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Evaluation of Antioxidant Properties of Some Indian Vegetable and Fruit Peels by Decoction Extraction Method

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ABSTRACT

Vegetable and fruit peels are generally thrown into the environment as waste material. If this waste can be exploited for some beneficial purpose it will be useful and helpful. With this idea it was thought of interest to evaluate the antioxidant potency of peels. Fourteen vegetables and six fruits belonging to nine different families were selected to evaluate their antioxidant potential. The extraction was done by decoction method which is a common traditional method. Antioxidant property was evaluated by superoxide anion radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP). The extractive yield was maximum in *Daucus carota*. Maximum Total Phenol Content (TPC) was in ripe peel of *Musa paradisiaca* while best and maximum superoxide anion scavenging activity was in *Terminalia catappa*. This activity was even better than standard gallic acid. *T. catappa* also showed highest FRAP. There was no correlation between TPC and antioxidant activity. The peel of *T. catappa* appears to be best agro waste which can be a promising source of natural antioxidants. The results confirm the belief that agro waste can be therapeutically used. However, further study need to be done using other antioxidant assays.

Key words: Peels, agro waste, antioxidant activity, FRAP, *Terminalia catappa*, *Musa paradisiaca*

INTRODUCTION

Nature has bestowed us with many different kinds of plants and all parts individually or totally exhibit therapeutic properties. The part may be leaf, bark, seed, stem, flowers, fruits, twigs and peel etc. each part showing different biological activity and antioxidant potency (Chanda *et al*., 2011, 2012; Kalpna *et al*., 2011; Rakholiya and Chanda, 2012a; Munir and Karim, 2013). The fruits and vegetables which are consumed daily contain many macronutrients, micro nutrients and non-nutrient compounds which play a protective role in the pathogenesis of life threatening human diseases and disorders. The search for natural antioxidants from fruits and vegetables for wide spread applications in various fields like medicine, cosmetics, food industry is going on with renewed interest. This is mainly because of their protective effect which is believed to play a significant role in the etiology and pathogenesis of various chronic diseases and the oxidative deterioration of cosmetics, foods and pharmaceutical preparations (Lagouri *et al*., 2011; Kaneria *et al*., 2012a; Rakholiya and Chanda, 2012b) by counteracting the effects of dangerous reactive oxygen species.

Oxidative stress is the root cause of all diseases and also causes food deterioration. Lipid peroxidation is very complicated processes which occur in aerobic cells and which interact with single molecular oxygen and ester of polyunsaturated fatty acids. Free radicals are known to take part in lipid peroxidation which are responsible for various chronic and degenerative diseases in organisms and also decreases nutritional quality of food; causes rancidity, discolouration and produces unpleasant flavours due to which consumption of such food becomes questionable regarding its safety. People who consume such food are prone to ill health (Zielinski *et al*., 2012; Moyo *et al*., 2013). Processed foods contain significant amounts of Polyunsaturated Fatty Acids (PUFAs) and it is necessary to add antioxidants to prevent oxidation, to increase product shelf life, decrease rancidity, discoloration, etc. Synthetic antioxidants like Butylated Hydroxyasinole (BHA) and Butylated Hydroxytoluene (BHT), Propyl Gallate (PG) are widely used in the food industry but it has raised serious objections because of their toxic nature, carcinogenetic and low solubility (Chanda and Nagani, 2010; Chanda *et al*., 2010, 2013; Adamez *et al*., 2012). Because of these detrimental consequences, the reduction of free radicals or reactive oxygen species in both human and food systems is highly desirable. Hence, the investigators have turned their attention to target and identify alternative novel antioxidants from natural sources which have therapeutic properties, lesser side effects and cost effective as compared to synthetic ones.

A Polyphenolic compound represents a rich source in fruits and vegetables. Polyphenolic compounds are important in terms of quality, influence the visual appearance and taste and are also important therapeutically. They are associated with the prevention of different degenerative diseases by reduction of oxidative stress as well as oxidative induced reactions in food products (Arancibia-Avila *et al*., 2012; Chanda *et al*., 2012; Dinis *et al*., 2012; Ravichandran *et al*., 2012; Shoko *et al*., 2013).

The vegetable and fruit peels are normally thrown away as waste into environment but different phytochemicals present in them like in any other part of the plant makes them important alternative source of natural antioxidants. Hence, attention should be paid for proper extraction of these compounds and check their suitability as therapeutics. This will increase the aggregate value of the agro industrial waste. The waste products from food, forest or agricultural industries are particularly interesting because they are inexpensive starting materials and their re-use makes them environmental friendly.

