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ORIGINAL ARTICLE

Antioxidant activity and phenolic content in genotypes of Indian jujube (*Zizyphus mauritiana* Lamk.)

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Abstract Indian jujube (*Zizyphus mauritiana* Lamk), an indigenous fruit crop of India has been widely used in traditional medicine for treating various kinds of diseases. Chinese jujube has been studied; however systematic study on Indian jujube is lacking. In this work, 12 commercial cultivars of *Z. mauritiana* were evaluated for their ascorbic acid (AA), total phenolics (TPH), flavonoids (TF), and total antioxidant activity (AOX). Results indicate that Indian jujube is a good source of ascorbic acid and total phenolics ranging from 19.54 to 99.49 mg/100 g and 172 to 328.6 mg GAE/100 g, respectively. Total AOX ranged from 7.41 to 13.93 and 8.01 to 15.13 μmol Trolox/g in FRAP and CUPRAC, respectively. Principal component analysis was performed to find a linear combination of the functional attributes which would account for most of the variance

Abbreviations: DNPH, dinitrophenylhydrazine; FRAP, ferric reducing antioxidant power; CUPRAC, cupric reducing antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FCR, Folin ciocalteu reagent; TPTZ, 2,4,6-tripyridyl-striazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; GAE, gallic acid equivalent; AOX, antioxidant activity; fx, fresh weight; TSS, total soluble solids; TA, titrable acidity; AA, ascorbic acid; TPH, total phenolic content; TF, total flavonoids; PC, principal component

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in the observed attributes. GGE biplots revealed that ZG-3, Elaichi and Gola, are promising genotypes in terms of total phenolics and flavonoids.

1. Introduction

Fruits and vegetables are rich source of antioxidants such as vitamin C, tocopherol, phenolics and β-carotene which contribute to their antioxidant or free radical or scavenging effects. Amongst these, phenolics serve as powerful antioxidants by virtue of the hydrogen-donating properties of their phenolic hydroxyl groups, as well as by donating electrons to stop free radical chain reactions emerging from oxidative stress (John and Shahidi, 2010). Incremental increase in scientific publications on phenolics strongly advocates the consumption of phenolic rich food for the management of degenerative diseases, particularly cardiovascular, cancer, Alzheimer's, diabetes and neurodegenerative diseases. In this regard there is a hectic search for plant sources rich in phenolics for improving human health.

Zizyphus species (Rhamanceae) are widely used as medicine predominantly in Asian countries, particularly in Taiwan and China for the treatment of allergies, constipation, urinary troubles, depression, chronic bronchitis, insomnia and liver diseases (Li et al., 2005). The seeds of *Zizyphus jujuba* Lamk are effective in the improvement of the blood glucose, lipid compositions in serum of dietary hyperlipidemic rats and have anti-inflammatory properties (Al-Reza et al., 2010). In this context, the jujuba fruit or red date has been described as the "fruit of life" and is a rich source of vital functional components such as polysaccharides, phenolics, flavonoids and saponins responsible for various biological activities including antiproliferation of cancer cell alleviation of brain nerve disorder, regulation of immune function and reduction of blood triglyceride (Dahiru and Obidoa, 2008; Li et al., 2011). Caffeic acid, *p*-hydroxybenzoic acid, ferrulic acid and *p*-coumaric acid are the most abundant phenolics reported in *Zizyphus* (Muchuweti et al., 2005) which account for its significant levels of AOX, reducing power and scavenging effect on free radicals (Kamiloglu et al., 2009; Zhang et al., 2010).

The fruit is popularly known as tsao in China, jujube or ber in India and nabk in Arabia. It is extensively grown in southern Asia, Syria, northern India, southern central China, and also in southeastern Europe. Indian jujube (*Zizyphus mauritiana* L.) is indigenous to India and grows under varying conditions of climate all over India even at elevations up to 1000 m above mean sea-level. Most of the information reported in the literature has been restricted to Chinese jujube and Indian genotypes still remain under researched. Despite being a cheap fruit, jujube still remains to be an underutilized fruit in average Indian diet in comparison to costlier yet popular fruits such as mango, apple and orange. This is mainly because the common consumer has no information about its health promoting effects. India has huge biodiversity of *Z. mauritiana* Lamk and more than 300 germplasms are available including commercial, exotic and indigenous cultivars. Unfortunately, no systematic information is available on antioxidant composition and antioxidant activity with regard to jujube grown under Indian conditions. Exploring biodiversity is essential for improving productivity, quality and nutritional value through breeding programmes. Nutritional and functional quality is strictly governed by not only genotypes but also by crop conditions (geographical origin, environmental conditions, maturity stage and cultural practices), ripening conditions and post harvest conditions (Capocasa et al., 2008).

