

Full Length Research Paper

Vegetable and fruit peels as a novel source of antioxidants

Rakholiya Kalpna, Kaneria Mital and Chanda Sumitra*

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India.

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Consumers are currently demanding less use of chemicals or minimally processed fruits and vegetables, so more attention had been paid to search for naturally occurring substances. This is particularly true for plant materials that act as alternative antioxidant sources. From this point of view, the present study was designed to evaluate the antioxidant potential of seven fruit and vegetable peels from India. Extraction was done individually by cold percolation method using various organic solvents (hexane, chloroform, acetone and methanol). Quantitative phytochemical analysis was done for total phenol and flavonoid content. Antioxidant testing assays were 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay and reducing capacity assessment. Amongst the seven plant peels, the acetone extract of *Mangifera indica* was the most potent and in some cases even better than the standard. The results obtained indicate that *M. indica* peel may become important as a cheap and noticeable natural source of compounds with health protective potential, which can be used in pharmaceutical, nutraceutical and food preparation.

Key words: Antioxidant activity, *Mangifera indica*, *Lagenaria siceraria*, peels, total phenol content, solvent extracts.

INTRODUCTION

Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane (Mantena et al., 2008). The body on the other hand, requires free radicals for immune system responses. However, an overload of these molecules had been linked to certain chronic diseases of heart, liver and some form of cancers (Temple, 2000; Prakash et al., 2007). Human body contains anti-free radical defense system, which includes antioxidant enzymes like catalase, peroxidase and superoxide dismutase and antioxidants like ascorbic acid and tocopherol (Oke et al., 2009). An antioxidant (free radical scavenger) is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than is the oxidized substrate. These free radical scavenger help in preventing stress induced diseases such as melanoma,

cardiac disorders, diabetes mellitus, inflammatory and neurodegenerative diseases, cancer (Prakash et al., 2007; Jing et al., 2008). Vegetables are a good source of dietary antioxidants, such as vitamin C, vitamin E and β -carotene. The antioxidative phytochemicals in grains, vegetables, fruits and medicinal plants have received increasing attention for their potential role in preventing human diseases (Pallauf et al., 2008).

Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages. The researchers opinion is that natural phenolic antioxidants are health-promoting substances and that their antioxidant mechanisms and their biological activity should be investigated at a fundamental scientific level (Antolovich, 2004). Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions (Bors et al., 1990; Liu, 2003). They are potent antioxidants and have free radical scavenging abilities.

The antioxidant constituents are present in all parts of

*Corresponding author. E-mail: svchanda@gmail.com.

the plant such as bark, stalks, leaves, fruits, roots, flowers, pods, seeds, stems, latex, hull (Baravalia et al., 2009; Kaneria et al., 2009; Rajaei et al., 2010; Golivand et al., 2010). Recent research revealed that fruit peels and seeds, such as grape seeds and peels (Negro et al., 2003), pomegranate peel (Singh et al., 2002), wampee peel (Prasad et al., 2010) and mango seed kernel (Kabuki et al., 2000) may potentially possess antioxidant properties.

The objectives of the present investigation is to determine the antioxidant property of peels of different fruits and vegetables, that are commonly available and readily consumed in India, and to indicate which of them can become a new source of natural antioxidants for food, nutraceutical and pharmaceutical industries. Therefore, in the present study, seven peels of fruits and vegetables (*Ananas comosus* (Linnaeus) Merr., *Mangifera indica* L., *Lagenaria siceraria* (Molina) Standl., *Luffa acutangula* L. (Roxb.), *Momordica charantia* L., *Moringa oleifera* Lam., *Solanum tuberosum* L.) were screened for their *in vitro* antioxidant potential.

MATERIALS AND METHODS

Reagents

The reagents used for the study are potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, sodium carbonate, Folin-Ciocalteu's phenol reagent, aluminium chloride, potassium acetate, quercetin, nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), Tris Buffer, 2-deoxy-D-ribose, ethylenediamine tetra acetic acid (EDTA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide, acetone, methanol, hexane, chloroform, obtained from Hi-media, Merck or sigma. All reagents used were of analytical grade.

Plant material

The aforementioned seven fresh fruits and vegetables were collected from Rajkot, Gujarat, India. The taxonomic identity of the plants was confirmed by Dr. N. K. Thakrar, Department of Biosciences, Saurashtra University, Rajkot, India. They were thoroughly washed with water and then peels were separated, washed again and dried under shade. The dried peels were homogenized to fine powder and stored in air tight bottles which were later used for solvent extraction. The ethnobotanical information (Anjaria et al., 2002; Mahattanatawee et al., 2006; Gouado et al., 2007) of the screened plants is given in Table 1.

Extraction method

The dried powder of peels was extracted individually by cold percolation method (Parekh and Chanda, 2007) using different organic solvents like hexane, chloroform, acetone and methanol. 10 g of dried powder was taken in 100 ml of hexane 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. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 ml of solvent (chloroform, acetone and methanol) 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 solvents were evaporated and the dry extract was stored at 4°C in air tight bottles. The residues were weighed to obtain the extraction yield.

