants (Varga *et al.,* 2001). Lipid peroxidation generates a number of degradation products, such as malondialdehyde, which is found to be an important cause of cell membrane destruction and cell damage (Yoshikawa *et al*., 1997). MEGO and METD demonstrated effective antioxidant activity in a concentration dependent manner on Fe^{+2}–ascorbate induced lipid peroxidation. It was found that methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* were to be less effective than Quercitin, a well-known antioxidant.

From these results, it can be considered that methanol extracts of *Glinus oppositifolius* and *Trianthema decandra* have good antioxidant activity.

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REFERECCES

Barua AK, Banerjee SK, Das Gupta C, Basu K, Bose L, Chakrabarti. (1980) The structure and stereochemistry of spergulagenol, a triterpene from *Mollugo pergula*. *Phytochemistry* **19**, 1551-1553.

Bishayee S, Balasubramanian AS. (1971) Lipid peroxide formation in rat brain. *J. Neurochem.* **18**, 909–920.

Blois MS. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **181**, 1199-1200.

Cai YZ, Sun M, Crorke H. (2003) Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric. Food Chem.* **51**, 2288-2294.

Chang WS, Chang YH, Lu FJ, Chiang HC. (1994) Inhibitory effects of phenolics on Xanthine Oxidase. *Anticancer Res*. **14**, 501-506.

Chatterjee A, Pakrashi SC. (1991) *The Treatise on Indian Medicinal Plants,* Vol. I, p. 78, Publication & Information Directorate, New Delhi.

Cuendet M, Hostettmann K, Potterat O. (1997) Iridoid glucosides with free radical scavenging properties from Fagraea blumei. *Helv. Chim. Acta* **80**, 1144–1152.

Dang MN, Takacsova M, Nguyen DV, Kristianova K. (2001) Antioxidant activity of essential oils from various species. *Nahrung* **45**, 64-66.

Dapkevicius A, Venskutonis R, Van Beek TA, Linssen JPH. (1998) Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.* **77**, 140-146.

Halliwell B. (1994) Free radicals, antioxidants and human disease – curiosity, cause or consequence. *Lancet* **344**, 721-724.

Hariharan V, Rangaswami S. (1971) Steroids and triterpenoids from the roots of *Mollugo spergula*. *Phytochemistry* **10**, 621-624.

Kirtikar KR, Basu BD. (1975) *Indian Medicinal Plants*, Vol. II, 2nd Ed. p. 1182, M/S Bishensingh, Mahendra pal Singh, New Connaught Place , Dehra Dun.

Kirtikar KR, Basu BD. (1975) *Indian Medicinal Plants,* Vol. II, 2nd Ed. p. 1184. M/S Bishensingh, Mahendra pal Singh, New Connaught Place, Dehra Dun.

Kitagawa I, Yamanaka H, Nakanishi, Yosioka I. (1976) Spergulatriol, a new bisnor-triterpenoid sapogenol from the root of *Mollugo spergula*. *Tetrahedron* **27**, 2327-2330.

Kunchandy E, Rao MNA. (1990) Oxygen radical scavenging activity of curcumin. *Int. J. Pharm.* **58**, 237-240.

Larson Ra. (1988) The antioxidants of higher plant. *Phytochemistry* **27**, 969-978.

Mayumi T, Schiller HJ, Bulkley G. (1993) *Free radicals – from basic science to medicine,* pp. 438-457. Switzerlands, Birkhauser, Verlag.

Michaels HB, Hunt JW. (1973) Hydroxyl radical scavenging activity of curcumin. *Radiat. Res.* **56**, 57–63.

Nakken KF, Pihl A. (1965) Hydroxyl radical scavenging properties of some plant extracts. *Radiat. Res*. **26**, 519-524.

Niki E. (1997) *Free radicals, antioxidants and cancer*. *Food Factors for Cancer Prevention,* 55-57. Springer, Tokyo.

Okhawa H, Oishi N, Yagi K. (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.

Pouison HE, Prieme H, Loft S. (1998) Role of oxidative DNA damage in cancer initiation and promotion. *Eur. J. Cancer Prev.* **7**, 9-16.

Repine JE, Fox RB, Berger EM. (1981) Fenton's reaction in the living systems. *J. Biol. Chem*. **256**, 7094-8005.

Velioglu YS, Mazza G, Gao L, Oomah BD. (1998) Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.* **46**, 4113-4117.

Vina J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardo FV, Cuesta A, Ferrero JA, Terada LS, Repine. (2000) Mechanism of free radical production in exhaustive exercise in humans and rats – role of xanthine oxidase and protection by allopurinol. *IUBMB Life* **49**, 539-544.

Yoshikawa T, Naito Y, Kondo M. (1997) *Food and diseases. Free radicals and diseases,* pp. 11-19, Plenum Press, New York.

Zheng W, Wang SY. (2001) Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **49**, 5165-5170.

Zsuzsa Varga, Andrea Czompa, Gyorgy Kakuk, Sader Antus. (2001) Inhibition of the superoxide anion release and hydrogen peroxide formation in PMNLs by flavonolignans. *Phytother. Res.* **15**, 608-612.