



## Phytochemical composition, antioxidant and antibacterial potential of underutilized parts of some fruits

<sup>1,2\*</sup>Sharma, G., <sup>2</sup>Vivek, <sup>1,2</sup>Gupta, A.K., <sup>2</sup>Ganjewala, D., <sup>3</sup>Gupta, C. and <sup>3</sup>Prakash, D.

<sup>1</sup>Amity Center for Cancer Epidemiology and Cancer Research, Amity University Uttar Pradesh, NOIDA, India

<sup>2</sup>Amity Institute of Biotechnology, Amity University Uttar Pradesh, NOIDA, India

<sup>3</sup>Amity Institute of Herbal Research and Studies, Amity University Uttar Pradesh, NOIDA, India

### Article history

Received: 27 April 2016

Received in revised form:

26 May 2016

Accepted: 27 May 2016

### Keywords

Fruit waste

Phytochemicals

Antioxidant

Antibacterial

Reducing Power

Free Radicals

### Abstract

The study was aimed to evaluate phytochemical composition, antioxidant and antibacterial properties from powder of various underutilized fruits. Antibacterial activities from extracts of the underutilized parts of various fruit samples against Gram+ve (*Staphylococcus aureus*) and Gram-ve (*Escherichia coli*) bacteria were determined by using ELISA reader and expressed as relative inhibition (%), antioxidant profiles by β-carotene and linoleic acid and ferric thiocyanate reducing ability. The phytochemical screening was done by standard methods and their composition revealed total phenols ranging from 119.5 (stone apple) to 139.0 (acacia, mg GAE/g). Mango waste (156 mg QE/g) contained the maximum amount of flavonoids while stone apple waste (13 mg QE/g) showed the least. Maximum and minimum amount of proanthocyanidins were shown by grape waste (468 mg CE/g) and mosambi waste respectively. Grape and acacia waste powder showed the highest reducing power while least amount of reducing power was observed in apple waste sample. The waste powders from stone apple and orange exhibited potent antibacterial activity against microorganisms tested. Study revealed strong antioxidant profiles in acacia fruits and antibacterial activity in stone apple and orange.

© All Rights Reserved

### Introduction

The use of natural substances generally known as bioactive phytochemicals is gaining interest in recent times. This can be easily understood in the light of questions concerning the safety, cytotoxicity, and side-effects of synthetic compounds, and the need to find new medicines, including new antibiotics to manage infectious diseases caused by multidrug resistant pathogens and substances to treat chronic diseases. Phytochemicals are broadly described as polyphenols, flavonoids, isoflavonoids, anthocyanidins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates and fibers. Phytochemicals acting individually or synergistically may help to reduce the risk for a variety of chronic and inflammatory disorders. These include atherosclerosis and stroke, myocardial infarction, certain types of cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, Alzheimer's disease, osteoporosis, psoriasis, septic shock, AIDS, menopausal symptoms, and neurodegeneration (Cseke *et al.*, 2006). They have tremendous impact on the health care system and may provide medical health benefits including the prevention and/ or treatment of diseases and

physiological disorders. Majority of foods, such as whole grains, beans, fruits, vegetables and herbs contain phytochemicals. Amongst these, fruits and vegetables contribute to the significant sources of phytochemicals (Sharma *et al.*, 2011).

The biologically active phytochemicals are normally present in leaves, roots, barks, flowers and stems, but the plant parts such as rind of the fruit, seed and fruit shell etc., normally treated as a waste and only a few reports are recorded on the waste parts. In general, no part of the plant is completely worthless (Sivakumar and Venkataraman, 2010). The by-products represent an important source of sugars, minerals, organic acids, dietary fibre, and various bioactive phytochemicals. Among these phenolics are a much diversified group of secondary metabolites, which includes simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), lignans, lignins, coumarins, flavonoids, stilbenes, flavonolignans and tannins (Dewick, 2002). Many of phenolic compounds have shown strong antioxidant properties as free radical scavengers, peroxide decomposers, metal and chelating agents (Van Acker *et al.*, 1998; Van Hoorn *et al.*, 2002).