Determination of total phenol content

The total phenol content was determined according to Folin-Ciocalteu's reagent method (Mc Donald et al., 2001). 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min. at room temperature and the absorbance was measured at 760 nm. Gallic acid was used as a positive control (Morsi et al., 2010). Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound).

Determination of flavonoid content

The flavonoid content was determined according to aluminium chloride colorimetric method (Chang et al., 2002). The reaction mixture consisting in a final volume of 3 ml, 1.0 ml of sample (1 mg/ml) 1.0ml methanol and 0.5 ml of (1.2%) aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance of all the samples was measured at 415 nm. Quercetin was used as positive control (Ghasemi et al., 2009; Kaneria et al., 2009). Flavonoid content is expressed in terms of Quercetin equivalent (mg g^{-1} of extracted compound).

Antioxidant testing assays

DPPH free radical scavenging activity

The free radical scavenging activity 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) 1 ml methanol 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 (Blois, 1958).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of different solvent extracts of peels 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-D-ribose (28 mM in 20 mM KH_2PO_4 -KOH buffer, pH 7.4), 500 μl of the various 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. 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 (Kunchandy and Rao, 1990).

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was measured

according to a described procedure (Robak and Gryglewski, 1988). Superoxide generated in phenazine methosulphate (PMS), nicotinamide adenine dinucleotide reduced (NADH) oxidation and assayed by nitroblue tetrazolium (NBT) reduction. The reaction mixture consisted in a final volume of 3 ml, 0.5 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 solvents extracts. The reaction 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 (Robak and Gryglewski, 1988).

Reducing capacity assessment

The reducing capacity assessment of different solvent extracts of peels was determined using the modified method of Athukorala et al. (2006). 1 ml of different concentrations of solvent extracts was mixed with phosphate buffer (2.5 ml, 200 mM, and pH 6.6) and potassium ferricyanide (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 FeCl₃ (0.5 ml, 6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control (Ksouri et al., 2009).

Statistical analysis

All experiments were repeated at least three times. Results were reported as mean ± S.E.M. (standard error of mean).

RESULTS AND DISCUSSION

Extractive yield

The extractive yield of different screened fruit and vegetable peels is given in Table 2. The extractive yield varied among different fruit and vegetable peels and among the solvents used. In all the plant peels, methanol extract showed highest extractive yield than the other extracts. In methanol extracts, it can be ranked from high to low in the following order *M. Oleifera* (21.05) > *L. siceraria* (19.69) > *A. comosus* (17.30) > *M. indica* (15.82) > *M. charantia* (11.25) > *S. tuberosum* (10.17) > *L. acutangula* (7.24). In acetone extracts, *M. indica* (5.64) showed highest, while *L. acutangula* (0.96) showed lowest extractive yield. In chloroform extract, *M. charantia* (3.87) showed maximum extractive yield, while minimum was in *A. comosus* (0.89). In hexane extract maximum yield was in *L. siceraria* (3.8), and minimum in *A. comosus* (0.89). There are many reports in the literature where extractive yield varied with different solvents (Vaghasiya and Chanda, 2007; Yang et al., 2007). Extraction is critical to the recovery of antioxidant phytochemicals; under the same time and temperature conditions, the solvents used and the chemical property of samples are the two most important factors (Shimada et al., 1992).

Total phenol and flavonoid content

Phenolic are the most wide spread secondary metabolites in the plant kingdom. Flavonoids are through scavenging or chelating process. Phenolic compounds are a class of antioxidant agents, which act as a free radical scavengers. It is believed that the phenolic and/or polyphenolic compounds biosynthesized in the plant sample might be responsible for antioxidant activity (Kessler et al., 2003).

In the present work, seven vegetable and fruit peels in various solvents were evaluated for their total phenol and flavonoid content (Table 2). In all the plant peels, total phenolic content was more than the flavonoid content. The acetone extract had maximum amount of phenols followed by methanol extract. The flavonoid content was maximum in hexane extract.

Among seven plants tested, acetone extract of *M. indica* contained high amount of phenol content while *L. siceraria* (acetone) and *M. oleifera* (hexane) contained high amount of flavonoid content. The results showed that acetone was superior to methanol in selective extraction of total phenols as also reported by Bensky et al. (2004).

Antioxidant testing assays

Many methods have been proposed to evaluate the antioxidant potential of natural sources of antioxidants. However, the antioxidant capacity of plant extracts cannot be evaluated by any one single assay because of the complex nature of phytochemicals present in them, solvent used for extraction and lastly the mechanism of different antioxidant assays is different. Therefore, it is essential that in order to assess the antioxidative capacity of any plant, more than one solvent and more than one antioxidant assays had to be performed (Singh et al., 2007; Chanda and Dave, 2009; Chanda and Nagani, 2010). In the present study, DPPH, superoxide anion and hydroxyl radical scavenging activities and reducing capacity assessment was evaluated in seven fruit and vegetable peels extracted in hexane, chloroform, acetone and methanol.