Traditionally decoction method was the most common form of extraction of herbal drugs. In this method medicinal plants are boiled in a specified volume of water for a definite time, then it is cooled and filtered and the extracts are consumed (Chanda and Dave, 2009). In this type of extraction, only water soluble and heat stable constituents are extracted but it is simple and convenient method and does not require any type of instrumentation and affordable by all people. From this point of view, the aim of this study was to evaluate antioxidant properties from Indian fruits and vegetable peels extracted by decoction methods.

MATERIALS AND METHODS

Chemicals and reagents: Nitroblue Tetrazolium (NBT), Phenazine Methosulphate (PMS), Nicotinamide Adenine Dinucleotide Reduced (NADH), gallic acid, ferrous sulphate ($FeSO_4$), Folin-Ciocalteu's reagent, sodium carbonate, potassium acetate, ferric chloride ($FeCl_3$), 2,4,6- tripyridyl-5-triazine (TPTZ), Tris-HCl, sodium acetate, Hydrochloric Acid (HCl), were obtained from Hi-media, Merck or Sigma. All reagents used were of analytical grade.

Collection and sample preparation

Collection: Different fresh fruits and vegetables were purchased from local market in Rajkot, Gujarat, India. The fruits were washed thoroughly with tap water and then the peels were separated and homogenized it to fine powder paste in deionized water and used for extraction. The traditional uses of studied (peels) fruits and vegetables and their family name are given in Table 1.

Decoction extraction method: For the decoction method (Li *et al*., 2007; Kaneria *et al*., 2012b, c), 5 g of fresh peels were extracted in 100 mL of deionized water at 100°C for 30 min in a water bath. It was filtered with eight layers of muslin cloth and centrifuged at 5000 rpm in centrifuge (Remi Centrifuge, India) for 10 min. The supernatant was collected and the solvent was evaporated to dryness. The residue was weighed to obtain the extractive yield and it was stored in air-tight bottle at 4°C.

Table 1: Plant name, family and traditional uses of fruits and vegetables studied

Family	Plant name	Traditional uses
Cucurbitaceae	*Lagenaria siceraria* (Molina) Standl.	It cures pain, ulcers, fever and used for pectoral cough, asthma and other bronchial disorders
	Momordica charantia L.	It used in constipation, intermittent fever, skin diseases, leprosy, ulcers, wounds and diabetes
	Cucumis sativus L.	It prevents haemorrhages, epitasis, jaundice and dehydration in the body; it also helpful in restoring the water loss
	Luffa cylindrica L.	It is used in vitiated kapha, pitta, jaundice, infective hepatitis, constipation, skin diseases, piles and tumours
	Coccinia indica W and A.	It is used in diabetes, eruption of skin, gonorrhoea, ring-worm, psoriasis and itch
	Luffa acutangula (L.) Roxb.	It is used in vitiated vata, pitta, skin diseases, jaundice, haemorrhoids and also used for general weakness
	Trichosanthes dioica Roxb.	It is used in biliousness, bronchitis, boils, heat troubles and blood diseases
	Momordica balsamina L.	It is used in ulcer, bronchitis, snake bite and recovering from fever. It is also used in urinary complaints
Solanaceae	*Solanum tuberosom* L.	It is used in chronic cough, constipation, hyper acidity, scabies and plaster for burns
	Solanum melongena L.	It is used in inflammations, asthma, cardiac debility, cholera, bronchitis and fever
Musaceae	*Musa paradisiaca* L.(ripe)	It is used in kapha, biliousness, pain in the ear, menstrual disorders, diseases
	Musa paradisiaca L.(unripe)	of the blood, leprosy
Combretaceae	*Terminalia catappa* L.	It is used in biliousness, bronchitis, leprosy, cutaneous diseases, headache, colic, dysentery and diarrhoea
Caricaceae	*Carica papaya* L.	It is used in diuretic, habitual constipation, piles and dyspepsia
Rutaceae	*Citrus raticulata* Blanco	It is used in abdominal distension, to enhance digestion and to reduce phlegm It is also used in Ayurveda
	Citrus limon L.	It is used as carminative, as flavouring liqueurs and also used in scurvy and in hypertrophy of spleen
	Aegle marmelos Correa ex roxb.	It is used in fever, abdominal pain, urinary troubles, inflammations, eye affections, vomiting, dysentery, pain, chronic diarrhoea and heart troubles
Chenopodiaceae	*Beta vulgaris* L.	It is used in inflammation, paralysis, headache, earache, diseases of spleen and liver
Apiaceae	*Daucus carota* L.Var. sativa DC.	It is used in colic, infestations of round worms and thread worms, diarrhoea, heartburn, hyperacidity, cough, asthma, bronchitis, wounds, ulcers, tumours, inflammations, diabetes and jaundice
Moringaceae	*Moringa oleifera* Lamk.	It is used in diarrhoea, colic, paralysis, inflammations, fever, cough, asthma, bronchitis, ringworm, scurvy, vata and kapha, wounds and tumours