Keeping this in mind, the objective of the present study was to determine the content of phenolics and flavonoids in 12 commercial genotypes of Indian jujube. In addition the antioxidant activity was assayed using three *in vitro* antioxidant assays. Detailed information about the health-promoting antioxidants could lead to a better understanding and an increased consumption of this fruit in fresh form for improved nutrition and increased food supply. Also this would provide a good rational for processing of Indian jujube for use in functional foods and as an ingredient in pharmaceuticals and nutraceuticals.

2. Materials and methods

2.1. Materials

Twelve commercial cultivars of *Zizyphus* sp., namely Chuhara, Mundia, Thornless, Jogia, Gola, Kathali, Umran, Seb, ZG-3, Sonaur-5, Rashmi and Elachi grown in the experimental fields of Central Institute for Arid Horticulture (CIAH), Bikaner, Rajasthan, India were taken for the study. Mature fruits were harvested manually at the commercial maturity stage in the month of March 2010. After sorting, the fruits were packed in cardboard fibre boxes and transported by road (approximate transition time ≈ 10–12 h). They were received at the analytical laboratory in the Division of Post Harvest Technology, IARI, New Delhi, subsequently stored at cold storage (4 °C) for a day.

For each replicate, twenty fruits from each cultivar were thoroughly homogenized in a domestic blender (Inalsa, India) into a pulp. The homogenized samples were then freeze dried in a freeze dryer (Labconco corporation, USA) for two days at −45 to −30 °C. Dry matter of sample was calculated by the weight difference before and after freeze drying. Freeze dried samples were ground into a fine powder in a domestic blender. Five gram of powder was extracted twice with 30 ml of ethanol (80%), by stirring and sonicating for 30 min in dark. The homogenate was then centrifuged for 15 min at 10,000*g* at 4 °C (Eppendorf, Westbury, USA). The supernatant was then vacuum concentrated at 40 °C in a rota-evaporator and stored at −20 °C. The concentrated sample was used as a sample extract for the estimation of TPH, TF and AOX.

2.2. Determination of ascorbic acid content

Ascorbic acid content was quantified in accordance with the dinitrophenylhydrazine (DNPH) method, modified by Nunes et al. (1995). Freeze dried powder (0.2 g) was homogenized in mortar pestle, with 20 mL of a mixture of 6% (w/v)

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metaphosphoric acid in 2 mol/L acetic acid. The mixture was centrifuged at 17,600*g* for 15 min at 4 °C. The supernatant was filtered through Whatman filter paper (no. 1). An aliquot of 0.05 mL of 0.2% (w/v) 2,6-dichlorophenolindophenol (DCPIP) was added to 1 mL of the supernatant and incubated at room temperature for 1 h. Thiourea solution (2%, w/v) in 5% metaphosphoric (w/v) acid and 0.5 mL of 2% (w/v) DNPH in 4.5 mol/L sulphuric acid were added and the solution was incubated at 60 °C for 3 h. Tubes were placed in an ice bath and 2.5 mL of ice cold 90% sulphuric acid were slowly added. Tubes were vortexed and total ascorbic acid was measured by absorbance at 540 nm. The concentration was calculated using a standard curve of ascorbic acid. Results were expressed as mg/100 g fw.

2.3. Determination of total phenolics and flavonoid content

TPH was estimated spectrophotometrically using Folin–Ciocalteu reagent (Singleton et al., 1999). To 100 μL of the sample extract (80% ethanol) 2.9 ml of deionized water, 0.5 ml of Folin–Ciocalteu reagent and 2.0 ml of 20% Na_2CO_3 solution were added. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against a reagent blank in UV–vis spectrophotometer (VARIAN Cary 50). Results were expressed as gallic acid equivalent (mg GAE/100 g). TF were analysed using Aluminium chloride method (Zhishen et al., 1999). An aliquot (1 ml) of *Zizyphus* extract in 10 ml of volumetric flask containing 4 ml of distilled water, 0.3 ml portion of 5% $NaNO_2$ and 0.3 ml portion of 10% $AlCl_3{\cdot}6H_2O$. The mixture was allowed to stand for 6 min at room temperature. Two millilitres of 1 N NaOH was added and the solution was diluted to 10 ml with distilled water. The absorbance of the solution versus a blank at 510 nm was measured immediately. The results were expressed as catechin equivalent (mg CE/100 g).

2.4. Determination of antioxidant activity

2.4.1. Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was performed according to the procedure described by Benzie and Strain (1996). The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in the ratio 10:1:1 (v/v/v). Three ml of the FRAP reagent was mixed with 100 μl of the sample extract in a test tube and vortexed in the incubator at 37 °C for 30 min in a water bath. Reduction of ferric-tripyridyltriazine to the ferrous complex formed an intense blue colour which was measured; at a UV–vis spectrophotometer (Varian Cary 50) at 593 nm at the end of 4 min. Results were expressed in terms of μmol Trolox/g.