DPPH free radical scavenging activity

There are different methods for estimation of antioxidant activity but the most widely used methods are those that involve generation of free radical species which are then neutralized by antioxidant compounds. DPPH radical is commonly used as substrate to evaluate antioxidant activity; it is a useful and stable free radical that can accept an electron or hydrogen radical to become a stable molecule. The reduction of DPPH free radical was determined by the decrease in its absorbance at 517 nm induced by different antioxidants. DPPH free radical reacts with antioxidants, consequentially, absorbance

Table 1. Ethnobotanical information of screened plants.

| No. | Botanical name | Vernacular name | Family | Medicinal/therapeutic use |
|-----|--------------------------------------|-----------------|---------------|--|
| 1 | <i>M. indica</i> L. | Ambo | Anacardiaceae | Peel as a source of dietary fiber and antioxidant, The peel and pulp rich in starch and pectins and source of antioxidants including ascorbic acid, carotenoids and phenolic compounds |
| 2 | <i>A. comosus</i> (Linnaeus) Merr. | Ananas | Bromeliaceae | Fruit, peel or juice is used in folk remedies for corns, tumors, and warts. Reported to be abortifacient, depurative, cholagogue, diaphoretic, digestive, emmenagogue, diuretic, hydragogue, discutient, estrogenic, intoxicant, parasiticide, purgative, laxative, refrigerant, styptic, and vermifuge, Many real or imagined pharmacological effects are attributed to bromelain: Burn debridement, antiinflammatory action, smooth muscle relaxation and stimulation of muscle contractions, cancer prevention and remission, ulcer prevention, appetite inhibition, enhanced fat excretion, and sinusitis relief |
| 3 | <i>L. siceraria</i> (Molina) Standl. | Dudhi | Cucurbitaceae | Fruits are used for cardio protective, cardio tonic, diuretic, antihepatotoxic activity, purgative and cooling effects. It also cures pain, ulcers, fever, and other bronchial disorders |
| 4 | <i>S. tuberosum</i> L. | Batata | Solanaceae | Peels are used in India to treat swollen gums and to heal burns and helpful in the treatment of peptic ulcers, bringing relief from pain |
| 5 | <i>L. acutangula</i> (L.) Roxb. | Turiya | Cucurbitaceae | Fruits: Diuretic, tonic, nutritive. Leaves used in splenitis, hemorrhoids, ring worm, leprosy, granular conjunctivitis |
| 6 | <i>M. charantia</i> L. | Karela | Cucurbitaceae | Fruits: Bitter, thermogenic, acrid, stimulant, purgative, antidiabetic, digestive, anti-inflammatory, hydrophobia, malarial |
| 7 | <i>M. oleifera</i> Lam. | Mitho Saragvo | Moringaceae | anti-inflammatory, rich in vitamin A and C, anodyne, anthelmintic, ophthalmic |

decreases and the DPPH free radical is converted into the DPPH-H form. The degree of discoloration indicates the scavenging potential of antioxidant compounds of extracts in terms of H₂ donating ability. Concentration of sample at which the inhibition percentage reaches 50% is its IC₅₀ value. IC₅₀ values are negatively related to the antioxidant activity, as it express the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC₅₀ value represents the higher antioxidant activity of the tested sample.

In the present work, seven plant species, extracted using various solvents were evaluated for their scavenging activity. Out of 28 extracts investigated, 19 extracts showed IC₅₀ value more than 1000 µg/ml (Table 3), the remaining 9 plant extracts showed a varied level of DPPH scavenging activity. IC₅₀ values ranged from 16.5 to 790 µg/ml (Table 3). Ascorbic acid was used as standard and its IC₅₀ value was 11.4 µg/ml.

The hexane extracts of all the studied plants showed low DPPH free radical scavenging activity (>1000 µg/ml) as compared to other solvent extracts. It appears that the free medical scavenging compounds are in polar solvents.

The IC₅₀ value of chloroform extract of *M. indica* was

140 µg/ml (Table 3). The acetone and methanol extracts of *M. indica* peels showed very good DPPH free radical scavenging activity, which were almost near to that of standard ascorbic acid (Table 3). The IC₅₀ values of acetone and methanol extracts of *M. indica* were 16.5 and 23.5 µg/ml, respectively (Table 3). The radical scavenging activity of *M. indica* peel could be related to the phenolics present in them, thus contributing to their electron transfer/ hydrogen donating ability. Saravana Kumar et al. (2008) reported similar results.

In nonpolar solvents, chloroform extract showed moderate activity, while hexane extract did not show any activity at all. Therefore, it can be concluded that extracting solvents play an important role in expressing antioxidant activity.

