Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(2): 200-203

Received: 22-01-2016
Accepted: 25-02-2016

Katike Umamahesh
Department of Biochemistry,
Sri Venkateswara University,
Tirupati, India.

Seelam Naga Sivudu
Department of Biochemistry,
Sri Venkateswara University,
Tirupati, India.

Obulam Vijaya Sarathi Reddy
Department of Biochemistry,
Sri Venkateswara University,
Tirupati, India.

Correspondence
Obulam Vijaya Sarathi Reddy
Department of Biochemistry,
Sri Venkateswara University,
Tirupati – 517502, India.

Evaluation of antioxidant activity, total phenolics and total flavonoids in peels of five cultivars of mango (*Mangifera indica*) fruit

Katike Umamahesh, Seelam Naga Sivudu, Obulam Vijaya Sarathi Reddy

Abstract
A comparative studies on the *in vitro* antioxidant activity of aqueous extracts of peels of five locally available cultivars of ripe mango (*Mangifera indica*) (Alphonso, Malgua, Rumani, Sindhura and Banisha) fruit has shown that antioxidant properties, total phenolics content (TPC) and total flavonoids content (TFC) differ significantly among selected cultivars. The results indicated that the peels of five types of mango cultivars had considerable amount of antioxidants, phenols and flavonoids, in that the aqueous extract of Sindhura had shown better antioxidant activity (IC 50 of DPPH was 65.34±0.62 µg/ml; IC 50 of ABTS was 28.29±0.43 µg/ml), and higher TPC (87.38±0.43 mg of GAE/g), as well as higher TFC (15.6±0.23 mg of QE/g) contents than of the remaining four aqueous extracts of mango fruit peels.

Keywords: Mango fruit peels, Antioxidant, Total phenolics, Total flavonoids

1. Introduction
Reactive oxygen species (ROS) are an integral part of metabolic and cell signaling pathways in living organisms (Muzembo *et al*., 2012) [1], that play an important role in some pathogenesis of serious diseases, such as cancer, liver cirrhosis, diabetes, neurodegenerative disorder, cardiovascular diseases and inflammation (Aruoma, 1998) [2]. Antioxidant primarily reduces this oxidative stress (Muzembo *et al*., 2012) [1]. Natural antioxidant compounds (poly phenols, vitamins, flavonoids and carotienoids) are available in dietary supplements that are rich in fruits (Dembitsky *et al*., 2011) [3]. The natural compounds are secondary metabolites during normal development in response to stress condition (Stahl and Sies, 2003: Close *et al*., 2005) [4, 5]. In recent trends natural compounds are used as food preservatives (Bruni, 2004) [6], because they avoid the toxic problem as compared to synthetic compounds like butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate (PG) (Amarowicz *et al*., 2000; Aruoma *et al*., 1992) [7,8].
The phyto-chemicals are present in all parts of plants (barks, leaves, flowers, roots, seeds and fruits), and they are used since time immemorial (Criagg and David, 2001) [9]. Fruits and vegetables have the properties like prevention of degenerative diseases, such as cancer and cardio-vascular diseases (Liu, 2003) [10]. These are having the phyto-chemicals, such as phenols, carotinoids, tocopherol and anthocyanin, as chemo-preventive (Dragsted *et al*., 1993) [11] and cardio-protective effects (Vita, 2005) [12], as well as protecting the human body against oxidative damage by free radicals (Halliwell, 1997) [13]. The seasonal fruits and vegetables are locally available at low cost.
Mango (*Mangifera indica* L.) is the national fruit of India. It has been cultivated in India since 4000 years, presently more than 1000 mango cultivars are available in the country (Mukherjee, 1953) [14]. This seasonal fruit is called as "king of fruits". In the world approximately 20% of the fruits are processed for products such as nectar, puree, pickle and canned slices (Loelillet, 1994) [15]. Peel and seed are the major by-products of fruit, and 7-10% of peels are discarded as waste from whole fruit, which leads to environmental pollution (Ajilla *et al*., 2007) [16]. Polyphenol content of peel was reported to be more than that of flesh (Lakshminarayana *et al*., 1979) [17]. The mango peels have been reported to possess the anti-proliferating activity (Hanna Kim *et al*., 2009) [18], anti-diabetic activity (Mahendranath, 2014) [19] and also having anti-inflammatory bioactive compounds like 5-(110Z-heptadecenyl) and 5-(80Z, 110Z-heptadecadienyl)-resorcinols (Matthias *et al*., 2008) [20].

Therefore, the object of the present study was to evaluate the antioxidant, total phenolics and total flavonoids contents of aqueous extracts of mango peels.

