Assessment of Nutritional And Free Radical Scavenging Activity of Selected Genotypes of *Cordia Myxa* L.: A Potential Underutilized Fruit Crop of Indian Arid Zone

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Abstract

Cordia myxa is one of the important underutilized fruit plants suitable for arid and semi-arid regions of India having potential for commercial exploitation in vegetable and pickle industry. Fruits of four improved genotypes of *Cordia myxa* were analyzed for nutritional value, total phenol content and free radical scavenging activity. Acetone extracts from dried fruit samples were prepared to determine total phenol content and free radical scavenging activity using DPPH essay. The fruits harvested at 20 or 30 days after fruit set recorded higher crude fibre and crude protein as compared to at full ripening stage. The total phenol content was found highest in fruits harvested earliest irrespective of genotypes. The correlation coefficient between total phenol content and free radical scavenging activity of fruit extract was found positive and significant. The results suggest that the fruits of *Cordia myxa* should be harvested at 20 to 30 days after fruit set to get maximum benefits of its anti-oxidant and other nutritional properties.

Introduction

Cordia myxa, known as *gonda* or *lasora* is an important multipurpose fruit plant belonging to the family Boraginaceae. It grows well in arid to semi-arid regions of north India and other isoclimatic regions the world over. Its distribution extends in tropical and subtropical regions of India especially in dry deciduous forest of Rajasthan and western ghat in Mynmar. The plant is a small to moderate sized deciduous tree with a short trunk and spreading crown. The flowers are short stalked, hermaphrodite and white in color which open in morning around 9.0 hours. It takes about three months from flowering to full ripening of the fruits; however, they are harvested at mature green stage much before full ripening upon turning the fruit colour from green to yellowish green or yellowish pink. Though, the fruits are very sweet at full ripening, they hardly have commercial value due to high content of mucilage. The fruit pericarp is edible part while endocarp is viscous. The unripe fruits of *C. myxa* are used as vegetable and pickled with raw mango. The Fruits can also be preserved after dehydration for subsequent uses. The picking of the fruits at right stage of maturity is necessary not only for better nutrient value, but also for getting higher yield and better market price. The fruit pulp was found rich in crude protein, fiber, carbohydrates and minerals (Aberoumand 2011). The fruits and other plant parts also have curative properties in skin diseases, dropsy, dysentery, dyspepsia, cholera and headache etc. Trees are mostly planted around farm as wind break and as shelter belt but for past two decades they are also planted as planned orchards as the fruits fetch high profits. Looking to its drought resistance, limited water requirement and multiple uses, systematic evaluation and ex-situ conservation were carried out at Central Arid Zone Research Institute, Jodhpur Rajasthan, India for further improvement of *Cordia myxa*. Collection was made on selective sampling strategy and each collection was allotted individual accession number. Out of various collections, fourteen diverse accessions were selected for characterization study (Meghwal et al. 2014). After long term evaluation of vegetatively propagated genotypes along with check population, four elite genotypes i.e. CZCM-2011, CZCM-2021, CZCM-2012, CZCM-2025 were selected as improved genotypes with respect to production and phytochemicals. Most of the accessions did not show significant

variations with respect to vegetative growth parameters such as plant height, canopy and morphological characteristics of leaves and flowers but significant variations were recorded in fruit yield in long term evaluation (Meghwal 2018). One of the accessions i.e. CZCM-2025 was released as Maru Samridhi, a new high yielding variety recently (Meghwal et al. 2019). The fruits of other three accessions used in this study were also consistently high yielder as compared to other germplasm accessions. In literature, little or no information exists on nutritional properties, total phenol content and antioxidant activity of fruits of *C. myxa* at different maturity stages. Hence, this study was undertaken to derive the information on these aspects.

Previous studies on nutritional composition of the fruits of *Cordia myxa* showed that they contained 8.32% crude protein,57.08% carbohydrate 6.7% ash, 25.7% fibre and 2.2% fat (Al-Snafi 2016). Mineral analysis of the fruits is reported to contain 1.62mg/g sodium, 7.83 mg/g potassium, 0.46mg/g calcium, 0.35mg/g zinc and 0.51mg/g iron (Aberoumand 2011)