2.4.2. Cupric reducing antioxidant power

The cupric ion reducing antioxidant capacity of berries was determined according to the method of Apak et al. (2008). Briefly, according to the protocol 0.1 mL of sample extract was mixed with 1 mL each of $CuCl_2$ solution (1.0×10^{-2} mol/L), neocuproine alcoholic solution (7.5×10^{-3} mol/L), and NH_4Ac(1 mol/L, pH 7.0) buffer solution and 1 mL of water to make the final volume 4.1 mL. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentration of Trolox. The results were expressed as μmol Trolox/g, using molar absorptivity of Trolox as 1.67×10^4 L mol^{-1} cm^{-1}.

2.4.3. Free radical scavenging activity using DPPH

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical (Brand-Williams et al., 1995). A 3.9 mL aliquot of a 0.0634 mM of DPPH solution, in methanol (95%) was added to 0.1 mL of each extract and shaken vigorously. Change in the absorbance of the sample extract was measured at 515 nm for 30 min till the absorbance reached a steady state. The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula: %inhibition = $100 \times (A_0 - A)/A_0$ where A_0 was the beginning absorbance at 515 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank. Results were expressed as μmol Trolox/g.

2.4.4. Free radical scavenging activity using ABTS

A modified procedure using ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)) as described by Re et al. (1999) was used. The $ABTS^{+\cdot}$ stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent. The working solution of $ABTS^{+\cdot}$ was obtained by diluting the stock solution in ethanol to give an absorption of 0.70 ± 0.02 at λ = 734 nm. Sample extract (10 μl) was added to 90 μl of $ABTS^{+\cdot}$ solution and absorbance readings at 734 nm were taken at 30 °C exactly 10 min after initial mixing. The percentage inhibition of $ABTS^{+\cdot}$ of the test sample and known solutions of Trolox were calculated by the following formula: %inhibition = $100 \times (A_0 - A)/A_0$ where A_0 was the beginning absorbance at 734 nm, obtained by measuring the same volume of solvent, and A was the final absorbance of the test sample at 734 nm. The calibration curve between %inhibition and known solutions of Trolox (100–2000 μM) was then established. The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC μmol Trolox/g).

2.5. Determination of quality attributes

Total soluble solids (TSS) was measured with a hand-held refractometer (Atago, Tokyo, Japan) and calibrated with distilled water. TA (titrable acidity) was determined from a 5 mL aliquot by titration with 0.1 mol L^{-1} NaOH with phenolphthalein as an indicator and results are given as grams of citric acid per 100 mL. The SSC/TA ratio was calculated.

2.6. Statistical analysis

Data represent the mean of two years. Values are presented as mean ± SD of three replicates. The results were statistically analysed by ANOVA. Statistical significance was accepted at a level of 5% level. Data were subjected to correlation analysis and principal component analysis (PCA). A graphical multivariate statistical analysis GGE biplot (Yan and Kang, 2003) was performed to obtain and visualize better and deeper relationship between functional constituents and genotypes. Biplot has been performed using scaling as standard deviation and data centring as tester centred.

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3. Results and discussion

3.1. Total phenolics and flavonoid content

Phenolic compounds are important contributors to functional quality and have important role to play in counteracting reactive oxygen species (ROS), this minimizing molecular damage. TPH content was also found to vary significantly ($p < 0.05$) among cultivars and ranged from 172 to 328.61 mg GAE/100 g depicting almost two-fold variations (Table 1). The hierarchy was: Umran ⩽ Kaithali ⩽ Seb ⩽ Thornless ≈ Jogia ⩽ Mundia ≈ Gola ⩽ Chuhara ≈ Rashmi ⩽ Elaichi ⩽ Sonaur-5 ⩽ ZG-3. Variation in TPH may be explained on the basis of difference in genotypic background of *Zizyphus* which shows rich genetic diversity mostly arising from natural cross pollination and self incompatibility (Bhargava et al., 2005). Our results are in agreement with the earlier reports (Kaur and Kapoor, 2005; Kamiloglu et al., 2009). It is interesting to note that TPH in Indian jujube was found to be comparable with fruits already reported to be high in TPH (mg/100 g) e.g. 126–247 in guava, 125–373 in plums (Thaipong et al., 2006). Plant phenolics have multifunctional properties and can act as singlet oxygen quenchers and scavenge free radicals, thus presence of substantial amounts of phenolics in Indian jujube indicates that they are a significant source of antioxidants which may provide health promoting advantages to the consumers.