2. Materials and methods

Preparation of peel powders: The five types of cultivars of ripe mangoes (Alphonso, Sindhura, Malgua, Rumani and Banisha) were purchased from local market in Tirupati. The mangoes were washed with warm water and peels were recovered by using sharp knife and underlying pulp was removed by scraping with blunt edge of the knife, and the peels were washed with distilled water, and air-dried. The dried peels were ground using mixer and grinder (Preethi Chef Pro-750W) individually up to the formation of a course powder. The mango peel powders were stored at 4ºC for further analysis.

Aqueous extracts of mango peel powders: Individually, the five cultivars of mango peel powders were immersed in distilled water for one day in aspirator bottles by stirring periodically and the filtrates were collected by using Whatmann No.1 filter paper repeatedly until colourless. The filtrates were evaporated by using rotary evaporator (Buchi R200 Rota vapor), and frozen samples were prepared by using lyophylizer (Technico Lyodel) at -50ºC, finally the sticky mango peel extracts were collected individually and labeled as Aq.E.M.P (Aqueous extract of Malgua peel), Aq.E.S.P (Aqueous extract of Sindhura peel), Aq.E.B.P (Aqueous extract of Banisha peel), Aq.E.A.P (Aqueous extract of Alphonso peel) and Aq.E.R.P (Aqueous extract of Rumani peel) upon those, and stored at 4ºC in refrigerator for further use.

***In vitro* antioxidant activities:** The following methods were used for evaluation of antioxidant activities

DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging activity: Free radical scavenging activity was determined by using the 2,2'-diphenly-1-picrylhydrazyl (DPPH) method of Burits and Bucar (2000) [21]. One ml of various concentrations of extracts (25, 50, 75, 100 and 500 µg) in methanol were added to 4 ml of 0.004% methanol solution of DPPH. They were then kept at room temperature in dark condition for 30-min. the absorbance was read against a methanol blank at 517 nm, and the ascorbic acid was used as standard for comparing with the test samples. The inhibition of free radicals by the extract in percentage terms (I %) was calculated using the following equation.

$$I\ \% = \{(A_c - A_s) / A_c\}\ X\ 100$$

Where, A_c is the absorbance of control against blank (containing all the reagents except the test sample) and as is the absorbance of test samples against blank.

ABTS (2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging activity: The antioxidant activity was determined using stable 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid cation (ABTS+) radical method by Re *et al*. (1999) [22]. ABTS (2 mM) was prepared by dissolving in 50 ml phosphate buffer saline (PBS). PBS contained 8.18 g of NaCl, 0.27 g of KH_2PO_4, 3.58 g of $NaHPO_4$, 0.15 g of KCl in 1000 ml of distilled water, and maintained pH 7.4). ABTS+ was produced by reacting 50 ml of stock solution with 200 µl of 70 mM potassium persulphate ($K_2S_2O_8$) water solution. The mixture was kept in dark condition at room temperature for 15-16 h, before every use. One ml of various concentrations of extracts (25, 50, 75, 100 and 500 µg) were prepared and mixed with 3 ml of ABTS solution and then they were kept at room temperature for 10-min, and then absorbance was read against blank at 734 nm. Here, phosphate buffed saline (PBS) was used as blank. Butylated hydroxytoluene (BHT) was used as standard.

$$I\ \% = \{(A_c - A_s) / A_c\}\ X\ 100$$

Where, A_c is the absorbance of control against blank (containing all the reagents except the test sample) and as is the absorbance of test samples against blank.

Reducing power assay: The reducing power assay was determined using Oyaizu method (Oyaizu, 1986) [23]. The aliquots of (25, 50, 75 and 100 µg/ml) extracts were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml 1% potassium ferricyanide ($K_3Fe\ (CN)_6$). The mixture was incubated at 50ºC for 20 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture, and then the mixtures was centrifuged at 3000 rpm for 10 min. Collected the 2.5 ml of upper layered solution which was mixed with 2.5 ml of distilled water and finally 0.5 ml of 0.1% ferric chloride solution, and the absorbance was measured at 700 nm. Increased reaction mixture indicated the increased reducing power. Ascorbic acid was used as standard.

Determination of total phenolics content: The total phenolics content of the extracts were determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton and Rossi (1965) [24], using gallic acid as a standard. Different aliquots of sample solution were made up to 1ml each with distilled water and added 1.5 ml of Folin–Ciocalteu reagent (previously diluted 10 folds with distilled water), kept at room temperature for 5 min and added 4 ml of 20% sodium carbonate (Na_2CO_3), and kept at room temperature for 30 min. The absorbance was read against the blank at 750 nm. Here, blank as except test sample (extracts, standard). The total phenolics content was represented as milligrams of gallic acid equivalent per gram of dry mass (mg GAE/ g) (Roy *et al*., 2011) [25].