The free radicals are molecular species having unpaired electron(s) in their atomic structure which can have independent existence (Jesburgar and Richardson 1991). The presence of an unpaired electron makes these species unstable and strongly reactive. The oxygen free radicals are most abundant free radicals in biological system. Singlet oxygen is another radical that owes its origin from oxygen. In biological system, the reactive oxygen species are generated enroute mitochondria and also as an intermediate product in several enzymatic reaction. These free radicals can be toxic to biomolecules such as lipid, protein and nucleic acids. The natural antioxidants present in the living systems try to neutralize them but if they are not effectively neutralized, they might harm to body leading to disease like conditions (Przedborski and Jackson-Lewis 1998). In recent years, antioxidant properties of fruits, vegetables and medicinal plant are studied with renewed interest for finding solution of several diseases in human beings. Phenolic compounds are naturally produced in the plants during secondary metabolism. The fruits and vegetables also exhibit strong antioxidant and antimicrobial properties due to phenolic substances (Alesiani 2010). Several other compounds such as flavonoids, tannins and proanthocyanidins might also act as antioxidant for protection against many diseases. The quantitative and qualitative composition of phenolic compounds in the fruits is determined by genotype, agro techniques and environmental conditions under which they are grown (Miletic et al. 2012). The extracts of different plant parts which are good source of phenolic compounds are finding uses in food and pharmaceutical industries because of their nutraceutical values. Some previous studies report the presence of phenolic and antioxidant activities from accessions from naturally occurring trees *C. myxa* without any background of fruit yield and growing conditions. However, in our study, we have quantified the total phenol content and free radical scavenging activity from well tested improved genotypes (developed and maintained at our research farm under similar agrotechniques) at different stages of fruit maturity.

Materials And Methods

Chemicals

Butylated Hydroxy Toluene (BHT), 2,2-Dipheny-1-pycril-hydrazyl (DPPH), Folin-Ciocalteu Reagent, Gallic acid, Sodium Carbonate (Na_2CO_3). All the chemicals and reagents were of analytical grade from CDH, New Delhi.

Sample collection and preparation

Fruits of *Cordia myxa* were obtained from central research farm, ICAR-Central Arid Zone Research Institute, Jodhpur during the month of April, 2019. So far, no information is available on standard maturity criteria for harvesting the fruits of *C. myxa* and generally, it is believed that less matured fruits have better culinary values. Therefore, simple criteria of harvesting the fruits after different days of fruit set was considered. The fruits of four accessions viz. CZCM-2011, CZCM-2012, CZCM-2021, and CZCM-2025 (Fig. 1) were harvested at 20, 30, and 40 days after fruit set to examine the presence of total phenolic content and free radical scavenging activities as influenced by stages of harvesting and genotypes.

These genotypes were promising selections identified after long-term evaluation from previous project based upon consistent higher yield. Flowering and fruit setting dates were recorded to harvest the fruits at specified stages. After separating the fruits from the stalks, the individual fruits were destoned and stones (seeds with hard coat) were discarded. The pericarp (the edible portion) was retained and dried in an oven at 55^0C till it become brittle or with 5–7% moisture. The dried samples were kept in a separate screw cap PVC container with its lid tightly closed and stored in deep freeze in dark till analysis.

Extraction procedure

Dried samples were grounded in a mixture to a fine powder. The extraction procedure for phenolic substances was slightly modified as per the available resources. 20 g of powdered fruit sample from each treatment was mixed with 100 ml of acetone (99.5% purity) in 200 ml conical flask and stirred thoroughly and kept overnight to allow solvent extraction. In the next morning, the mixture was stirred over magnetic stirrer for 30 minutes. The mixture of each sample was then filtered. The filtrates were kept in known weight of glass beakers under room temperature for 24 hours to allow evaporation of the solvent. The dried acetone extract was weighed to calculate the yield of total phenolic extracts from each sample on the basis of empty beaker weight.

Determination of free radical scavenging activity

The solutions of fruit extracts (100 & 500 ppm) and synthetic antioxidant i.e. BHT (200 ppm) were prepared in pure methanol. The DPPH solution (0.1mM) was prepared in methanol fresh each time. Free radical scavenging activities of the fruit extracts and synthetic antioxidant (BHT) were determined according to Shimada et al. (1992) which is based on the principle of scavenging the DPPH. In this assay, a volume of 2 mL of methanolic solution of fruit extract was mixed with 2 mL DPPH (0.1 mM). An equal amount of methanol and DPPH was taken as control. The samples were then kept in dark at room temperature for 30 minutes and the absorbance measured at 517 nm using a UV-VIS spectrophotometer

(Model TS2080PLUS, Analytical Technologies Limited). The free radical scavenging activity was expressed as percent inhibition calculated using the following formula:

DPPH radical scavenging activity in % = $[(A_0 - A_1)/A_0] \times 100$

Where, A_0 is the absorbance of the DPPH solution and A_1 is the absorbance of the sample.