Total flavonoid content was determined using the aluminium complexation method. The content of total flavonoids was also found to vary significantly ($p < 0.05$) and content ranged from 8.36 to 21.97 mg CE/100 g. The TF in increasing order was: Chuhara ⩽ Umran ≈ Sonaur-5 ≈ Thornless ⩽ Mundia ≈Kaithali ⩽ ZG3 ≈ Rashmi ≈ Seb ≈ Elaichi ⩽ Jogia ⩽ Gola. Recently, Lamien-Meda et al. (2008) reported high TF in range of 56.88–92.55 mg/100 g in *Zizyphus* extracts. Quercetin, kaempferol, and phloretin derivatives have been reported from *Zizyphus jujube* Lamk and *Zizyphus spina-chisti* (Pawlowska et al., 2009). Flavonoids have been reported to have beneficial health effects including anti-inflammatory, inhibition of platelet aggregation, inhibition of mast cell histamine release, and antimicrobial activities.

Table 1 Total phenolics, flavonoids and ascorbic acid content of some *Zizyphus* genotypes.

Cultivar	Total phenolics[1]	Total flavonoids[2]	Ascorbic acid[3]
Chuhara	258.06 ± 37.99^{cb}	8.36 ± 1.47^{e}	99.49 ± 1.53^{a}
Mundia	243.13 ± 41.59^{dcb}	12.7 ± 1.11^{edc}	93.88 ± 1.02^{a}
Thornless	237.83 ± 37.09^{dc}	12.38 ± 0.45^{ed}	94.9 ± 2.04^{a}
Jogia	241.26 ± 57.76^{dc}	18.47 ± 3.03^{b}	59.69 ± 0.51^{d}
Gola	252.23 ± 18.29^{dcb}	21.97 ± 2.09^{b}	57.65 ± 4.59^{d}
Kaithali	187.48 ± 34.16^{ed}	13.09 ± 3.93^{edc}	71.94 ± 1.53^{e}
Umran	172.08 ± 31.77^{e}	10.76 ± 0.85^{ed}	19.54 ± 1.85^{f}
Seb	203.23 ± 2.82^{edc}	15.62 ± 1.92^{dc}	21.95 ± 0.5^{f}
ZG-3	328.65 ± 13.98^{a}	14.58 ± 0.59^{dc}	83.16 ± 0.51^{b}
Sonaur-5	309.51 ± 46.73^{ba}	11.47 ± 1.83^{ed}	36.22 ± 0.51^{e}
Rashmi	261.21 ± 43.68^{cb}	14.71 ± 1.85^{dc}	39.29 ± 3.57^{e}
Elaichi	267.28 ± 18.39^{cba}	16.07 ± 2.58^{dc}	71.56 ± 1.15^{c}

Values represent the mean of three replicates. Mean followed by the same superscripts are not significantly different ($p < 0.05$).
[1] Total phenolics expressed as mg GAE/100 g.
[2] Total flavonoids expressed as mg CAE/100 g.
[3] Ascorbic acid expressed as mg/100 g.

3.2. Ascorbic acid

Ascorbic acid content was determined using the DNPH and values are presented in Table 1. There was significant difference ($p < 0.05$) in AA content among *Zizyphus* genotypes. The content ranged from 19.54 to 99.49 mg/100 g depicting five-fold variations. The AA in increasing order was: Umran ≈ Seb < Sonaur-5 ≈ Rashmi < Gola ≈ Jogia < Elaichi ≈ Kaithali < ZG3 < Mundia ≈ Thornless ≈ Chuhara. High variation in AA content among genotypes could be due to the existing differences in growing conditions and maturity levels. The values are in agreement with the previous reports of (Abbas et al., 1988; Zhang et al., 2010). The extent of AA was found to be very high as compared to other fruit crops commonly consumed in Indian diet such as mango, papaya and guava. The range of AA (mg/100 g) was 60.5 in mango, 92.9 in papaya and 72.2 in guava (Luximon-Ramma et al., 2003; Thaipong et al., 2006). Ascorbic acid acts as a reducing and a chelating agent and has been shown to scavenge free radicals and is an important component of the antioxidative defence mechanism in cells and tissues. Increased levels of ascorbate also protect the lungs against the oxidizing agents present in cigarette smoke (Bendich et al., 1986). It also helps to detoxify the hazardous effects of stannous chloride commonly used as a preservative in soft drinks (Yousef et al., 2007).