Determination of total flavonoids content: The total flavonoids content was estimated colorimetrically by using aluminum chloride as described by Woisky and Salatino (1998) [26]. Quercetin was used as standard. The 10 mg quercetin was dissolved in 80% ethanol and then diluted to 25, 50, 75 and 100 µg/ml. The diluted standard solution (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water, incubated at room temperature for 30 min. The absorbance was estimated against blank at 415 nm. Here, blank as except test samples (extracts, standard). The total flavonoids content was represented as milligrams of Quercetin equivalent per gram of dry mass (mg of QE/g).

Statistical analysis: All experiments were carried out in triplicate and results were expressed as mean ± standard deviation (SD). The statistical analysis was carried out by using one-way analysis (ANOVA), and P value significant at $P<0.05$.

3. Results and discussion

Scavenging activity on 2,2-diphenyl-1-picrylhydrazyl radical: The free radical scavenging activity of extracts was determined by synthetic DPPH (2,2-Diphenyl-1-picrylhydrazyl). The color changes from purple color to yellow

color, when reduced the hydrogen or electron donation (Shimada *et al*., 1992) [27]. The purple color of DPPH was bleached rapidly to form yellow color in all concentrations of aqueous extracts of mango peels. Sindhura peel extract (89.24±0.57%) has more radical scavenging activity at 500 µg/ml than that of the other extracts (Fig.1). And all the extracts have shown the lower activity than the standard (Ascorbic acid). It was found that the IC50 value of Aq.E.S.P (65.34±0.73 µg/ml) was at the lowest concentration than the others Aq.E.R.P, Aq.E.M.P, Aq.E.A.P and Aq.E.B.P, respectively (Table 1).

Scavenging activity on 2, 2-azinobis (3-ethylbenzothiozolin-6-sulphonic acid): The aqueous extracts of mango peel showed a steady increase in the percentage of inhibition of $ABTS^{+}$ radicals in all concentrations (Fig 2), and the Sindhura (91.16± 0.81%) showed the maximum scavenging activity when compared with other extracts at 500 µg/ml. And all the extracts showed lower activity, when compared with standard (BHA). It was found that the IC50 value of Aq.E.S.P (28.29±0.62 µg/ml) was at the lowest concentration, followed by Aq.E.R.P, Aq.E.M.P, Aq.E.A.P and Aq.E.B.P, respectively (Table 1).

Reducing power: The reducing power method reflects the electron donation ability of the antioxidant present in the extracts to convert the Fe^{3+} into Fe^{2+}. The amount of Fe^{2+} complex (Pearl's Prussian blue color) was measured at 700 nm (Amarowicz *et al*., 2010) [28], and the increase in absorbance indicates the increase in the reducing power activity. The antioxidant characteristics of aqueous extracts of mango peel and standard compound (Ascorbic acid) are shown in Figure 3. Generally in this assay the reducing power increased when the concentration of aqueous extracts of mango peel increased and also the standard, which was represented as absorbance at 700 nm versus sample concentration on plot (Fig. 3). The reducing power was showed to be in the following order: Aq.E.S.P > Aq.E.R.P > Aq.E.M.P >Aq.E.A.P > Aq.E.B.P, respectively. The 100 µg of aqueous extract of Sindhura peel has shown highest absorbance at 700 nm, as compared to other aqueous extracts.

Total phenolics content: The quantification of total phenolics compounds by Folin-Ciocalteu's phenol reagent is shown in Table 1 and found that the phenolics content of aqueous extracts of Sindhura peel showed the highest phenolic content (TPC) as compared to the other cultivars peels extracts. The solvent polarity plays a major role for extraction of TPC (Lee *et al*., 2007) [29]. The acetone and ethanol extracts of mango peel have shown the higher TPC value (Ajila *et al*., 2007; Hana Kim *et al*., 2010) [16,18] than the presented aqueous extracts, because of the capacity of water to dissolve residual substances such as organic acid and sugars that could interfere in the quantification of phenolics (Chirinos *et al*., 2007; Mohammedi, 2011) [30,31].

Total flavonoids content: The quantification of total flavonoids by aluminum chloride is shown in Table 1, The results shown that the flavonoids content of aqueous extract of Sindhura peel has shown the higher TFC value (15.6±0.23 mg of QE/g), than the aqueous extracts of Rumani peel (14.4±0.15 mg of QE/g), Malgua peel (12.8±0.47 mg of QE/g), Alphonso peel (11.5±0.19 mg of QE/g) and Banisha peel (8.7±0.32 mg of QE/g). According to Hana Kim *et al*. (2010) [18] the mango peel extract has shown the higher TPC and TFC values than the flesh extract of even the ripe mango.