Antioxidant testing assays

Superoxide anion radical scavenging assay (SO): The superoxide anion radical scavenging activity was measured by the method as described by (Robak and Gryglewski, 1988). Superoxide radicals are generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture (3.0 mL) consisted of 1.0 mL of different concentrations (20-1000 μg mL^{-1}) of different solvent extracts and fractions diluted by distilled water, 0.5 ml Tris-HCl buffer (16 mM, pH 8), 0.5 mL NBT (0.3 mM), 0.5 ml NADH (0.936 mM) and 0.5 mL PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solution to the mixture. The reaction mixture was incubated at 25°C for 5 min and then the absorbance was measured at 560 nm using a UV-VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. Gallic acid (50-225 μg mL^{-1}) was used as a positive control (Robak and Gryglewski, 1988). Percentage of inhibition was calculated using the following formula:

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

where, A is the absorbance of the test and B is the absorbance of the control.

Ferric reducing antioxidant power (FRAP): The reducing ability was determined by Ferric Reducing Antioxidant Power (FRAP) assay of Benzie and Strain (1996). FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The extract (0.1 mL) was added to 3.0 mL FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ in 40 mM HCl and 1 part 20 mM $FeCl_3$) and the reaction mixture was incubated at 37°C for 10 min. And then, the absorbance was measured at 593 nm using a UV-VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. The calibration curve was made by preparing a $FeSO_4$ (100 to 1000 μM mL^{-1}) solution in distilled water (Thondre *et al*., 2011). The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as M $FeSO_4$ equivalents per gram of extracted compounds.

Quantitative phytochemical analysis:

Determination of total phenol content (TPC): The amount of Total Phenol Content (TPC) was determined by Folin-Ciocalteu's reagent method (McDonald *et al*., 2001). The extract (0.5 mL) and 0.1 mL Folin-Ciocalteu's reagent (0.5 N) were 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 using a UV-VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. The calibration curve was made by preparing gallic acid (10 to 100 μg mL^{-1}) solution in Distilled water (Chanda and Kaneria, 2012). Total phenol content is expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compounds).

Statistical analysis: Each sample was analyzed individually in triplicate and the results are expressed as the mean value (n = 3) ±Standard Error of Mean (SEM).

RESULTS AND DISCUSSION

Extractive yield: The extractive yield of decoction extracts of fruits and vegetable peels are given in Table 2. The extractive yield varied amongst all the plant peels investigated. Amongst the peel extracts screened, the highest extractive yield was in *D. carota* (8.57) and lowest was in *L. siceraria* (1.44). There are many reports in the literature where extractive yield varied in aqueous extracts of different plants (Zhou *et al*., 2011; Kaneria *et al*., 2012c; Rakholiya and Chanda, 2012a). There is no universal criterion for maximum yield in a particular solvent, or particular plant or plant part. It varies from plant to plant because of the nature of secondary metabolites present in them.

Antioxidant properties: The antioxidative phytochemicals in vegetables, fruits and medicinal plants have received increasing attention because of their potential role in preventing human diseases. Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides (Wang *et al*., 2012). Owing to the complex reactive nature of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Chanda and Dave, 2009; Schlesier *et al*., 2002). Therefore, in the present study, Ferric Reducing Antioxidant Power (FRAP) and superoxide anion radical scavenging activity was evaluated in all the twenty plant peels studied.

Superoxide anion free radical scavenging assay: Superoxide anion radical scavenging activity of standard Gallic acid, *S. tuberosum* and *T. catappa* is shown in Fig. 1. Out of 20 plant peels of 9 families screened, only the peels of *S. tuberosum* and *T. catappa* showed superoxide anion scavenging activity and it was concentration dependent activity. None of the other peel extracts showed superoxide anion radical scavenging activity and their EC_{50} values were >1000 μg mL^{-1}. *S. tuberosum* could scavenge superoxide anion radical in concentration range between

Table 2: Extractive yield, total phenol content and ferric reducing power of decoction extracts of different fruits and vegetable peels