3.3. Antioxidant activity

Evaluation of AOX is becoming increasingly relevant in the field of nutrition as it provides useful information with regard to health promoting and functional quality of raw material without the analysis of each antioxidant compound (Scalfi et al., 2000). The parameter accounts for the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds and their synergistic and/or antagonistic effects. In this study we have used four *in vitro* assays, namely ferric reducing antioxidant power (FRAP), Cupric reducing antioxidant power (CUPRAC), Trolox equivalent antioxidant capacity (TEAC) and free radical scavenging assay using DPPH assay. The assays were used because they are quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present. Moreover they require a spectrophotometer unlike sophisticated equipment in the case of ORAC assay (Awika et al., 2003). Although FRAP, DPPH and ABTS have been very popular among researchers, CUPRAC assay is a relatively new assay developed by Apak et al. (2008). It utilizes the copper(II)–neocuproine [Cu(II)–Nc] reagent as the chromogenic oxidizing agent and is based on the cupric reducing ability of reducing compounds to cuprous. Total AOX in Indian jujube ranged from 7.41 to 13.93 μmol Trolox/g and 8.59–15.11 μmol Trolox/g in FRAP and CUPRAC assays, respectively (Table 2). The order of hierarchy was Sonaur-5 < Rashmi ≈ Elaichi < ZG3 < Chuhara ≈ Jogia ≈ Mundia ≈ Thornless ⩽ Gola ⩽ Seb ⩽ Kaithali ⩽ Umran. A somewhat similar trend was observed in CUPRAC assay as well, although the values were slightly higher than FRAP assay. High AOX in CUPRAC may be attributed to the presence of flavonoids such as

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Table 2 Antioxidant activity of some *Zizyphus* genotypes.

Cultivar	FRAP[1]	CUPRAC[2]	DPPH[3]	TEAC[4]
Chuhara	9.96 ± 1.6^{ba}	13.57 ± 2.7^{ba}	15.42 ± 3.2^{d}	15.76 ± 2.7^{d}
Mundia	9.25 ± 2.7^{b}	13.45 ± 3.4^{ba}	19.24 ± 2.7^{c}	18.93 ± 3.4^{c}
Thornless	9.13 ± 1.6^{b}	12.67 ± 1.5^{c}	14.18 ± 1.6^{e}	15.65 ± 1.5^{d}
Jogia	9.94 ± 1.9	13.89 ± 1.6^{ba}	18.72 ± 1.9^{c}	17.12 ± 1.6^{c}
Gola	8.58 ± 2.5^{b}	12.56 ± 1.3^{bc}	23.19 ± 2.5^{bc}	22.94 ± 1.3^{ba}
Kaithali	8.25 ± 2.1^{b}	9.12 ± 1.5^{cd}	14.45 ± 2.1^{d}	13.55 ± 1.5^{de}
Umran	7.41 ± 1^{b}	8.01 ± 1.2^{d}	14.56 ± 1^{d}	12.74 ± 1.2^{e}
Seb	8.37 ± 4.5^{b}	12.14 ± 2.5^{c}	28.65 ± 4.5^{b}	27.75 ± 2.5^{a}
ZG-3	11.65 ± 3.7^{ba}	15.1 ± 1.7^{a}	39.64 ± 3.7^{a}	29.45 ± 1.7^{a}
Sonaur-5	13.93 ± 2.65^{a}	15.13 ± 3.4^{a}	18.26 ± 2.65 ^{cd}	12.59 ± 3.4^{e}
Rashmi	11.95 ± 1.46^{ba}	13.01 ± 3.65^{b}	18.65 ± 1.46^{c}	19.54 ± 3.65^{bc}
Elaichi	11.95 ± 2.9^{ba}	12.21 ± 1.8^{c}	21.82 ± 2.9^{bc}	21.1 ± 1.8^{b}

Values represent the mean of three replicates. Mean followed by the same superscripts are not significantly different ($p < 0.05$).
[1] FRAP – ferric reducing antioxidant power.
[2] CUPRAC – cupric ion antioxidant reducing capacity.
[3] DPPH – 2,2-diphenyl-1-picrylhydrazyl.
[4] TEAC – Trolox equivalent antioxidant capacity. Activity in each method was expressed in common unit μmol Trolox/g.