Table 1: Total flavonoids and phenolic content of aqueous extract of five mango cultivars along with their antioxidant activity measured by DPPH and ABTS

Extracts	TFC (mg of QE/g)	TPC (mg of GAE/ g)	IC50 (DPPH) (µg/ml	IC50 (ABTS) (µg/ml)
Aq.E.M.P	12.8±0.47	62.13±0.27	69.15±0.76	30.32±0.68
Aq.E.B.P	8.7±0.32	48.9±0.35	169.83±0.89	84.88±0.79
Aq.E.A.P	11.5±0.19	58.48±0.72	95.11±0.57	51.11±1.21
Aq.E.S.P	15.6±0.23	87.38±0.43	65.34±0.62	28.29±0.43
Aq.E.R.P	14.4±0.15	84.39±0.28	67.28±0.78	31.79±0.81

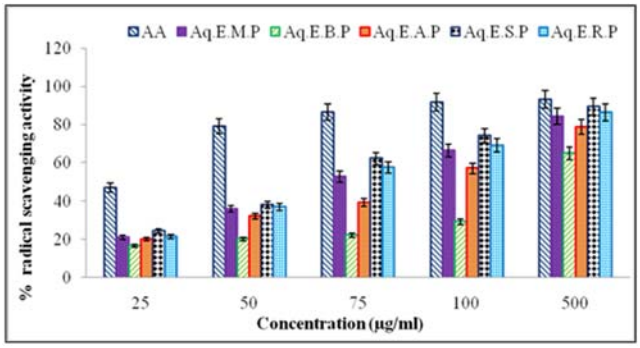

Fig 1: Scavenging activity of DPPH on various concentrations of Aq.E.M.P (Aqueous extract of Malgua peel), Aq.E.S.P (Aqueous extract of Sindhura peel), Aq.E.B.P (Aqueous extract of Banisha peel), Aq.E.A.P (Aqueous extract of Alphonso peel), Aq.E.R.P (Aqueous extract of Rumani peel) and Ascorbic acid (AA).

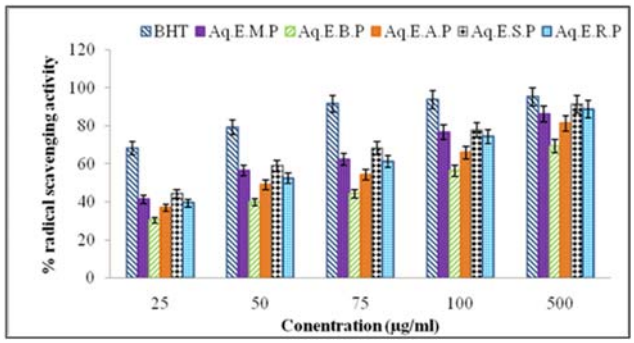

Fig 2: Scavenging activities of ABTS on various concentrations of Aq.E.M.P (Aqueous extract of Malgua peel), Aq.E.S.P (Aqueous extract of Sindhura peel), Aq.E.B.P (Aqueous extract of Banisha peel), Aq.E.A.P (Aqueous extract of Alphonso peel), Aq.E.R.P (Aqueous extract of Rumani peel) and Butylated hydroxyl toluene (BHT).

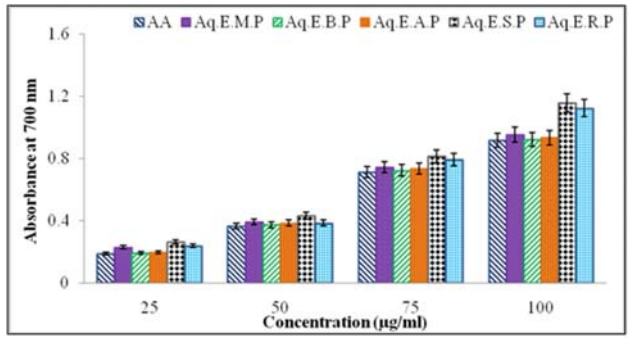

Fig 3: Reducing power of various concentrations of Aq.E.M.P (Aqueous extract of Malgua peel), Aq.E.S.P (Aqueous extract of Sindhura peel), Aq.E.B.P (Aqueous extract of Banisha peel), Aq.E.A.P (Aqueous extract of Alphonso peel), Aq.E.R.P (Aqueous extract of Rumani peel) and Ascorbic acid (AA).

4. Conclusion

The present study indicates that all the aqueous extracts of mango peels have shown the good contents of antioxidant activity, phenolics and flavonoids. More particularly the Sindhura cultivar had maximum phenols, flavonoids and antioxidant activity as compared to other mango cultivars. Hence its potential antioxidant, it may also imply that eating mango along with its peel would impart more health benefits.

5. Acknowledgement

We thank Prof. Ch. Appa Rao, Dept. of Biochemistry, S.V.University, Tirupati for providing the Rota Vapour and Lyophilizer and Dr.S.C.Basappa, former Deputy Director, CFTRI, Mysore for giving valuable comments and suggestions on this manuscript.

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