In recent times, there is an increasing interest

\*Corresponding author.

Email: [sharmagi03@gmail.com](mailto:sharmagi03@gmail.com), [gsharma3@amity.edu](mailto:gsharma3@amity.edu)

in the role of free radical-mediated damage in the etiology of human diseases. In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions (Thompson, 1994; Temple, 2000). Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, proteins and several diseases (Liu, 2002).

Besides antioxidant activity, phenolic compounds have a wide range of action which includes antitumor, antiviral, antibacterial, cardioprotective and antimutagenic activities. Thus, new aspects concerning the use of these wastes as by-products for further exploitation on the production of food additives, supplements with high nutritional value and medicinally important phytochemicals have gained significant importance (Sivakumar and Venkataraman, 2010). Infectious diseases are leading cause of death worldwide due to multidrug resistant strains of bacteria, reduced susceptibility to antimicrobials and increase in untreatable bacterial infections. Natural products provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to increasing threat of infectious diseases, the need of the hour is to find natural agents with novel mechanism of action. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of bioactive phytochemicals. It may be economic, eco-friendly and reduce pollution (Chanda *et al.*, 2010). The present studies were undertaken with the objectives of qualitative and quantitative estimation of phytochemicals, their antioxidant and antibacterial activities in waste parts of some fruits.

## Materials and Methods

### *Extraction of plant materials*

For present study, eight plants were used, namely mosambi (*Citrus sinensis*), orange (*Citrus reticulata*), grape (green variety Sonaka seedless, *Vitis vinifera*), pineapple (*Ananas comosus*), mango (*Mangifera indica*), stone apple (*Aegle marmelos*), acacia (*Acacia auriculiformis*) and apple (*Malus baccata*). The selected fruit peels were collected from the fruit juice shops and vicinity of Delhi-NCR (India) region. After collection, the peels were shade dried at room temperature (32-35°C), powdered (40-Mesh) and stored at room temperature separately in polybags.

Dried and powdered fruit waste (1 g) was soaked separately in 30 ml of 50% methanol and distilled water at ambient temperature for 24 hour under

shaking condition at 130 rpm on a mechanical shaker. The extract was then filtered using Whatman filter paper No. 1. Each extracts were transferred to sterile plastic vials and kept at 4°C till further use. All the experiments were conducted in triplicates and results are their mean values.

### *Qualitative analysis of phytochemicals*

The extracts of fruit peels were subjected to phytochemical screening by following the methodology as described by Harborne (1998). 1 ml of the extract was mixed with few drops of Wagner's reagents. A reddish-brown precipitate indicated the presence of alkaloids. Similarly, 1 ml of the extract was treated with 10% ammonium hydroxide. Yellow color indicated the presence of flavonoids. For detection of tannins, 1 ml of the extract was treated with few drops of 0.1% ferric chloride and observed for brownish green or a blue-black coloration. While to test phlobatannins, when 1 ml of the extract of each plant sample was boiled with 1% aqueous hydrochloric acid, deposition of a red precipitate was taken as evidence for its presence.

Bornträger's test was performed to detect the presence of anthraquinones. Briefly, 1 ml of the extract solution was hydrolyzed with diluted sulphuric acid and extracted with benzene. 1 ml of dilute ammonia was added to the extract. Rose pink coloration suggested the positive response for anthraquinones. Whereas to test saponins, froth test was used. Briefly, 2.5 ml extract was added to 10 ml of sterile distilled water in a test tube. The test tube was closed with cap and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins. Likewise, to detect the presence of terpenoids, Salkowski's test was performed. Briefly, 1 ml of extract was mixed in 0.5 ml of chloroform, and concentrated Sulphuric acid (0.5 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

To detect the presence of cardiac glycosides, Keller-Killani's test was performed. Concisely, 1 ml of extract was treated with 0.5 ml of glacial acetic acid containing one drop of ferric chloride solution followed by addition of 0.5 ml of concentrated sulphuric acid. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer. Similarly, to detect chalcones, 2 ml of ammonium hydroxide was added to 1 ml of extract of each sample. Appearance of reddish color showed the presence of chalcones. On the other hand, to test

anthocyanosides, to 1 ml of the extract 5 ml of dilute hydrochloric acid was added. Appearance of pale pink color showed the presence of anthocyanosides.