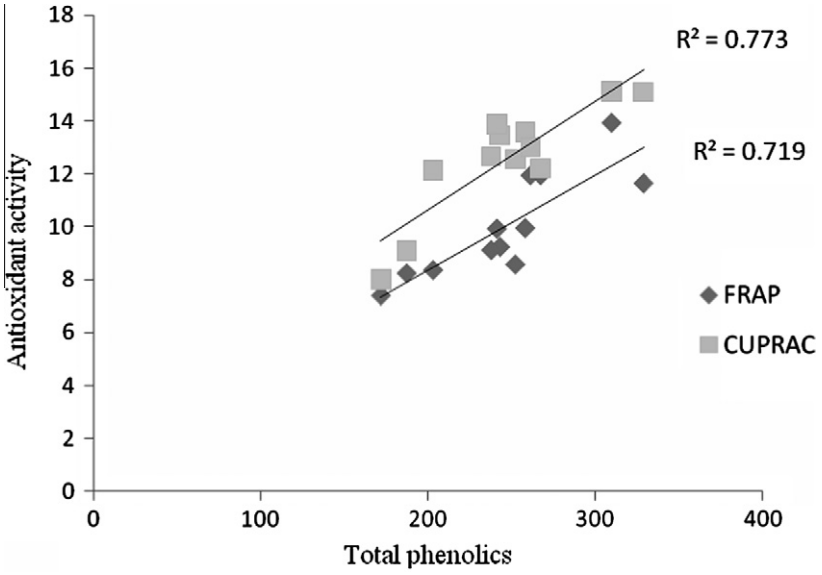

Figure 1 Correlation between total phenolics and antioxidant activity. Total phenolics expressed as mg GAE/100 g, Antioxidant activity in both methods was expressed as μmol Trolox/g.

quercetin, and kaempferol. The antioxidant potency of flavonoids is roughly proportional to the total number of –OH groups and is positively affected by the presence of an *o*-dihydroxy moiety in the B-ring (Apak et al., 2008). Evaluation of AOX in *Zizyphus* sp using FRAP assay has been reported by several workers (Li et al., 2005, Kamiloglu et al., 2009; Zhang et al., 2010). However our values are relatively lower than reported by earlier workers. These differences in results may be explained on the basis of genotypic differences in cultivars grown under different environmental conditions. The content of other constituents such as ascorbic acid or flavonoids can also contribute to large differences in AOX. Since ascorbic acid and flavonoids are strong reducing agents, they may reduce the oxidized state of antioxidant compounds, causing an increase in AOX.

Free radical scavenging activity in *Zizyphus* extracts was measured using DPPH and TEAC. DPPH assay is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenolics. The bleaching of DPPH solution increases regularly with increasing amount of fruit in a given volume. The TEAC assay measures the relative antioxidant ability of fruits to scavenge the radical-cation $ABTS^{+\cdot}$ produced by the oxidation of 2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate. Free radical scavenging activity ranged from 15.18 to 29.69 and 12.74 to 29.45 μmol Trolox/g in DPPH and TEAC, respectively (Table 2). The order of trend was somewhat similar to that found in FRAP and CUPRAC assays and genotypes ZG-3 and Seb scored over the rest. It is difficult to compare the results of DPPH assay with those reported by earlier workers

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Table 3 Physicochemical composition of some *Zizyphus* genotypes.

Cultivar	TA[1]	TSS[2]	TSS/TA	pH
Chuhara	0.22 ± 0.005^{c}	15 ± 0.58^{c}	66.96c	4.58 ± 0.06^{ab}
Mundia	0.22 ± 0.011^{c}	13 ± 0.58^{b}	58.03^{c}	5.09 ± 0.12^{de}
Thornless	0.10 ± 0.001^{a}	11 ± 0.58^{a}	100.91^{a}	5.07 ± 0.06^{de}
Jogia	0.25 ± 0.005^{d}	17 ± 0.58^{e}	66.40^{e}	4.76 ± 0.12^{bc}
Gola	0.25 ± 0.005^{d}	15 ± 0.29^{c}	58.59^{b}	4.35 ± 0.06^{a}
Kaithali	0.16 ± 0.005^{b}	13 ± 0.58^{b}	81.25^{b}	5.26 ± 0.06^{ef}
Umran	0.22 ± 0.005^{c}	15 ± 0.58^{c}	66.96^{c}	5.37 ± 0.12^{f}
Seb	0.224 ± 0.003^{c}	15 ± 0.33^{c}	66.96^{c}	4.83 ± 0.06^{bcd}
ZG-3	0.32 ± 0.002^{f}	15 ± 0.00^{c}	46.87^{bc}	4.72 ± 0.12^{bc}
Sonaur-5	0.32 ± 0.002^{f}	15 ± 0.00^{c}	46.87^{c}	4.92 ± 0.12^{cd}
Rashmi	0.30 ± 0^{e}	19 ± 0.58^{e}	63.33^{c}	5.5 ± 0.03^{f}
Elaichi	0.28 ± 0.001^{e}	10 ± 0.00^{a}	34.72^{c}	4.99 ± 0.12^{cde}

Values represent the mean of three replicates per cultivar. Mean followed by the same superscripts are not significantly different ($p < 0.05$).
[1] TA – total acidity.
[2] TSS – total soluble solids.