Determination of total phenolics

The total phenol content from the extract was determined using Folin-Ciocalteu Reagent (McDonald et al., 2001) with slight modification. Different concentrations of gallic acid i.e. 25, 50, 75, and 100 ppm were prepared in methanol for preparation of standard curve. To each concentration of gallic acid, 5 mL of Folin-Ciocalteu Reagent was mixed and of 2 mL of Na_2CO_3 (75g/L) was added after 5 minutes. The solution was kept in dark for two hours and the absorbance measured at 760 nm using a UV-VIS spectrophotometer. The standard curve was plotted from the absorbance values of gallic acid. One mL (1000 ppm) of dried fruit extract was mixed with 5 mL of Folin-Ciocalteu Reagent for determination of total phenol. After 5 minutes, 2 mL of sodium carbonate (75g/L) was added and the mixture was kept in dark for two hours at room temperature and the absorbance was measured at 760 nm. The total phenols in the extracts were measured in terms of gallic acid equivalents (GAE) by the following equation, T = CV/M; where, T = total phenolic contents (GAE) in milligram per gram extract; C = the concentration of gallic acid established from the calibration curve mg/mL; V = the volume of extract in milliliter and M = the weight of sample extract (g).

Determination of nutritional properties

The fruits were harvested at different maturity stages, destoned and dried were grounded in mixer for analyzing of nutritional and mineral analysis. The samples were analyzed on dry matter basis (AOAC 2007) for Ca, Fe, Zn, Cu, Mn, Cr, Pb and Mo using Graphite furnace P.G (PG Instruments Ltd.,UK).

Statistical analysis

The experiments were laid out in Completely Randomized Block Design with three replications and the results were presented as mean ± standard error with CD at 5 % significance level. The data in all the experiments were analyzed in Microsoft Excel 2016 for statistical significance. The correlation coefficients between total phenol content and free radical scavenging activity were determined using Pearson’s correlation.

Results And Discussion

Nutritional and mineral assessment of selected genotypes

Result of important nutritional quality traits of four selected genotypes at three maturity stages were analysed (Tables 1 and 2). Wide variability among genotypes vis-a-vis within the genotypes at different maturity stages was recorded for all proximate traits. The range of various parameters recorded are ether

extractives (2.16–4.03%), crude protein (6.5-12.21%), total ash (8.8–13.10%), silica (0.50 to 1.3%), crude fibre (11.76–18.52 %), N.D.F. (26.45–70.16 %) and A.D.F(26.22–56.79%). Fruit growth and development stages have shown distinct variations on concentrations of different nutrients in different genotypes. Ether extract and crude protein noticed increasing trend up to 30 days after fruit set followed by declining with lowest value in fully ripened fruits. Total ash and crude fibre showed increasing trend with highest value in fully ripened fruits. However, silica, NDF and ADF content showed decreasing trend with maturity stage and recoded minimum in fully ripened fruit in all the genotypes. Amongst genotypes, ether extract, crude protein, crude fibre and ADF content was recorded highest in CZCM-2011 while total ash content in CZCM-2025, silica in CZCM-2021 and NDF content in CZCM-2012. Ali Aberoumand (2011) reported that *Cordia myxa* fruits contained ash-6.7%, crude protein-8.32%, crude lipid-2.2%, crude fibre-25.7% and carbohydrates-57.08%.

Data contained in Table 4 revealed that both genotypes and stage of maturity has significant effect on macro and micro nutrients element of *Cordia myxa* fruits. Calcium content varied from 139.23 to 239.45 mg/100g and showed perceptible differences among the accessions at different maturity stages. However, at particular stage of maturity different genotypes showed non-significant effect. It was clearly noticed that calcium content was higher in unripe fruits compared to fully ripened fruits in all the genotypes. In contrast to calcium, phosphorus content was found relatively higher in mature and fully ripened fruit and it ranged from 250.25 to 379.65 mg/100g. Micro nutrients viz copper, manganese molybdenum and chromium showed wide variations among the genotypes and ranged from 342.23 to 776.29 µ/100g, 357.07 to 524.72 µ/100g, 52.12 to 140.26 µ/100g and 290.6 to 459.8 µ/100g, respectively. Moreover, major mineral nutrient was found higher in green fruits compared to fully ripened fruits except phosphorus content which was recorded more in fully ripened fruits irrespective of genotypes. Earlier studies also reported quite good quantity of biochemical and nutritional properties of *Cordia* species (Ali Aberoumand 2011, Rathore 2009, Bouby et al. 2011).