#### *Quantitative analysis of phytochemicals*

The amount of phenol in the fruit peel extracts was determined with Folin-Ciocalteu reagent using the modified method of Ragazzi and Veronese (1973) and Spanos and Wrolstad (1990). 0.5 ml of 10% Folin-Ciocalteu reagent and 0.5 ml of Sodium bicarbonate (2% w/v) was added to 0.5 ml of each sample extract (1 mg/ml). The resulting mixture was incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at  $\lambda_{\text{max}}$  765 nm. Results were expressed as milligrams of gallic acid equivalent (mg GAE/g). Similarly, total flavonoid contents were measured by the method of Jia *et al.* (1999). Aliquots (250 µl) of each extract (1 mg/ml) were added to a test tube containing 1.25 ml of distilled water. To this, 75 µl of 5% sodium nitrate solution was added and left for 5 min. Then, 150 µl of 10% ammonium chloride was added. After 6 min, 500 µl of 1 M sodium hydroxide was added. The content was diluted with 275 µl of distilled water. Absorbance of the solution was measured at  $\lambda_{\text{max}}$  510 nm. Total flavonoids content was expressed as milligrams of quercetin equivalent (mg QE/g) dry weight. For determination of total proanthocyanidins, the procedure of Sun *et al.* (1998) was used. The mixture of 3 ml of vanillin-methanol (4% v/v), 1.5 ml of hydrochloric acid was added to 0.5 ml (1 mg/ml) of extract and vortexed. The resulting mixture was allowed to stand for 15 min at room temperature followed by the measurement of the absorbance at  $\lambda_{\text{max}}$  500 nm. Total proanthocyanidins content was expressed as milligrams of catechin equivalent (mg CE/g) dry weight.

#### *Test for antioxidant activity*

Antioxidant activity (AOA) was estimated as described by Emmons and Peterson (1999) by monitoring the coupled autoxidation of  $\beta$ -carotene and linoleic acid. 10 mg of powdered sample was suspended in 10 ml of 50% methanol/water at room temperature followed by filtration and used to test AOA. 2 mg  $\beta$ -carotene was dissolved in 20 ml chloroform and 3.0 ml of this solution was added to 40 mg of linoleic acid and 400 mg of tween-40. Aliquots (3 ml) of the  $\beta$ -carotene and linoleic acid emulsion were mixed with 40 µl of sample extracts and incubated in a water bath at 50°C. Oxidation of this emulsified reaction mixture was monitored on a spectrophotometer by measuring absorbance at  $\beta/\lambda_{\text{max}}$  470 nm at 15 min intervals for 60 minutes and

calculated for AOA.

#### *Determination of reducing power*

The reducing power of the extract was evaluated according to the method of Oyaizu (1986). The mixture containing 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanite (1%w/v) was added to 1.0 ml of the extract. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloro acetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%, w/v). The absorbance was then measured at  $\lambda_{\text{max}}$  700 nm against blank sample.

#### *Bacterial inhibition (%)*:

Antibacterial activities of the extracts of underutilized parts of various fruits were assessed against Gram+ve (*Staphylococcus aureus*) and Gram-ve (*Escherichia coli*) bacteria. A sterile 96-well plate was labelled. The 20 µL of bacterial suspension of *E. coli* and *S. aureus* was added to respective well. The wells in the periphery of the plate were added with 250 µL sterile distilled water and placed in an incubator at 37°C for 18–24 h. Then absorbance was measured at  $\lambda_{\text{max}}$  595 nm using an ELISA plate reader and percentage of bacterial inhibition was calculated as described by Patel *et al.* (2011).