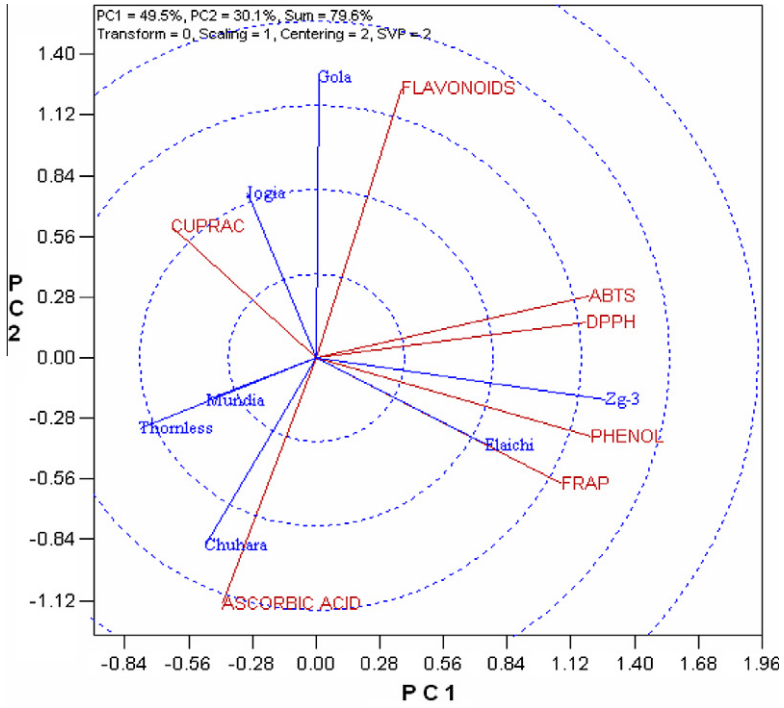

Figure 2 Biplot of the relationship between quality attributes and *Zizyphus* genotype variables are phenolics, flavonoids, ascorbic acid, FRAP = ferric reducing antioxidant power, CUPRAC = cupric ion antioxidant reducing capacity, DPPH = 2,2-diphenyl-1-picrylhydrazyl and ABTS = 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid).

since the activities were expressed as percent inhibition and not in terms of an equivalent standard. Overall the AOX values for *Zizyphus* were found to be comparable with anthocyanin rich fruits such as plum and strawberry (Wang et al., 1996).

3.4. Correlation

To explore the effect of the AOX in jujube, correlation coefficients of the total phenolics and flavonoids with AOX were also worked (Fig. 1). There was a positive correlation (R^2 = 0.719 in FRAP and 0.773 in CUPRAC) between total phenolics content and AOX then with flavonoids and ascorbic acid (data not shown). Similar strong relationship has been demonstrated in peaches and plums reflecting the fact that total phenolics are the major determinant of AOX in *Zizyphus* (Cevallos-casals et al., 2005).

3.5. TSS, TA, TSS/TA ratio and pulp yield

The results of general quality attributes viz, TSS, TA, pH and pulp percentage in *Zizyphus* sp. are presented in Table 3. TSS and TA in *Zizyphus* cultivars ranged from 10% to 19% and 0.11% to 0.32.0%, respectively. The TSS/TA ratio is also an important parameter, related with sensory quality

