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In vitro Antioxidant Property of Some Indian Medicinal Plants

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ABSTRACT

The main goal of this study was to determine the antioxidant activity of twelve medicinal plants (*Averrhoa carambola* (Oxalidaceae), *Buchanania lanzan* (Anacardiaceae), *Calophyllum inophyllum* (Clusiaceae), *Celastras peniculatus* (Celastraceae), *Clerodendron multiflorum* (Verbenaceae), *Luffa acutangula* (Cucurbitaceae), *Morinda citrifolia* (Rubiaceae), *Ocimum gratissimum* (Lamiaceae), *Paltophorum ferrugineum* (Papillionaceae), *Phyllanthus fraternus* (Euphorbiaceae), *Triumfetta rotundifolia* (Tilliaceae), *Ziziphus nummularia* (Rhamnaceae) belonging to different families. Antioxidant activity was determined by using different methods like DPPH (2,2'-Diphynyl, 1-picrylhydrazyl) free radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay and reducing capacity assessment. The plants were extracted individually by cold percolation method using different organic solvents (petroleum ether, acetone and methanol) depending on their polarity. Ascorbic acid was used as standard to determine DPPH free radical scavenging activity and reducing capacity assessment. Gallic acid was used as standard to determine hydroxyl radical scavenging activity and superoxide anion radical scavenging activity. Amongst the twelve plants studied, acetone and methanolic extract of *Paltophorum ferrugineum* showed the best radical scavenging activity and reducing capacity assessment.

Key words: DPPH, antioxidant activity, superoxide anion radical, reducing capacity, *Paltophorum ferrugineum*, medicinal plants

INTRODUCTION

Reactive oxygen species (ROS) (e.g., superoxide anion ($O_2^{\bullet -}$)), hydroxyl radicals ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are formed as a result of normal metabolic activity and due to exogenous sources (Halliwell and Gutteridge, 1986). In pathological conditions, the antioxidant mechanisms are often inadequate, as excessive quantities of ROS can be generated. The ROS formed may cause cellular and subcellular damage by peroxidation of membrane lipids, by denaturing cellular proteins and by breaking DNA strands, disrupting cellular functions (Halliwell and Gutteridge, 1986).

The use of traditional medicine is widespread in India (Jeyachandran and Mahesh, 2007). Many medicinal plants contain large amounts of antioxidants, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The medicinal value of plants has assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and

primary metabolites but also a diverse array of secondary metabolites with antioxidant potential (Akinmoladun *et al*., 2007; Ashawat *et al*., 2007). Antioxidants are a group of compounds that facilitate survival in plants and may promote the health of humans that consume a variety of plant foods (Connor *et al*., 2002; Lampart-Szczapa *et al*., 2001). In plants, the term antioxidant often refers to a wide range of phenolic compounds that vary from simple phenolic acids to highly polymerized compounds such as tannins.

The human body synthesizes endogenous antioxidants such as superoxide dismutases, glutathione peroxidases alpha-tocopherol and melatonin to counteract cellular damage by active oxygen and free radicals (Mojzisova and Kuchta, 2001; Oktay *et al*., 2003). Many studies suggest that endogenous antioxidants, or exogenous antioxidants present in diet, can function as free radical scavengers and improve human health. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process (Aruoma, 2003). Aging and aging-related diseases may be due to the long term effects of oxidative damage to the cells and tissues of the body that arises primarily as a result of aerobic metabolism (Balaban *et al*., 2005). Radical scavenging antioxidants are particularly important in anti oxidative defense in protecting cells from the injury of free radicals. It is therefore promising to develop functional foods with plants containing naturally occurring compounds uncontaminated and with few side effects. As a matter of fact, the commercial development of plants as sources of antioxidants, that can be used to enhance the properties of food and play roles in the development of cancer and atherosclerosis remedies are currently of major interest (Ou *et al*., 2002; VanderJagt *et al*., 2002).

The therapeutic effects of several plants and vegetables, which are used in traditional medicine, are usually attributed to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns (Akinmoladun *et al*., 2007). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Jayaprakasha *et al*., 2001; Gulcin *et al*., 2002). A profile of the chemical composition of a plant together with knowledge of its antioxidant property will give a fair estimate of its therapeutic potential (Akinmoladun *et al*., 2007).