## **Results and Discussion**

#### *Qualitative screening of phytochemical constituents*

Qualitative screening of powders from various fruit wastes are summarized in Tables 1A and 1B. A reddish-brown precipitate indicated the presence of alkaloids. All the fruit waste samples contained alkaloids. Yellow colour indicated the presence of flavonoids. All the fruit waste samples contained flavonoids but highest amount was observed in mango followed by orange, grapes and mosambi. Brownish green or a blue-black coloration was observed which indicated the presence of tannins. All the fruit waste samples contained trace amounts of tannins except grape waste. Deposition of a red precipitate was observed which indicated the presence of phlobatannins. Only acacia showed appreciable amount of phlobatannins. Rose pink coloration suggested the positive response for anthraquinones. Grape, acacia and apple waste samples showed appreciable amount of anthraquinones. Honeycomb froth indicated the presence of saponins. All the samples except mango contained appreciable amount of saponins. A reddish brown coloration at the

Table 1. A: Qualitative phytochemical screening of underutilized parts of various fruits.

Samples	Tannins	Phlobatannins	Anthra-quinones	Terpenoids	Chalcones
<i>Citrus sinensis</i>	+	nd	nd	nd	nd
<i>Citrus reticulata</i>	+	nd	nd	nd	nd
<i>Vitis vinifera</i>	nd	nd	++	nd	nd
<i>Ananas comosus</i>	+	nd	nd	nd	nd
<i>Mangifera indica</i>	+	nd	nd	nd	nd
<i>Aegle marmelos</i>	+	nd	nd	nd	nd
<i>Acacia auriculiformis</i>	+++	++	+++	++	++
<i>Malus baccata</i>	++	nd	++	++	+

+ = Traces, ++ = Moderate, +++ = High, nd = not detected

Table 1. B: Qualitative phytochemical screening of underutilized parts of various fruits

Samples	Anthocyanoside	Saponins	Cardiac Glycosides	Alkaloids	Flavanoids
<i>Citrus sinensis</i>	nd	+++	nd	++	++
<i>Citrus reticulata</i>	nd	+	nd	++	++
<i>Vitis vinifera</i>	+++	++	nd	+++	++
<i>Ananas comosus</i>	nd	++	nd	++	+
<i>Mangifera indica</i>	nd	nd	nd	++	+++
<i>Aegle marmelos</i>	nd	+++	nd	++	+
<i>Acacia auriculiformis</i>	nd	+++	++	++	+
<i>Malus baccata</i>	nd	++	nd	++	+

+ = Traces, ++ = Moderate, +++ = High, nd = not detected

interface was formed to show positive results for the presence of terpenoids. Only acacia and apple waste samples showed appreciable amount of terpenoids. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer indicated the presence of cardiac glycosides. Only acacia waste showed positive for cardiac glycosides. Appearance of reddish color showed the presence of chalcones. Only acacia and apple waste samples showed appreciable amount of chalcones. Appearance of pale pink color showed the presence of anthocyanosides. Among all the samples only acacia, apple and grape waste contained appreciable amount of anthocyanosides.

#### Quantitative estimation of some phytochemical constituents

Nearly all the samples showed good amount of total phenolic contents (TPC) (Figure 1A) ranging

from 119.5 (stone apple) to 139.0 (acacia, mg GAE/g). Mango waste powder (156 mg QE/g) contained the maximum amount of flavonoids which was followed by orange, grape, mosambi, apple and acacia (Figure 1B). The least amount of flavonoids was present in stone apple waste powder (13 mg QE/g). Grape waste (468 mg QE/g) contained the maximum amount of proanthocyanidins which was followed by apple, mango, acacia, pineapple, stone apple and orange. The least amount of proanthocyanidins was present in mosambi (Figure 2). Earlier studies on TPC in 16 different fruits and their under-utilized parts showed a wide variation ranging from 6.1 to 136.5 mg GAE/g (Prakash et al., 2011, 2013). These phytochemical compounds are known to support bioactive activities in fruit wastes and thus may be responsible for their antioxidant activities.