Family	Plant name	% Extractive yield (w w^{-1})	TPC (mg g^{-1})*	FRAP (M g^{-1})*
Cucurbitaceae	*L. siceraria*	1.44	32.86±0.27	6.57±0.09
	M. charantia	2.33	26.60±0.27	2.47±0.15
	C. sativus	2.36	9.30±0.74	1.69±0.10
	L. cylindrica	2.70	17.22±0.10	2.93±0.04
	C. indica	2.36	20.74±0.09	1.91±0.02
	L. acutangula.	2.34	25.11±0.29	2.58±0.05
	T. dioica	3.19	29.22±0.16	3.78±0.15
	M. balsamina	2.95	31.79±0.27	8.60±0.10
Solanaceae	*S. tuberosom*	2.69	29.37±0.42	4.74±0.03
	S. melongena	3.97	31.71±0.06	3.69±0.19
Musaceae	*M. paradisiaca* (ripe)	2.93	55.43±0.61	9.02±0.13
	M. paradisiaca (unripe)	2.21	18.82±0.95	13.18±0.24
Combretaceae	*T. catappa*	4.40	27.21±0.49	78.48±0.88
Caricaceae	*C. papaya*	4.51	43.59±0.29	4.63±0.07
Rutaceae	*C. raticulata*	5.96	8.65±0.93	1.87±0.06
	C. limon	4.80	22.40±0.65	1.88±0.08
	A. marmelos	4.15	18.46±0.16	6.36±0.04
Chenopodiaceae	*B. vulgaris*	6.60	34.09±0.67	5.45±0.66
Apiaceae	*D. carota*	8.57	11.32±0.69	0.61±0.25
Moringaceae	*M. oleifera*	4.40	20.76±0.85	1.75±0.31

*Values are expressed in Mean±Standard error of the mean (n = 3), TPC = Total phenol content, FRAP: Ferric reducing antioxidant power

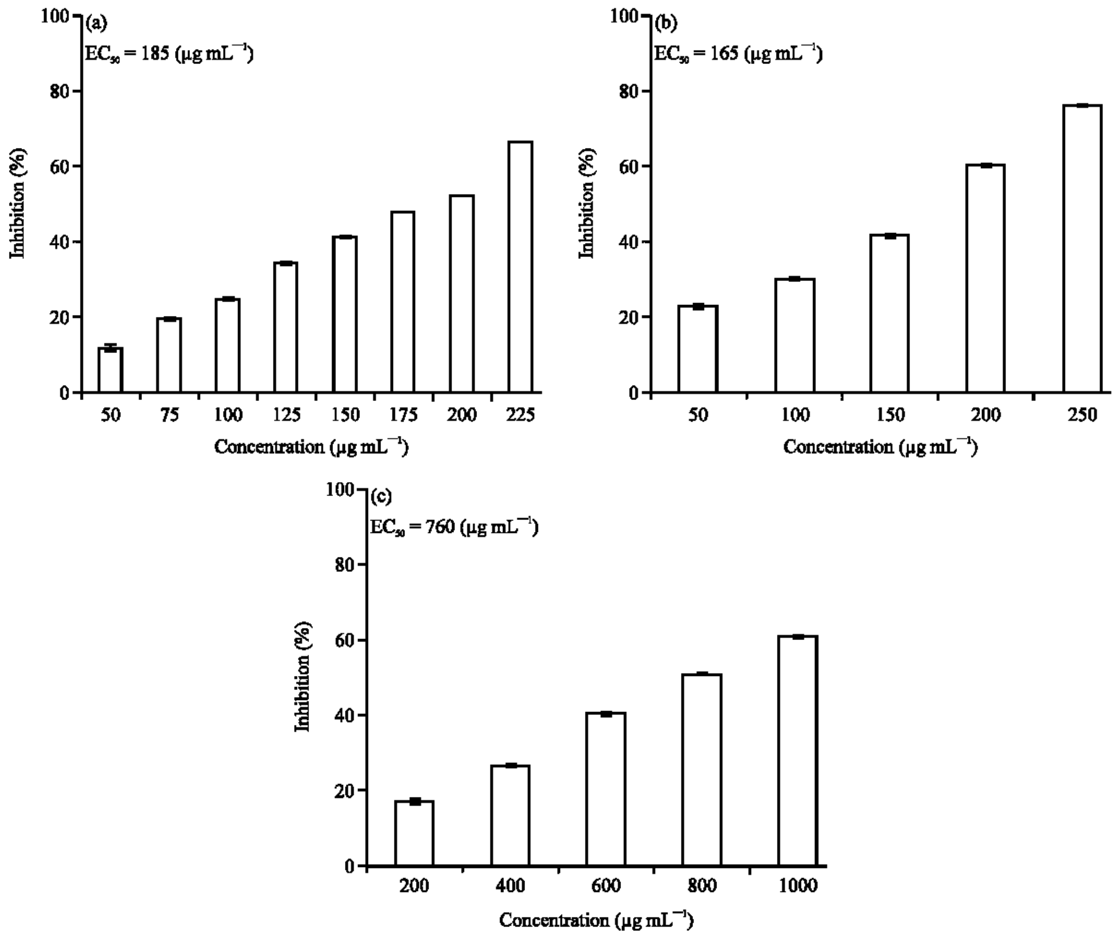

Fig. 1(a-c): Superoxide anion radical scavenging activity of standard gallic acid and decoction extracts of *T. catappa* and *S. tuberosum*. Values represent Mean±SEM (n = 3)