Considering the above, the main aim of the present study was to screen 12 plants for their antioxidant property by different radical scavenging methods.

MATERIALS AND METHODS

Collection of the plant materials: The plants or plant parts were collected from Gir forest of Saurashtra region, Gujarat, India in the year 2008. All the plants were identified by taxonomist Dr. N.K. Thakrar at Department of Biosciences, Saurashtra University, Rajkot. The lists of twelve plants which showed antioxidant activity, the botanical name, vernacular name, family name, part used and traditional use are presented in Table 1 (Anjaria *et al*., 2002). The plants were thoroughly washed with tap water, shade dried, crushed in a homogenizer to fine powder and stored in air tight bottles.

Extraction: The plants were extracted by cold percolation method (Parekh and Chanda, 2007) individually using different organic solvents like petroleum ether, acetone and methanol. Ten gram

of dried powder was taken in 100 mL of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvent was evaporated. The residue was then taken in 100 mL of solvent (acetone and methanol) in a conical flask. Then the same procedure as above was followed and the dry extract was stored at 4°C in air tight bottles. The residues were weighed with the help of a balance (Sartorius) to obtain the extractive yield. The varied level of extractive yield of all the twelve plants in three different solvents viz. petroleum ether, acetone and methanol is given in Table 2.

Table 1: Ethnobotanical information of studied plants

Plant name	Vernacular name	Family	Parts used	Traditional use
Averrhoa carambola L.	Kamrakha	Oxalidaceae	Leaves	Scabies, antipruritic and antipyretic
Buchanania lanzan Spr.	Charoli	Anacardiaceae	Leaves	Biliousness, fever, thirst, ulcer and blood diseases
Calophyllum inophyllum L.	Nagchampo	Clusiaceae	Leaves	Ulcer, wound and painful joints
Celastrus paniculatus Willd.	Malkangla	Celastraceae	Leaves	Beriberi, enriching blood, abdominal complaints, cough, asthma, headache, leucoderma
Clerodendron multiflorum L.	Arani	Verbenaceae	Leaves	Leaf juice is alterative
Luffa acutangula (L) Roxb.	Tuliya	Cucurbitaceae	Aerial part	Leprosy, granular conjunctivitis, ring worm and dermatopathy
Morinda citrifolia L.	Noni	Rubiaceae	Leaves	Coughs, nausea and colic, possibly due to its anti-inflammatory activity
Ocimum gratissimum L.	Jangli tulsi	Lamiaceae	Aerial part	Cephalgia, colic, rheumatoid arthritis, leucoderma, diarrhea
Peltophorum ferrugineum (Decne.) Benth.	Paltopham	Papillionaceae	Leaves	Edible, emetic, used in diarrhea
Phyllanthus fraternus Webster Syn.	Bhoi ambli	Euphorbiaceae	Whole plant	Ulcer, fever and gonorrhea
Triumfetta rotundifolia Lam.	Gipto	Tilliaceae	Fruit	Plant is mucilaginous, astringent and used in gonorrhea
Ziziphus nummularia (Burm.F.) Wight and Arn.	Moti bordi	Rhamnaceae	Aerial part	Edible fruit, bark contains tannin

Table 2: Extractive yield of studied plants

	Yield (w/w) (%)		
Plant name	Petroleum ether	Acetone	Methanol
Averrhoa carambola	1.35	3.79	10.91
Buchanania lanzan	0.93	1.98	9.28
Calophyllum inophyllum	1.49	2.42	10.45
Celastras peniculatus	2.10	1.72	8.07
Clerodendron multiflorum	1.04	1.70	9.66
Luffa acutangula	0.82	0.70	4.11
Morinda citrifolia	3.50	3.12	5.29
Ocimum gratissimum	1.53	1.83	9.04
Paltophorum ferrugineum	1.36	4.36	15.32
Phyllanthus fraternus	4.64	2.14	10.76
Triumfetta rotundifolia	1.52	1.37	2.28
Ziziphus nummularia	0.86	1.70	9.66

Antioxidant activities:

DPPH free radical scavenging activity: The free radical scavenging activity of various solvent extracts was measured by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) by the modified method of McCune and Johns (2002). The reaction mixture consisting of DPPH in methanol (0.3 mM, 1 mL) and different concentrations of the solvent extracts (1 mL) was incubated for 10 min. in dark, after which the absorbance was measured at 517 nm. Ascorbic acid was used as positive control. The percentage inhibition was determined by comparing the result of the test and the control. Percentage of inhibition was calculated by using the formula:

$$\text{Inhibition}(\%) = [1-(A/B)]\times 100$$

Where:
A = Absorbance of sample
B = Absorbance of control

The inhibiting effect of all the 24 extracts showed varied levels of DPPH radical scavenging activity, expressed as IC_{50}, is presented in Table 3.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity of different solvent extracts was measured by studying the competition between deoxyribose and test compound for hydroxyl radical generated by Fe^{3+}-Ascorbic acid-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao (1990). The reaction mixture containing (1.0 mL), 100 μL of 2-deoxy-2-D - ribose (28 mM in 20 mM KH_2PO_4 -KOH buffer, pH 7.4), 500 μL of the various

Table 3: IC_{50} values of DPPH free radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity of acetone and methanol extracts of studied plants

	IC_{50} values					
	DPPH		OH		SO	
Plant name	Ac	Me	Ac	Me	Ac	Me
Averrhoa carambola	46	560	A	A	600	A
Buchanania lanzan	32	36	500	450	420	340
Calophyllum inophyllum	156	330	625	A	A	A
Celastras peniculatus	310	235	A	A	A	A
Clerodendron multiflorum	200	60	A	550	A	A
Luffa acutangula	600	620	A	A	A	A
Morinda citrifolia	450	360	900	A	A	A
Ocimum gratissimum	162	225	A	775	A	A
Paltophorum ferrugineum	20	20.5	575	825	156	175
Phyllanthus fraternus	37.5	82	525	A	320	A
Triumfetta rotundifolia	26.5	17	775	775	270	215
Ziziphus nummularia	35	41	625	750	360	340
Std Ascorbic acid	11.4		ND		ND	
Gallic acid	ND		140		185	

Ac: Acetone extract; Me: Methanol extract; A: >1000; Std : Standard; ND: Not done; DPPH: DPPH free radical scavenging activity, OH: Hydroxyl radical scavenging activity; SO: Superoxide anion radical scavenging activity

solvent extracts, 200 µL EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µL 1.0 mM H_2O_2 and 100 µL ascorbic acid (1.0 mM) was incubated at 37°C for 1 h. The 1.0 mL of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8 %) were added and incubated at 100°C for 20 min. After cooling, absorbance of pink color was measured at 532 nm, against a blank sample. Gallic acid was used as a positive control.

Superoxide anion radical scavenging activity: The superoxide anion radical scavenging activities of various solvent extracts were described by Robak and Gryglewski (1988). Superoxide generated in phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH) oxidation was assayed by nitroblue tetrazolium (NBT) reduction. The reaction mixture consisted of a final volume of 3 mL Tris-HCl buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3 mM), 0.5 mL NADH (0.936 mM) solution and 1.0 mL of various concentrations of different solvent extracts. The reaction mixture was initiated by adding 0.5 mL PMS solution (0.12 mM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample. Gallic acid was used as a positive control.

Reducing capacity assessment: The reducing capacity assessment of different solvent extracts was determined using the modified method of Athukorala *et al.* (2006). One milliliter of different concentrations of solvent extracts was mixed with phosphate buffer (2.5 mL, 200 mM and pH 6.6) and potassium ferricynide (2.5 mL, 30 mM). The mixture was then incubated at 50°C for 20 min. There after Trichloroacetic acid (2.5 mL, 600 mM) was added to the reaction mixture and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control.

Statistical analysis: Each of the measurements was carried out in triplicates and the results were expressed as Mean±SEM.