The polar solvents appear to be better than non-polar solvents; based on the present results, the polar solvent acetone appears to be the best.

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and had been implicated as a highly damaging species in free radical pathology,

Table 2. Extractive yield (%) and total phenol and flavonoid contents of different peels in different solvent extracts.

| No. | Plant species | Extract | % yield (w/w)# | Total phenol content (mg/g)* | Flavonoid content (mg/g)* |
|-----|----------------------|------------|----------------|------------------------------|---------------------------|
| 1 | <i>A. comosus</i> | Hexane | 0.56 | 1.87 ± 0.23 | 19.00 ± 0.22 |
| | | Chloroform | 0.89 | 12.02 ± 1.39 | 25.50 ± 1.25 |
| | | Acetone | 2.13 | 10.34 ± 0.77 | 4.25 ± 0.60 |
| | | Methanol | 17.3 | 18.66 ± 0.30 | 3.19 ± 0.23 |
| 2 | <i>M. indica</i> | Hexane | 2.4 | 1.53 ± 0.16 | 26.29 ± 0.82 |
| | | Chloroform | 2.24 | 53.58 ± 1.17 | 10.24 ± 0.42 |
| | | Acetone | 5.64 | 490.64 ± 0.43 | 11.25 ± 0.20 |
| | | Methanol | 15.82 | 216.74 ± 0.30 | 4.83 ± 0.08 |
| 3 | <i>L. siceraria</i> | Hexane | 3.80 | 2.78 ± 0.29 | 8.37 ± 0.67 |
| | | Chloroform | 1.38 | 30.13 ± 0.74 | 14.69 ± 0.14 |
| | | Acetone | 4.09 | 304.44 ± 12.01 | 36.08 ± 0.50 |
| | | Methanol | 19.69 | 144.11 ± 2.55 | 22.10 ± 0.27 |
| 4 | <i>L. acutangula</i> | Hexane | 0.69 | 3.11 ± 0.40 | 13.30 ± 0.65 |
| | | Chloroform | 1.32 | 10.32 ± 0.32 | 2.47 ± 0.99 |
| | | Acetone | 0.96 | 19.39 ± 0.57 | 8.02 ± 3.97 |
| | | Methanol | 7.25 | 28.10 ± 0.30 | 4.25 ± 0.20 |
| 5 | <i>M. charantia</i> | Hexane | 0.69 | 5.37 ± 0.03 | 2.71 ± 1.10 |
| | | Chloroform | 3.87 | 5.50 ± 0.15 | 6.21 ± 0.47 |
| | | Acetone | 2.30 | 5.44 ± 0.44 | 7.15 ± 1.36 |
| | | Methanol | 11.25 | 8.22 ± 0.21 | 2.75 ± 0.24 |
| 6 | <i>M. oleifera</i> | Hexane | 1.29 | 1.05 ± 0.09 | 11.54 ± 1.01 |
| | | Chloroform | 2.06 | 7.34 ± 0.23 | 10.32 ± 0.17 |
| | | Acetone | 0.97 | 29.94 ± 0.85 | 31.90 ± 4.06 |
| | | Methanol | 21.05 | 16.87 ± 0.03 | 7.91 ± 0.18 |
| 7 | <i>S. tuberosum</i> | Hexane | 2.28 | 4.45 ± 0.28 | 2.20 ± 0.23 |
| | | Chloroform | 2.39 | 9.60 ± 0.51 | 8.93 ± 0.41 |
| | | Acetone | 1.34 | 47.79 ± 0.71 | 12.61 ± 0.54 |
| | | Methanol | 10.17 | 46.94 ± 0.34 | 5.78 ± 0.17 |

The values are mean (n = 3); * The values are mean ± standard error mean (n = 3).

capable of damaging almost every molecules found in living cells (Hochstein and Atallah, 1988). The most reactive free radical is the hydroxyl radical which is known to initiate lipid peroxidation and cause fragmentation of DNA leading to mutation. The chloroform extract of all the studied plants showed poor hydroxyl radical scavenging activity (>1000 µg/ml, Table 3), therefore only the results of acetone and methanol extracts is presented in the present work. Gallic acid was used as standard (140 µg/ml). Best hydroxyl radical scavenging activity was shown by acetone extract of *M. indica*; its IC₅₀ value was 350 µg/ml.