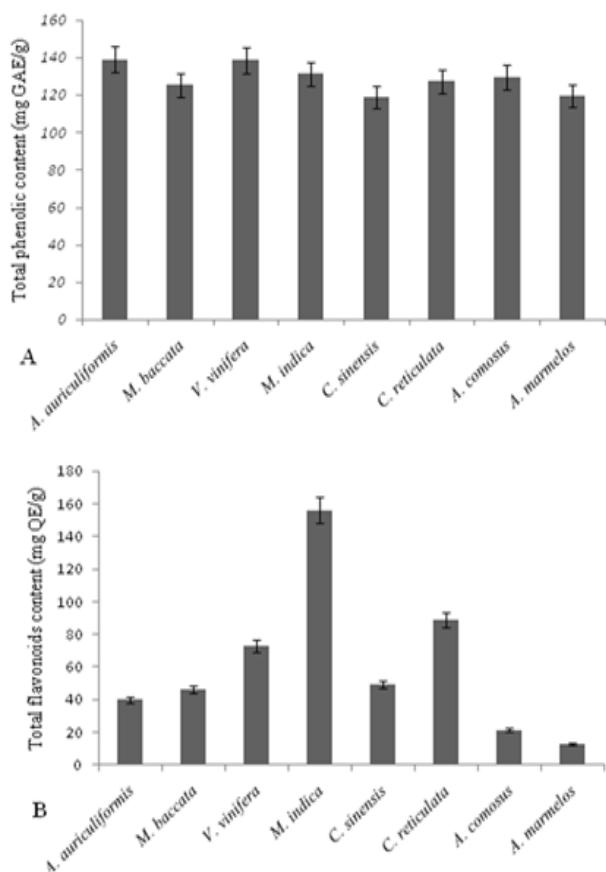


Figure 1. A: Total phenols in extracts of underutilized parts of various fruits. B: Total flavonoids in extracts of underutilized parts of various fruits.

#### Quantitative estimation of antioxidant activity and reducing power

Acacia waste showed the highest AOA (75.6%) followed by grapes (65.8%), apple (54.6%), stone apple (53.6%), mango (48.9%), orange (43.5%), pineapple and mosambi (Table 2). Grape and acacia waste showed the highest reducing power which was followed by mango, orange, mosambi, pineapple and stone apple. The least amount of reducing power was observed in apple waste sample (Figure 3). The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable (Prakash *et al.*, 2011, 2013). In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron. The amount of  $\text{Fe}^{2+}$  complex can then be monitored by measuring the formation of Perl's blue at  $\lambda_{\text{max}}$  700 nm. Increasing absorbance indicates an increase in reductive ability.

#### Estimation (%) of bacterial inhibition against *E. coli* and *S. aureus*

The extract of orange waste sample showed the maximum amount of inhibition of *E. coli* which was followed by mango, mosambi and apple. The least