RESULTS AND DISCUSSION

Extractive yield: In petroleum ether extracts of all the plants, the maximum extractive yield was obtained in *P. fraternus* and minimum yield was obtained in *L. acutangula* followed by *Z. nummularia*. In the acetone extracts of all the plants, the maximum extractive yield was obtained in *P. ferrugineum* and minimum yield was obtained in *L. acutangula*. In the methanol extracts of all the plants, the maximum yield was obtained in *P. ferrugineum* and minimum was obtained in *T. rotundifolia* which shown in Table 2. In all plants extracts, the extractive yield was maximum in methanol extract of *P. fraternus* and minimum in acetone extract of *L. acutangula*. The extraction yields with different solvents varied from 15.32 to 0.70% per 100 g dry powder (Table 2) and can be ranked from high to low in the following order: methanol extract > petroleum ether extract > acetone extract. The higher yield of the methanol extracts suggests higher proportion of methanol soluble plant components.

Antioxidant activities: The antioxidant reactions involve multiple steps including the initiation, propagation, branching and termination of free radicals. The antioxidants which inhibit the formation of free radicals from their unstable precursors are called preventive antioxidants and those which interrupt the radical chain reaction (propagation and branching) are the chain-breaking antioxidants (Ou *et al.*, 2002).

DPPH free radical scavenging activity: Various assays are used to test antioxidant activity but the most widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds (Arnao *et al*., 2001; Masoko and Eloff, 2007). The DPPH radical is commonly used as a substrate to evaluate antioxidant activity; it is a stable free radical that can accept an electron or hydrogen radical to become a stable molecule. The reduction of DPPH radical was determined by the decrease in its absorbance induced by antioxidants at 517 nm. Concentration of sample at which the inhibition percentage reaches 50% is its IC_{50} value. IC_{50} value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample. In the present work, twelve plant species, in acetone and methanolic solvents were evaluated for their DPPH free radical scavenging activity. All the 24 extracts investigated showed varied levels of DPPH scavenging activity (Table 3). IC_{50} values ranged from 17 to 620 μg mL^{-1} (Table 3). Ascorbic acid was used as standard and its IC_{50} value was 11.4 μg mL^{-1}. Acetone and methanol extracts of all the plants showed DPPH free radical scavenging activity. The IC_{50} value of acetone extract of all twelve plants ranged between 20 and 600 μg mL^{-1} (Table 3). Among all the acetone extracts, lowest IC_{50} value was of *P. ferrugineum* (20 μg mL^{-1}) and highest IC_{50} value was of *L. acutangula* (600 μg mL^{-1}). The IC_{50} value of methanol extract of all twelve plants ranged between 17 and 620 μg mL^{-1} (Table 3). Among all the methanol extracts, lowest IC_{50} value was of *T. rotundifolia* and highest IC_{50} value was of *L. acutangula* (620 μg mL^{-1}). Amongst all the plant studied, acetone and methanol extracts of *P. ferrugineum* and *T. rotundifolia* showed better DPPH free radical scavenging activity.

Hydroxyl radical scavenging activity: Hydroxyl radical formation can occur in several ways by far the most important mechanism *in vitro* is the fenton reaction where a transition metal is involved as a prooxidant in the catalysed decomposition of superoxide and hydrogen peroxide (Stohs and Bagchi, 1995). Hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism among the ROS (Waling, 1975), which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause the ageing of human body and some diseases (Siddhuraja and Becker, 2007). The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Attalah, 1988).

In the present study, twelve plant species, in acetone and methanolic solvents, were evaluated for their hydroxyl radical scavenging activity. Out of 24 extracts investigated, 11 extracts showed IC_{50} value more than 1000 μg mL^{-1} (Table 3) while the remaining 13 extracts showed varied levels of hydroxyl radical scavenging activity. IC_{50} values ranged from 450 to 900 μg mL^{-1} (Table 3). Gallic acid was used as standard and its IC_{50} value was 140 μg mL^{-1}. The acetone and methanolic extracts of *B. lanzan*, *P. ferrugineum*, *T. rotundifolia* and *Z. nummularia* showed an IC_{50} value of less than 1000 μg mL^{-1}; while only acetone extracts of *C. inophyllum*, *M. citrifolia* and *P. fraternus* and methanol extracts of *C. multiflorum* and *O. gratissimum* showed an IC_{50} value less than 1000 μg mL^{-1}. Among all the extracts, the lowest IC_{50} value was of methanolic extract of *B. lanzan* (450 μg mL^{-1}) and the highest IC_{50} value was of acetone extract of *M. citrifolia* (900 μg mL^{-1}). Amongst all the plants studied, methanolic and acetone extract of *B. lanzan* showed better hydroxyl radical scavenging activity.