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 the oxidative stress. Superoxide anion, which is a reduced form of molecular oxygen, had been implicated in the containing oxidation reactions associated with aging (Cotelle et al., 1996). Antioxidant properties of flavonoid are effective mainly via the scavenging of superoxide anion radical. In the PMS/NADH-NBT system, superoxide anion derived from

Table 3. DPPH free radical, hydroxyl radical and superoxide anion radical scavenging activities of different solvent extracts of screened plants.

| No. | Plant name | IC ₅₀ value (µg/ml) | | | | | | | | | | | |
|-----|----------------------------|--------------------------------|-----|------|------|----|----|------------|------------|----|----|-----|-------|
| | | DPPH | | | | OH | | | | SO | | | |
| | | HE | CH | AC | ME | HE | CH | AC | ME | HE | CH | AC | ME |
| 1 | <i>Ananas comosus</i> | A | A | A | A | ND | A | 700 | A | ND | A | A | A |
| 2 | <i>Mangifera indica</i> | A | 140 | 16.5 | 23.5 | ND | A | 350 | A | ND | A | 86 | 187.5 |
| 3 | <i>Lagenaria siceraria</i> | A | 790 | 58 | 160 | ND | A | 335 | 660 | ND | A | 750 | A |
| 4 | <i>Solanum tuberosum</i> | A | A | 200 | 380 | ND | A | 910 | A | ND | A | A | A |
| 5 | <i>Luffa acutangula</i> | A | A | A | A | ND | A | A | A | ND | A | A | A |
| 6 | <i>Momordica charantia</i> | A | A | A | A | ND | A | A | 750 | ND | A | A | A |
| 7 | <i>Moringa oleifera</i> | A | A | 520 | A | ND | A | A | A | ND | A | A | A |

DPPH: 2,2-diphenyl-1-picryl-hydrazyl free radical; OH: hydroxyl radical; SO: superoxide anion radical; A: >1000; ND: not done; HE: hexane extract; CH: chloroform extract; AC: acetone extract; ME: methanol extract.

dissolved oxygen by PMS/NADH coupling reaction reduces NBT. Antioxidants are able to inhibit the blue NBT formation (Cos et al., 1998). The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion radical in the reaction mixture. Table 3 shows the IC₅₀ values of superoxide anion radical scavenging activity.

The chloroform extract of all the studied plants showed poor superoxide anion radical scavenging activity (>1000 µg/ml, Table 3). The IC₅₀ value of acetone extract of *M. indica* peel was 86 µg/ml while that of methanol extract was 187.5 µg/ml. The acetone extract appears to be a better scavenger than the standard gallic acid (IC₅₀ = 185 µg/ml) and methanol extract is as good as that of the standard.

Reducing capacity assessment

The reducing capacity assessment 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 activity and reducing capacity assessment of components of medicinal herbs (Yildirim et al., 2001). Therefore reducing capacity assessment may be used as an indicator of potential antioxidant activity. In this method, antioxidant compounds form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride that is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the increase in the reducing capacity assessment of the sample. Ascorbic acid was used as a standard (Ksouri et al., 2009).

In the present work, seven vegetable and fruit peels in various solvents were evaluated for their reducing capacity assessment (Figures 1 to 2). Out of seven studied vegetable and fruit peels, *M. indica* (Figure 1a), *L. siceraria* (Figure 1b) and *L. acutangula* (Figure 2b) peels showed reducing capacity assessment, while other peel extracts showed poor reducing capacity.

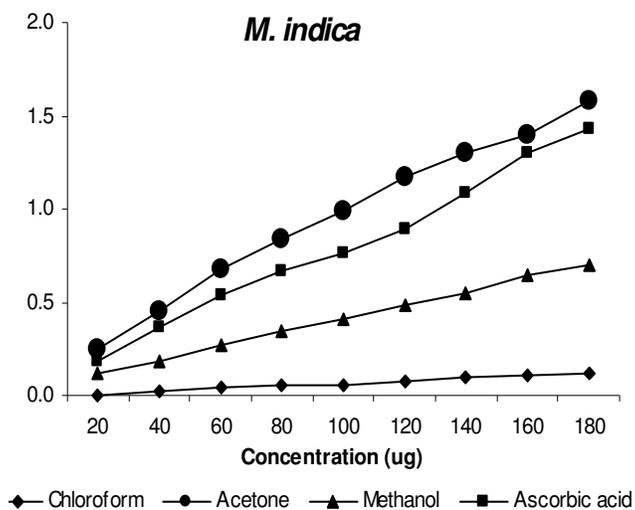
In *M. indica*, there was concentration dependent increase in the absorbance of reaction mixture for all the three extracts and standard ascorbic acid (Figure 1a). The acetone extract showed maximum absorbance and hence maximum capacity assessment among its various solvent extracts. In fact, the reducing capacity assessment of acetone extract was more than that of the standard ascorbic acid (Figure 1a).

The reducing capacity assessments of other extracts were in the order: acetone extract > ascorbic acid > methanol extract > chloroform extract. The ability of *M. indica* peel to exhibit significant reducing capacity assessment and to scavenge DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reaction.