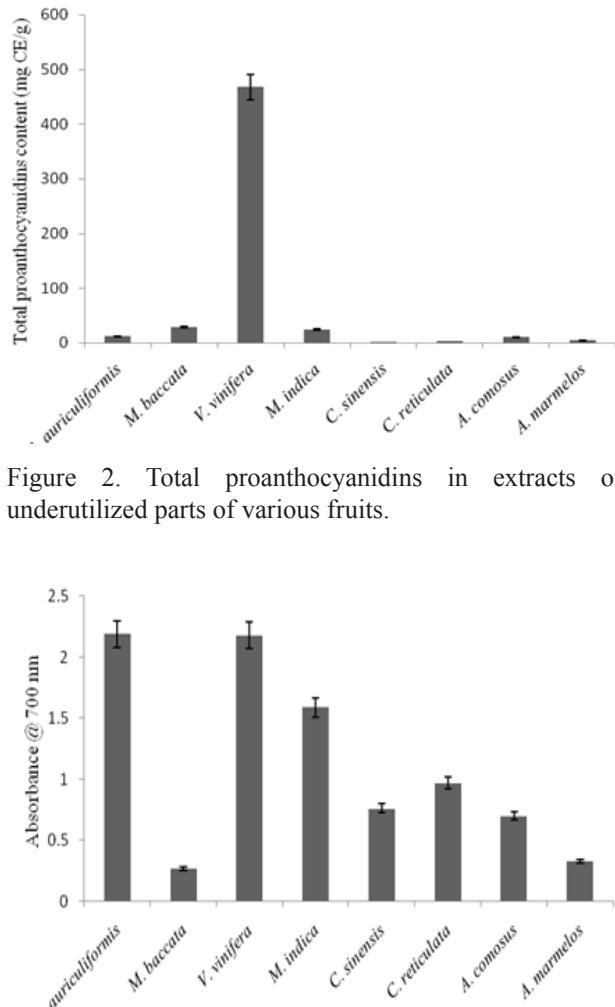


Figure 2. Total proanthocyanidins in extracts of underutilized parts of various fruits.

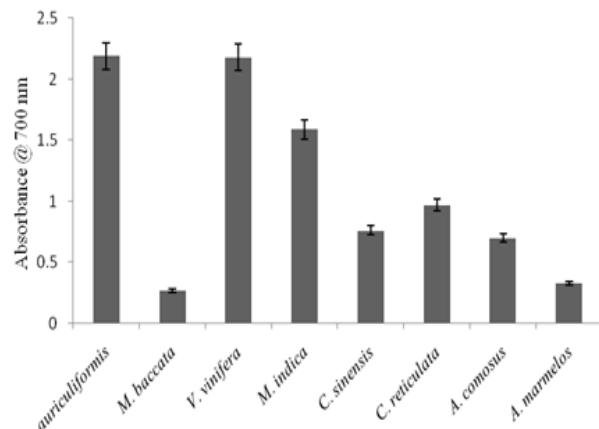


Figure 3. Reducing power of extracts of underutilized parts of various fruits

amount of inhibition was observed in grape waste sample. Stone apple, pineapple and acacia showed no inhibition against *E. coli* (Table 2). Similarly for *S. aureus* extract of apple waste sample showed the maximum inhibition followed by stone apple, orange, acacia, mango and pineapple. The least amount of inhibition was observed in grape waste while mosambi showed no inhibition (Table 2).

Waste products of a number of fruits correspond to vital source of sugars, minerals, dietary fiber, organic acid and phenolics which contributes to a variety of their properties, including antioxidant, antiviral, antibacterial, cardioprotective, antitumoral and antimutagenic activities. Consequently, uses of the wastes therapeutically are gaining importance. Preliminary qualitative analysis in present studies revealed that all the fruit waste samples contained alkaloids and flavonoids. Tannins were present in all samples except grape. Only acacia showed positive appreciable amount of phlobatannins. Grape, acacia and apple waste samples showed appreciable amount of anthraquinones. All the samples except mango

Table 2. Antioxidant activity (AOA, %) and Relative inhibition (%) of growth of *E. coli* and *S. aureus* by extracts of underutilized parts of various fruits.

Samples	Family	AOA (%)	Relative inhibition (%)	
			<i>E. coli</i>	<i>S. aureus</i>
<i>Citrus sinensis</i>	Rutaceae	26.7	1.1	45.0
<i>Citrus reticulata</i>	Rutaceae	43.5	14.7	68.1
<i>Vitis vinifera</i>	Vitaceae	65.8	11.5	3.8
<i>Ananas comosus</i>	Bromeliaceae	28.7	26.3	42.8
<i>Mangifera indica</i>	Anacardiaceae	48.9	23.7	3.1
<i>Aegle marmelos</i>	Rutaceae	53.6	70.3	59.0
<i>Acacia auriculiformis</i>	Fabaceae	75.6	1.7	38.3
<i>Malus baccata</i>	Rosaceae	54.6	3.5	64.6