Superoxide anion radical scavenging activity: Superoxide anion radical is a weak oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Dahal and Richardson, 1978). Superoxide anion, which is a reduced form of molecular oxygen, has been implicated in the containing oxidation reactions associated with aging (Cotelle *et al.,* 1996).

In the present study, twelve plant species, in acetone and methanolic solvents were evaluated for their superoxide anion radical scavenging activity. Out of 24 extracts investigated, 14 extracts showed an IC_{50} value of more than 1000 μg mL^{-1} (Table 3) while the remaining 10 extracts showed varied levels of superoxide anion radical scavenging activity. IC_{50} values ranged from 156 to 600 μg mL^{-1} (Table 3). Gallic acid was used as standard and its IC_{50} value was 185 μg mL^{-1}. Acetone and methanolic extracts f *B. lanzan*, *P. ferrugineum*, *T. rotundifolia* and *Z. nummularia* showed an IC_{50} value of less than 1000 μg mL^{-1}. Only acetone extract of *A. carambola* and *P. fraternus* showed an IC_{50} value of less than 1000 μg mL^{-1}. Among all the extracts, the lowest IC_{50} value was of acetone extract of *P. ferrugineum* (156 μg mL^{-1}) and the highest IC_{50} value was of acetone extract of *A. carambola* (600 μg mL^{-1}). Amongst all the plants studied, acetone and methanol extracts of *P. ferrugineum* showed best superoxide anion radical scavenging activity, which is better than standard.

Reducing capacity assessment: A good correlation between antioxidant activity and reducing power in some plant extracts has been established. Therefore, reducing power may be used as an indicator of potential antioxidant activity (Yen *et al.*, 2001). The presence of reductants (antioxidants) in the herbal extracts causes the reduction of Fe (III) to Fe (II). Therefore the Fe (II) complexes can be monitored by measuring the formation of Perl's Prussian blue color (Chung *et al.*, 2002).

The reducing power increased with an increase in extract concentration. The data show that all the samples increased their reducing ability when the concentration of extracts was increased. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds (Shimoda *et al.*, 1992) which is also related to the presence of reducing agent. In addition, the number and position of hydroxyl group of phenolic compounds also rule their antioxidant activity (Rice-Evans *et al.,* 1995).

The reducing capacity of *A. carambola*, *B. lanzan*, *C. inophyllum*, *C. paniculatus*, *C. multiflorum* and *L. acutangula* of two different solvent extracts is given in Fig. 1a-f. The reducing capacity of *A. carambola* was maximum in methanolic extract and minimum in acetone extract. The reducing capacity of *B. lanzan* was almost the same in methanolic and acetone extracts. The reducing capacity of *C. inophyllum* was maximum in methanolic extract and minimum in acetone extract. The reducing capacity of *C. paniculatus* was almost same in methanolic and acetone extracts. The reducing capacity of *C. multiflorum* was maximum in acetone extract and minimum in methanolic extract. The reducing capacity of *L. acutangula* was almost the same in methanolic and acetone extracts.

The reducing capacity of *M. citrifolia*, *O. gratissimum*, *P. ferrugineum*, *P. fraternus*, *T. rotundifolia* and *Z. nummularia* of two different solvent extracts is given in Fig. 2a-f. The reducing capacity of *M. citrifolia* was almost the same in methanolic and acetone extracts. The reducing capacity of *O. gratissimum* was almost the same in methanolic and acetone extracts. The reducing capacity of *P. ferrugineum* was almost the same in methanolic extract and acetone extract. The reducing capacity of *P. fraternus* was maximum in methanolic extract and minimum in acetone