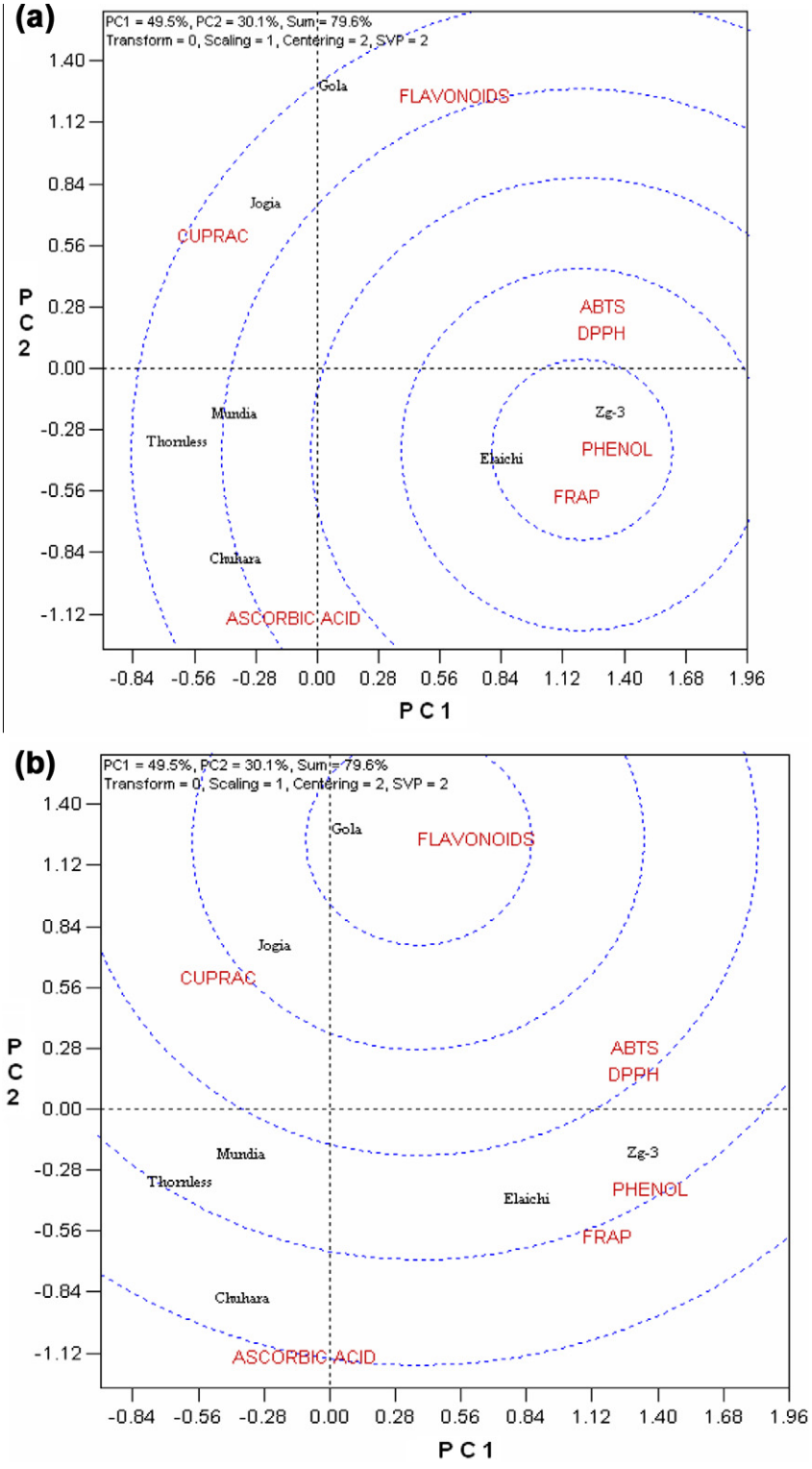

Figure 3 Biplot using (a) phenolics and (b) flavonoids as major quality attribute (only the promising genotypes are represented) variables are phenolics, flavonoids, ascorbic acid, FRAP = ferric reducing antioxidant power, CUPRAC = cupric ion antioxidant reducing capacity, DPPH = 2,2-diphenyl-1-picrylhydrazyl and ABTS = 2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid.

and ranged from 34.72 to 100.91. Pulp percentage an important economic trait in processing industry was found to range from 84% to 96.5% data not shown. Based on general quality characteristics, Rashmi, Seb, ZG-3 and Sonaur-5 appear to be promising cultivars with good organoleptic quality.

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3.6. Multivariate analysis

Principal component analysis is a variable reduction procedure and to develop a smaller number of artificial variables (called principal components) that will account for most of the variance in the observed variables. If the variables are highly related, they can be combined into a component that accounts highest quantity of variance in the sample (observations). The second component explains the second highest quantity of variance and will not be correlated to the first component (Favero et al., 2009). Based on the theoretical arguments of the PCA described by Hair et al. (2005) the significant factor loading values higher than or equal to 0.7 were used to identify the most important variables and attributes in each dimension, or principal components (PCs). Factor loading values are the correlation of each variable (attributes) with the PC. They are represented as vectors (positions) in the space resulted by the axes of the biplot graphic. In the graphic, variables (Fig. 2) and attributes (that are close to each other, and in the same geometric plane of the biplot, are interrelated, and distant from variables and observations to which they are not related, or even negatively related. The greater is the vector (distance from the origin of the axis), the greater is the correlation of the variable with the PC represented in that dimension (axis). First two PCs explained approximately 79.6% of total data variability, called PC1 (49.5%) and PC2 (30.1%) (Fig. 2). In general, the genotypes located in the right hand of the biplot between attribute vectors, indicates a higher antioxidant activity than those located on the left. So ZG-3 and Elaichi seem to be promising candidates with high functional properties. Considering the high AOX associated with total phenolics and flavonoids, biplots were plotted by taking them as reference compounds (Fig. 3a and b). The figures clearly indicate that genotypes ZG-3 and Elaichi were found to be most promising genotypes when total phenolics was selected as reference compound and Gola and Jogia in terms of flavonoids.

In this regard, identification of nutritionally superior cultivars of *Zizyphus* could potentially increase the intake of health promoting compounds through diet and aid in the prevention of chronic human diseases. These cultivars may be highly desirable in germplasm breeding programs to breed quality varieties of *Z. mauritiana* Lamk with high antioxidant potential.

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