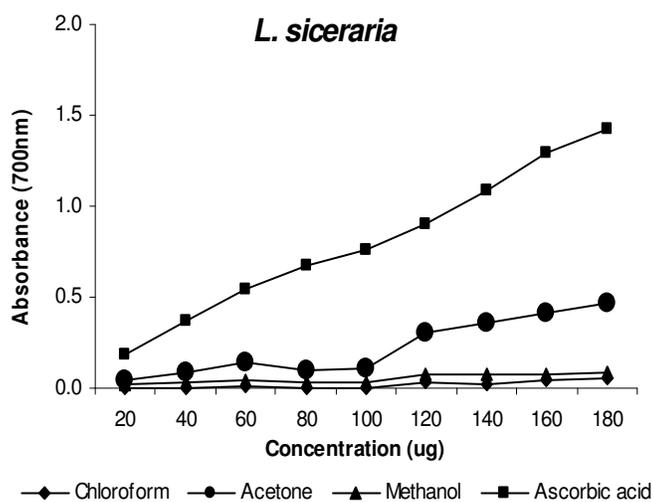
Plant extracts and plant-derived antioxidants can elicit a number of *in vivo* effects such as promotion of increased synthesis of endogenous antioxidant defenses or themselves acting directly as antioxidants (Halliwell, 1990). However, antioxidant activity of a compound can also be assessed *in vitro* by testing it on biologically relevant reactive oxygen species. The ability of *M. indica* peel to exhibit significant reducing capacity assessment and to scavenge DPPH radicals suggests that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reaction.

Conclusion

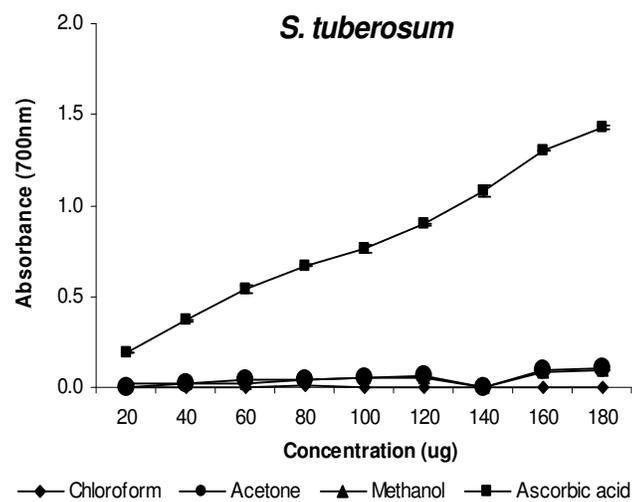
In this study, several *in vitro* assays were applied to evaluate the antioxidant potential of seven fruits and vegetables peels. The results of the present study would certainly help to ascertain the potency of the crude extract of peels of *M. indica* as a potential source of natural antioxidants. The author think they are the first to report about the strong antioxidant capacity of acetone extract of *M. indica* peel. However, further studies are



a



b



c

Figure 1. Reducing capacity assessment of different solvents extracts.