contained appreciable amount of saponins. Acacia and apple waste samples showed appreciable amount of terpenoids. Only acacia waste showed positive for cardiac glycosides. Acacia and apple waste samples showed appreciable amount of chalcones. Majority of health beneficial effects of apple are mostly due to the presence of phenolic compounds which are strong antioxidants and therefore capable of counterbalancing free radical activities that may cause mutagenic changes resulting in onset of several diseases. It was observed that samples with high phenols showed high AOA. A wide variation in total phenols ranging from 10.5 (*Carissa carandas*, fruit peel) to 343.2 mg/g (*Caesalpinia mexicana*, fruits) and from 2.12 to 69.4 g/100g in different parts of *Cassia fistula* had been reported (Prakash et al., 2011, 2013). The AOA of *Acacia auriculiformis* (78.9%), fruit pericarp of *Aegle marmelos* (65.2%), fruit peel of *Malus sylvestris* (51.7%) and *Mangifera indica* (54.8%) have been reported (Prakash et al., 2011, 2013).

Among all the samples only grape waste contained appreciable amount of saponins. Acacia waste sample contained the maximum amount of phenols. Mango waste sample contained the maximum amount of flavonoids. Certain flavonoids have been reported to impart a variety of biological activity including anticancer and antimicrobial activities (Ortuno et al., 2006; Parashar et al., 2014). Citrus fruits have peculiar fragrance partly due to flavonoids and limonoids present in the peel and these fruits are good sources of vitamin C and flavonoids (Sawalha et al., 2009; Parashar et al., 2014). Grape

waste samples contained the maximum amount of proanthocyanidins as well as reducing power.

The fruit peel extracts showed antibacterial activity. Extract of stone apple waste sample showed the maximum amount of inhibition of *E. coli* whereas mosambi, apple and acacia showed least inhibition against *E. coli*. Extract of orange waste sample showed the maximum amount of inhibition of *S. aureus* whereas mango showed least inhibition against *S. aureus*. However in another study, *M. indica* has been reported with strong antimicrobial activity (Chanda et al., 2010) which may be attributed to the agro-climatic conditions.

It has been described earlier that Gram-ve bacteria were more susceptible than Gram+ve bacteria which contradict the previous reports that plant extracts are more active against Gram+ve than Gram-ve bacteria (Rabe and Van Staden, 1997). The observed difference may be due structural differences in cell wall of these bacteria. In Gram-ve bacteria, the cell wall is complex and multilayered structure having an outer phospholipid membrane consisting of the structural lipopolysaccharide components, which makes a barrier to many environmental substances including synthetic and natural antibiotics. Whereas in Gram+ve bacteria comprises a single outer peptidoglycan layer that is not an effective permeability barrier (Costa et al., 2008).

## Conclusion

The present investigation focuses on the possibility of using fruit peel wastes as a source of

low-cost antioxidants and natural antibacterials. Some of the fruit peels, usually a waste product, that are thrown into the environment have a great antioxidant and antimicrobial potential. Therefore, present study may open new avenues for future utilization of the waste for therapeutic purpose.

### Acknowledgments

Authors are grateful to Dr. Ashok K. Chauhan, Founder President, and Dr. Atul Chauhan, Chancellor, Amity University Uttar Pradesh for their valuable guidance, kind support and providing research facilities.