200-1000 µg mL^{-1} and its EC_{50} value was 760 µg mL^{-1} (Fig. 1c). The EC_{50} value of *T. catappa* was 165 µg mL^{-1} which was lower than that of standard Gallic acid (185 µg mL^{-1}; Fig. 1a, b), indicating better antioxidant capacity of *T. catappa*. The possible mechanism by which *T. catappa* acts as an antioxidant may be attributed to its electron donation power to the free radicals, thereby terminating the radical chain reactions (Lai *et al*., 2010).

Ferric reducing antioxidant power: The FRAP assay treats the antioxidants contained in the samples as reductant in a redox linked colorimetric reaction and the value reflects the reducing power of antioxidants (Benzie and Strain, 1996). The antioxidant potentials of different samples were estimated by their ability to reduce the TPTZ-Fe^{3+} complex to the TPTZ-Fe^{2+} complex with a maximum absorption at 593 nm. The reduction of absorbance is proportional to the antioxidant content (Fan *et al*., 2012; Kaneria *et al*., 2012c).

The reducing ability of fresh peels of fruits and vegetables determined by FRAP assay is shown in Table 1. Amongst peels of 9 families studied, of *T. catappa* of Combretaceae family showed maximum ferric reducing antioxidant power as compared to other peels investigated (78.48 M g^{-1}; Table 1) while lowest FRAP was in Apiaceae family i.e., in *D. carota* (Table 1).

Total phenol content: Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins and phenolic acids (Lee and Lee, 2010; Chanda and Kaneria, 2012). Polyphenols are generally known as the antioxidant agents in plant extracts (Bernardi *et al*., 2008). It is generally reported that total phenolic content is a good indicator of antioxidant capacity of a plant and this is a very easy and convenient method to analyze plant antioxidative property before further studies are carried out.

The total phenol content of fruits and vegetables peels belonging to 9 different families is given in Table 2. In Cucurbitaceae family, the total phenol content was highest in *L. siceraria* (32.86 mg g^{-1}) and minimum was in *C. sativas* (9.30 mg g^{-1}). In Solanaceae family, the total phenol content was more in *S. melongena* while in Rutaceae family, total phenol content was more in *C. limon*. Amongst peels of 9 families studied, *M. paradisiaca* (ripe) peel of Musaceae family had highest phenolic content (55.43 mg g^{-1}) while lowest was in *C. raticulata* of Rutaceae family (8.65 mg g^{-1}; Table 2).

There are many correlation studies which demonstrated a link between antioxidant activities in plants and their phenolic content, underlining the significant contribution which phenolics can make to antioxidant activities (Park and Jhon 2010; Kaneria and Chanda, 2011, 2012; Razali *et al*., 2012). However, according to these results there is no correlation between phenol content and antioxidant activity, suggesting that non phenolic compounds also contribute to the antioxidant property of plants. No correlation between phenol content and antioxidant activity of plants is also reported (Chanda and Nagani, 2010; Sulaiman *et al*., 2011; Shabir *et al*., 2011). The results also revealed that the higher and/or lower values of antioxidant activities (evaluated using different assays) were detected from different plant parts and were not restricted towards certain part.

CONCLUSION

From above results, it can be concluded that highest total phenol content was present in *M. paradisiaca* ripe peel but decoction extracts of *T. catappa* showed best antioxidant activity even better than that of the standard used. Thus there was no correlation between total phenol content and antioxidant activity. However, it is not out of place to state that *T. catappa* extracted by simple decoction method showed best antioxidant activity and *T. catappa* peel may be useful in maintaining health and preventing degenerative diseases such as cancer, diabetes, coronary heart disease, mountain sickness that are exacerbated by generation of reactive oxygen species in the body as well as in food industry. Therefore, it can be stated that *T. catappa* peel could be an excellent natural source of antioxidants. *In vivo* study, involving animal models, will provide a better insight into the antioxidative potential of *T. catappa* peel, including its influence on the cellular antioxidant defence system.

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