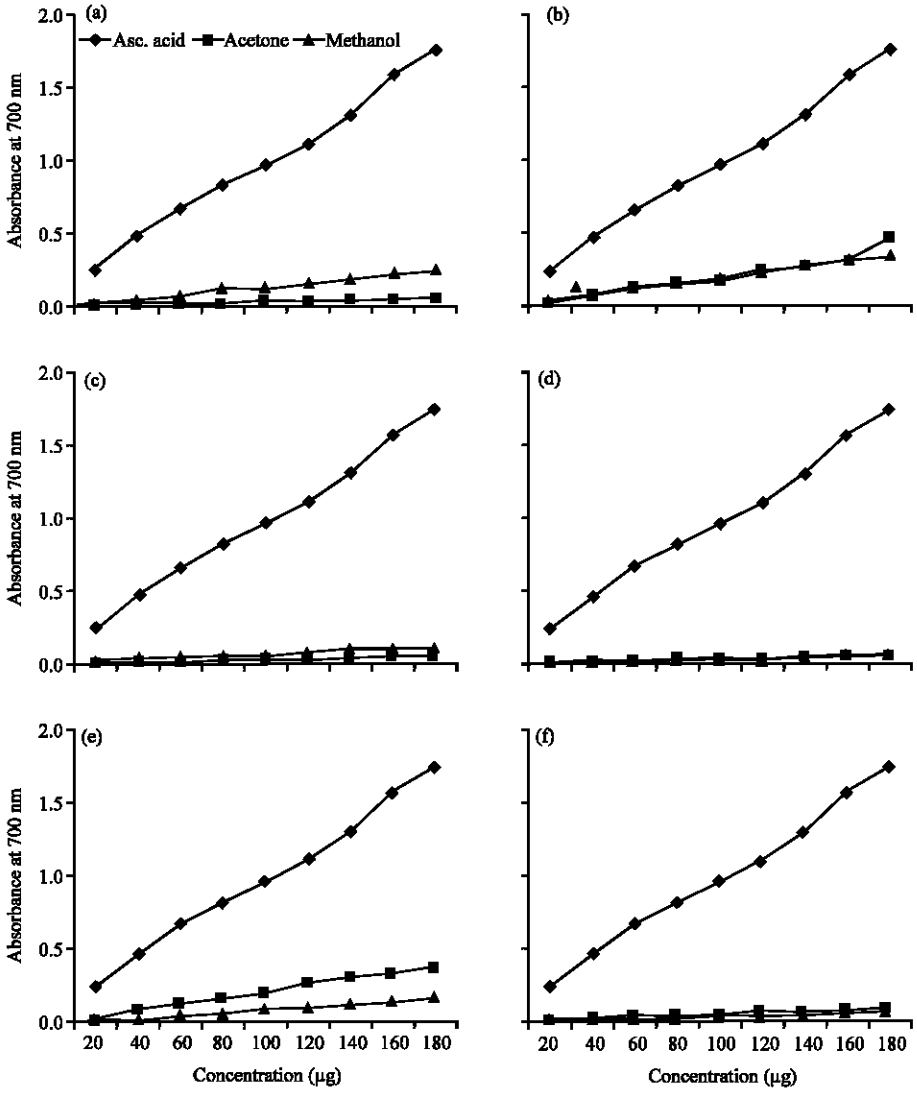

Fig. 1: Reducing capacity assessment of acetone and methanol extracts of studied plants. (a) *Averrhoa caramboia*, (b) *Buchanania lanzan*, (c) *Calophylium inophyllum*, (d) *Celastras peniculatus*, (e) *Clerodendron multiflorum* and (f) *Luffa acutangula*

extract. The reducing capacity of *T. rotundifolia* was maximum in acetone extract and minimum in methanolic extract. The reducing capacity of *Z. nummularia* was maximum in methanolic extract and minimum in acetone extract.

In all the twelve plants, methanolic and acetone extracts of *P. ferrugineum* have shown better reducing capacity when compared with other plants investigated. The results of the determination of the antioxidant capacity of an extract depend greatly on the methodology used. That is the oxidant and the oxidisable substrate used in the measurement. Therefore, it is important to compare different analytical methods varying in them oxidation initiators and targets in order to understand the biological activity of an antioxidant. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of components of some plants.