### References

- Chanda, S., Baravalia, Y., Kaneria, M. and Rakholiya, K. 2010. Fruit and Vegetable Peels—Strong Natural Source of Antimicrobics. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 2: 444–450.
- Costa, E. S., Hiruma-Lima, C. A., Lima, E. O., Sucupira, G. C., Bertolin, A. O., Lolis, S. F., Andrade, F. D. P., Vilegas, W. and Souza-Brito, A. R. M. 2008. Antimicrobial activity of some medicinal plants of the Cerrado, Brazil. Phytotherapy Research 22: 705-707.
- Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A. and Brielmann, H. L. 2006. Natural products from plants. New York: CRC Press.
- Dewick, P. M. 2002. Medicinal natural products: a biosynthetic approach. New York: John Wiley and Sons.
- Emmons, C. L. and Peterson, D. M. 1999. Antioxidant activity and phenolic contents of oats, groats and hulls. Cereal Chemistry 76: 902-906.
- Harborne, J. B. 1998. Phytochemical methods: a guide to modern techniques of plant analysis. 3<sup>rd</sup> edn. London: Chapman and Hall.
- Jia, Z., Tang, M. and Wu, J. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64: 555-559.
- Liu, R. H. 2002. Supplement quick fix fails to deliver. Food Technology International 1: 71-72.
- Ortuno, A. A., Baidez, P., Gomez, M. C., Arcas, I., Porras, A. G. and Del Rio, J. A. 2006. *Citrus paradise* and *Citrus sinensis* flavonoids: Their influence in the defence mechanism against *Penicillium digitatum*. Food Chemistry 98: 351-358.
- Oyaizu, M. 1986. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine. Journal of Nutrition 44: 307-315.
- Parashar, S., Sharma, H. and Garg, M. 2014. Antimicrobial and Antioxidant activities of fruits and vegetable peels: A review. Journal of Pharmacognosy and Phytochemistry 3: 160-164.
- Patel, J. P., Gami, B., Patel, K. and Solanki, R. 2011. Antibacterial activity of methanolic and acetone extract of some medicinal plants used in Indian folklore. International Journal of Phytomedicine 3: 261-269.
- Prakash, D., Upadhyay, G. and Pushpangadan, P. 2011. Antioxidant potential of some under-utilized fruits. Indo-Global Journal of Pharmaceutical Sciences 1: 25-32.
- Prakash, D., Upadhyay, G. and Gupta, C. 2013. Total phenol and antioxidant activity of some fruits and their under-utilized parts. International Food Research Journal 20: 1717-1724.
- Rabe, T. and Van Staden, J. 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56: 81-87.
- Ragazzi, E. and Veronese, G. 1973. Quantitative analysis of phenolic compounds after thin layer chromatographic separation. Journal of Chromatography 77: 369-375.
- Sawalha, S. M. S., Arráez-Román, D., Segura-Carretero, A. and Fernández-Gutiérrez, A. 2009. Quantification of main phenolic compounds in sweet and bitter orange peel using CE- MS/MS. Food Chemistry 116: 567-574.
- Sharma, G., Srivastava, A. K. and Prakash, D. 2011. Phytochemicals of nutraceutical importance: their role in health and diseases. Pharmacologyonline 2: 408-427.
- Sivakumar, N. T. and Venkataraman, R. 2010. Phytochemical and pharmacological studies on plant waste materials. Der Pharmacia Sinica 1: 1-6.
- Spanos, G. A. and Wrolstad, R. E. 1990. Influence of processing and storage on the phenolic Composition of Thompson seedless grape juice. Journal of Agricultural Food Chemistry 38: 1565-1571.
- Sun, J. S., Tsuang, Y. W., Chen, J. J., Huang, W. C., Hang, Y. S. and Lu, F. J. 1998. An ultra- weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns 24: 225-231.
- Temple, N. J. 2000. Antioxidants and disease: more questions than answers. Nutrition Research 20: 449-459.
- Thompson, L. U. 1994. Antioxidant and hormone-mediated health benefits of whole grains. Food Science and Nutrition 34: 473-497.
- Van Acker, S. A., van Balen, G. P., van den Berg, D. J., Bast, A. and van der Vijgh, W. J. 1998. Influence of iron chelation on the antioxidant activity of flavonoids. Biochemical Pharmacology 56: 935-943.
- Van Hoorn, D. E., Nijveldt, R. J., Van Leeuwen, P. A., Hofman, Z., M'Rabet, L. and De Bont, D. B. 2002. Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. European Journal of Pharmacology 451: 111-118.