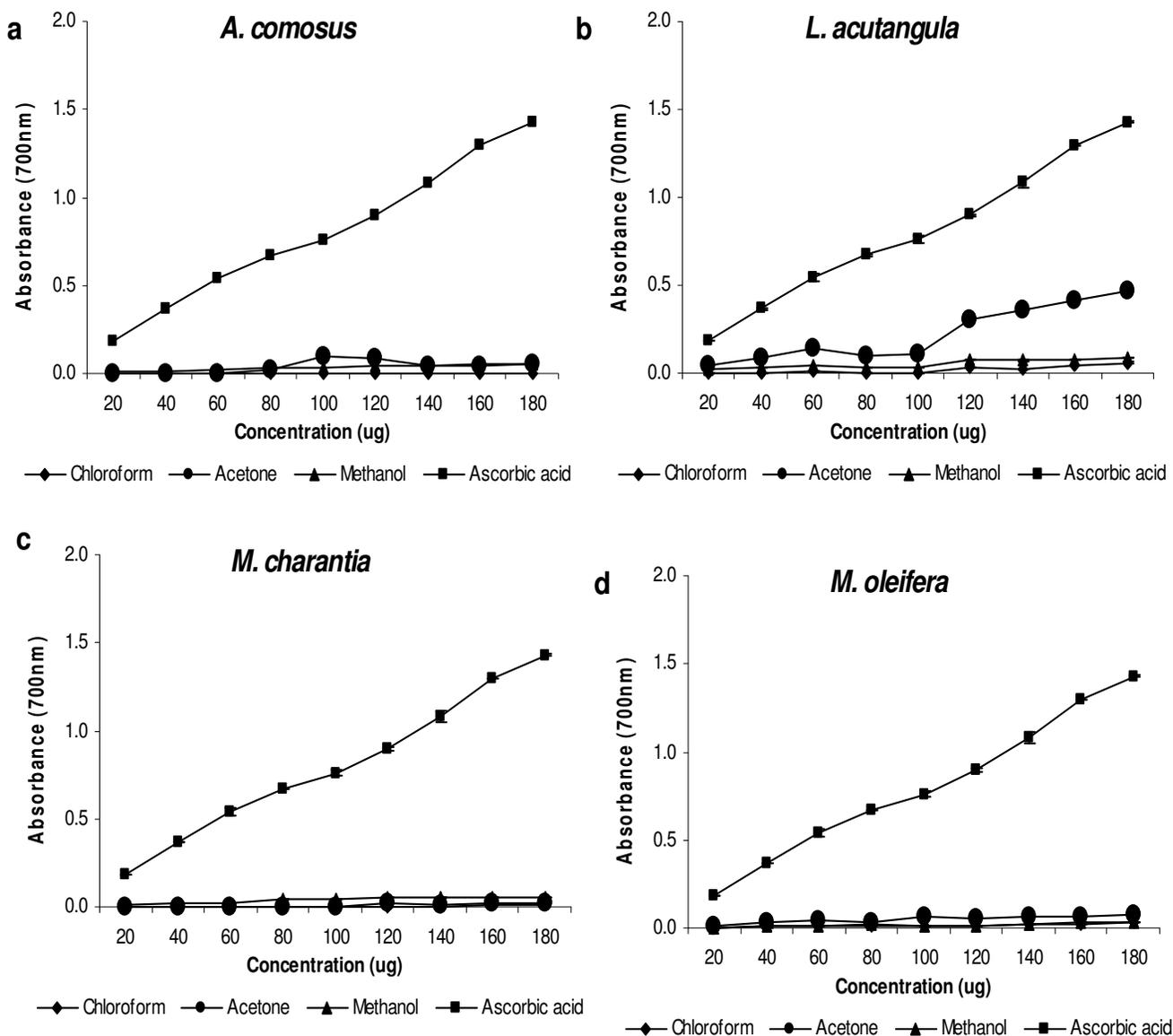


Figure 2. Reducing capacity assessment of different solvent extracts.

required before it can be used as a source of antioxidant. These are novel, natural and economic sources of antioxidants, which can be used in the prevention of diseases caused by free radicals. Therefore, our study will definitely open, scope for future utilization of these waste products for therapeutic purpose. Our results also indicate that selective extraction from natural materials, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity. The acetone extract of *M. indica* peel showed best antioxidant capacity may be because of its higher phenolic content which normally is the major determinant of antioxidant potential of food plants. Therefore, *M. indica* peel can be a good source of natural antioxidants.

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REFERENCES

Anjaria J, Parabia M, Dwivedi S (2002). Ethnovet Heritage Indian Ethnoveterinary Medicine – An Overview, pp 420. Pathik Enterprise, Ahmedabad, India.