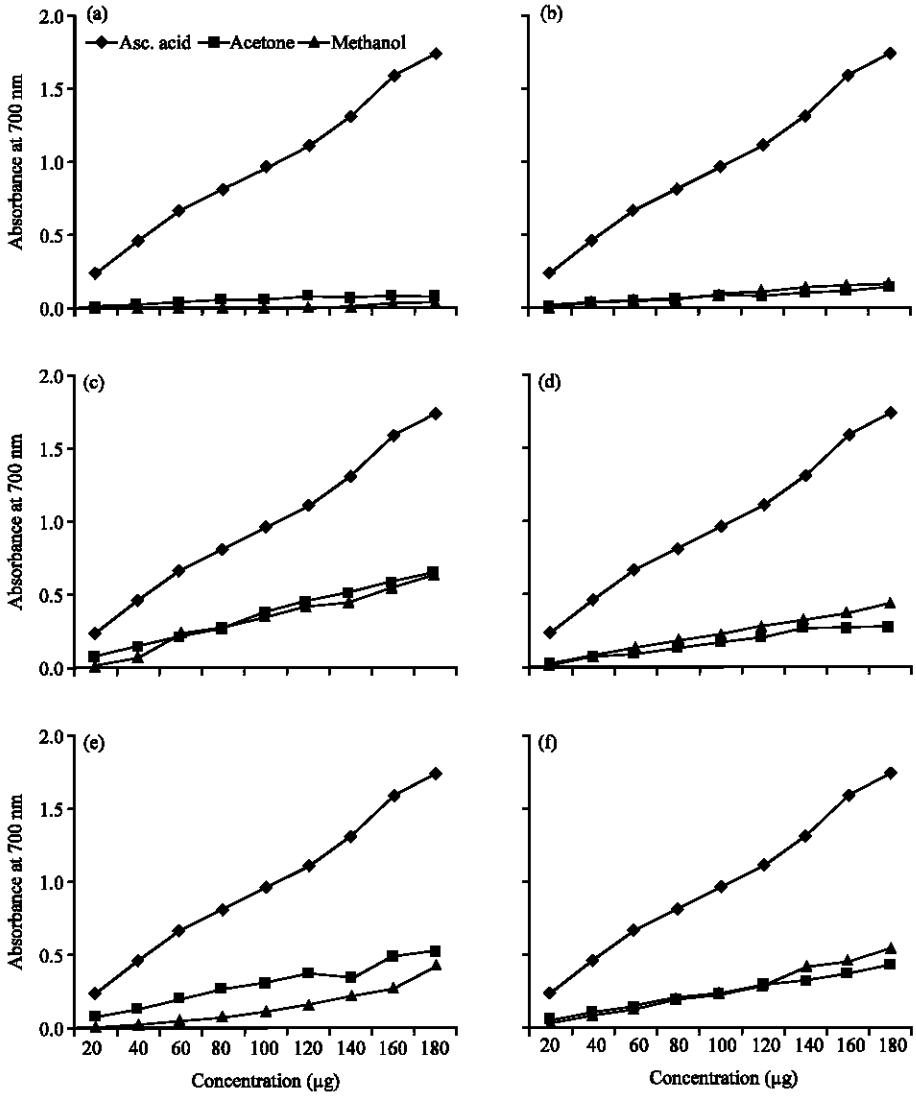

Fig. 2: Reducing capacity assessment of acetone and methanol extracts of studied plants. (a) *Morinda citrifolia*, (b) *Ocimum gratissimum*, (c) *Paltophorum ferrugineum*, (d) *Phyllanthus fraternus*, (e) *Triumfetta rotundifolia* and (f) *Ziziphus nummularia*

CONCLUSION

In the present study, the acetone and methanol extracts of *P. ferrugineum* and *T. rotundifolia* showed maximum DPPH free radical scavenging activity. The methanolic and acetone extract of *B. lanzan* showed maximum hydroxyl radical scavenging activity, while the acetone and methanol extracts of *P. ferrugineum* showed maximum superoxide anion radical scavenging activity better than standard and better reducing capacity. Among the twelve plants used in the antioxidant study, acetone and methanol extracts of *P. ferrugineum* showed maximum and best activity.

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