- Antolovich A, Bedgood DR, Bishop AG, Jardine D, Prenzler PD, Robards K (2004). LC-MS Investigation of oxidation products of phenolic antioxidants. *J. Agric. Food Chem.*, 52: 962-971.
- Athukorala Y, Kim KN, Jeon YJ (2006). Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Ecklonia cava*. *Food Chem. Toxicol.*, 44: 1065-1074.
- Baravalia Y, Kaneria M, Vaghasiya Y, Parekh J, Chanda S (2009). Evaluation of antioxidant and antibacterial activity of *Diospyros ebenum* Roxb. Leaf extracts. *Turk. J. Biol.*, 33: 159-164.
- Bensky D, Claveys Stoger E (2004). (Eds.) *Materia Medica*, Eastland Press, Inc. USA, 193-194.
- Bors W, Heller W, Michel C, Saran M (1990). Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods Enzymol.*, 186: 343-355.
- Blois MS (1958). Antioxidant determination by the use of a stable free radical. *Nat.*, 181: 1199-1200.
- Chanda S, Dave R (2009). *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *Afr. J. Microbiol. Res.*, 3: 981-996.
- Chanda SV, Nagani KV (2010). Antioxidant capacity of *Manilkara zapota* L. leaves extracts evaluated by four *in vitro* methods. *Nat. Sci.*, 8: 260-266.
- Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid content in *propolis* by two complementary colorimetric methods. *J. Food Drug Anal.*, 10: 178-182.
- Cos P, Ying LY, Calomme M, Hu JP, Cimanga K, Van Poel B, Pieters L, Vlietinck AJ, Berghe DV (1998). Structure activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J. Nat. Prod.*, 61: 71-76.
- Cotelle N, Berier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM (1996). Antioxidant properties of hydroxy flavones. *Free Rad. Biol. Med.*, 20: 35-43.
- Ghasemi K, Ghasemi YD, Ebrahimzadeh MA (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak. J. Pharm. Sci.*, 22: 277-281.
- Golivand MB, Nasrabadi MR, Batooli H, Ebrahimabadi AH (2010). Chemical composition and antioxidant activities of the essential oil and methanolic extracts of *Psammogeton canescens*. *Food Chem. Toxicol.*, 48: 24-28.
- Gouado I, Schweigert FJ, Ejoh RA, Tchouanguep MF, Camp JV (2007). Systemic levels of carotenoids from mangoes and papaya consumed in three forms (juice, fresh and dry slice). *Eur. J. Clin. Nutr.*, 61: 1180-1188.
- Halliwell B (1990). How to characterize a biological antioxidant. *Free Radic. Res. Commun.*, 9: 1-32.
- Hochstein P, Atallah AS (1988). The nature of oxidant and antioxidants systems in the inhibition of mutation and cancer. *Mutat. Res.*, 202: 363-375.
- Jing Z, Nan H, Ya-lin W, Yuan-jiang P, Cui-rong S (2008). Preliminary studies on the chemical characterization and antioxidant properties of acidic polysaccharides from *Sargassum fusiforme*. *J. Zhejiang. Univ. Sci. B.*, 9: 721-727.
- Kabuki T, Nakajima H, Arai M, Ueda S, Kuwabara Y, Dosako S (2000). Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. *Food Chem.*, 71: 61-66.
- Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S (2009). Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra region, India. *Indian J. Pharm. Sci.*, 71: 406-412.
- Kessler M, Ubeaud G, Jung L (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J. Pharm. Pharmacol.*, 55: 131-142.
- Ksouri R, Hanen Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magne C, Abdely C (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem. Toxicol.*, 47: 2083-2091.
- Kunchandy E, Rao MNA (1990). Oxygen radical scavenging activity of curcumin. *Int. J. Pharmaceut.*, 58: 237-240.
- Liu RH (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.*, 78: 517-520.
- Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner K, Baldwin EA (2006). Total antioxidant activity and fiber content of select Florida-grown tropical fruits. *J. Agric. Food Chem.*, 54: 7355-7363.
- Mantena RKR, Wijburg OLC, Vindurampulle C, Bennett-Wood VR, Walduck A, Drummond GR, Davies JK, Robins - Browne RM, Strugnell RA (2008). Reactive oxygen species are the major antibacterial against *Salmonella typhimurium* purine auxotrophs in the phagosome of RAW264.7 cells. *Cell Microbiol.*, 10: 1058-1073.
- Mc Donald S, Prenzler PD, Autolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chem.*, 73: 73-84.
- McCune LM, Johns T (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J. Ethnopharmacol.*, 82: 197-205.
- Morsi RMY, Tahan NREL, El-Hadad AMA (2010). Effect of aqueous extract *Mangifera Indica* leaves, as functional foods. *J. Appl. Sci. Res.*, 6: 712-721.
- Negro C, Tommasi L, Miceli A (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technol.*, 87: 41-44.
- Pallauf K, Rivas-Gonzalo JC, Castillo MD, Cano MP, Pascual-Teresa S (2008). Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo* L.) fruits. *J. Food Compos. Anal.*, 21: 273-281.
- Parekh J, Chanda S (2007). *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae). *Braz. J. Microbiol.*, 38: 204-207.
- Prakash D, Upadhyay G, Singh BN, Singh HB (2007). Antioxidant and free radical - scavenging activities of seeds and agri - wastes of some varieties of soyabean (*Glycine max*). *Food Chem.*, 104: 783-790.
- Prasad KN, Xie H, Hao J, Yang B, Qiu S, Wei X, Chen F, Jiang Y. (2010). Antioxidant and anticancer activities of 8 - hydroxypsoralen isolated from wampee [*Clausena lansium* (Lour.) Skeels] peel. *Food Chem.*, 118: 62-66.
- Oke F, Aslim B, Ozturk S, Altundag S (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chem.*, 112: 874-879.
- Rajaei A, Barzegar M, Mobarez AM, Sahara MA, Esfahani ZH (2010). Antioxidant, antimicrobial and antimutagenicity activities of pistachio (*Pistachia vera*) green hull extract. *Food Chem. Toxicol.*, 48: 107-112.
- Robak J, Gryglewski RJ (1988). Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.*, 37: 837-841.
- Saravana Kumar A, Mazumder A, Vanitha J, Venkateshwaran K, Kamalakannan K, Sivakumar T (2008). Evaluation of antioxidant activity, phenol and flavonoid contents of some selected Indian medicinal plants. *Phcog Mag.*, 4: 143-147.
- Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.*, 40: 945-948.
- Singh RP, Murthy KNC, Jayaprakasha GK (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J. Agric. Food Chem.*, 50: 81-86.
- Singh R, Singh S, Kumar S, Arora S (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Food Chem. Toxicol.*, 45: 1216-1223.
- Temple NJ (2000). Antioxidants and disease: more questions than answers. *Nutr. Res.*, 20: 449-459.
- Vaghasiya Y, Chanda S (2007). Screening of methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. *Turk. J. Biol.*, 31: 243-248.
- Yang D, Wang Q, Ke L, Jiang J, Ying T (2007). Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* Gaertn) rhizome. *Asia Pac. J. Clin. Nutr.*, 16: 158-163.
- Yildirim A, Mavi A, Kara AA (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J. Agric. Food Chem.*, 49: 4083-4089.