Extract yield from fruits

The total content of phenolics and free radical scavenging activity might differ in fruits of different genotypes as well as according to fruit maturity stages. Therefore, fruits of four genotypes of *C. myxa* at three maturity stages were tested for phenolic content and free radical scavenging activity. The acetone extract yield of phenolic substances differed significantly with highest value in the fruits harvested at 20 days after fruit set irrespective of genotypes and this declined as the harvesting was delayed with lowest yield in fruits harvested at 40 days after fruit set (Table 3). The difference between extraction yield of phenolic compounds were not significant when compared with different genotypes at same stage of harvesting. This shows that extraction yield is a species-specific character not influenced significantly due to genotypes/varieties. The extraction yield was lowest in fruits harvested after 40 days of fruit set which indicated lower content of these substances with advancement of fruit maturity. Less matured fruits are considered good for making vegetables or pickles because of lower content of mucilaginous substances. The fact established from this study that fruits harvested at 20 days after fruit set recorded highest value of phenolic compounds which might also have higher anti-oxidant activity.

Table 1
Proximate nutritional composition of fruits of different accessions of *cordia myxa* at various maturity stages

Accessions	Maturity stage	Ether extract (%)	Crude Protein (%)	Total Ash %	Silica (%)	Crude Fibre (%)	N.D.F %	A.D.F %
CZCM-2011	20 DAFS	3.577	12.120	8.900	1.200	18.527	67.406	56.799
	30 DAFS	4.033	12.210	9.100	0.800	17.874	65.018	54.778
	Fully Ripened	2.527	6.950	11.500	0.850	11.769	29.846	27.389
CZCM-2012	20 DAFS	3.550	11.560	8.900	0.900	14.470	70.161	56.193
	30 DAFS	3.703	11.680	9.000	0.900	17.523	66.671	51.544
	Fully Ripened	2.160	6.800	13.100	0.850	13.255	28.652	26.227
CZCM-2021	20 DAFS	3.750	11.970	8.800	1.300	15.063	67.589	55.991
	30 DAFS	3.837	12.110	8.900	1.100	14.058	66.212	55.081
	Fully Ripened	2.380	6.500	12.120	0.790	13.255	27.660	27.389
CZCM-2025	20 DAFS	2.340	11.060	9.400	0.500	14.360	65.018	56.193
	30 DAFS	3.370	11.320	8.900	0.600	12.853	65.385	55.688
	Fully Ripened	2.183	6.620	12.900	0.800	12.773	26.448	26.884
SEm		0.134	0.12	0.148	0.013	0.268	6.007	1.146
CD (0.01)		0.393	0.35	0.434	0.037	0.786	17.637	3.366

Table 2
Some major and micronutrient content of *cordia myxa* fruits at different maturity stages (on dry weight basis)

Accessions	Maturity stage	Calcium mg $100g^{-1}$	Phosphorus mg $100g^{-1}$	Cu (µg $100g^{-1}$	Mn (µg $100g^{-1}$)	Mo µg 100 g^{-1}	Cr µg $100g^{-1}$
CZCM-2011	20 DAFS	179.10	349.50	342.239	500.96	113.95	401.5
	30 DAFS	139.23	349.45	471.615	397.55	140.26	404.3
	Full ripening stage	199.45	339.10	461.568	385.28	54.12	318.7
CZCM-2012	20 DAFS	199.06	250.25	454.927	524.72	116.58	459.8
	30 DAFS	189.35	379.65	776.292	357.07	135	318.4
	Full ripening stage	179.08	359.20	465.775	471.57	51.88	317.6
CZCM-2021	20 DAFS	239.45	280.70	555.094	380.83	111.32	290.6
	30 DAFS	229.25	270.20	675.123	480.72	117	311.5
	Full ripening stage	219.32	359.32	466.897	485.69	52.12	317.6
CZCM-2025	20 DAFS	209.63	300.08	351.585	382.27	115.2	316.5
	30 DAFS	199.02	349.40	421.531	358.82	121	382.1
	Full ripening stage	189.48	349.20	459.094	475.88	53.42	315.6
SEm		5.0	6.02	10.946	6.58	2.4	6.58
CD (0.01)		NS	0.018	32.14	19.321	7.048	19.321

Table 3
Acetone extract yield from dried fruit samples of different genotypes of *Cordia myxa* harvested at different stages of maturity

Genotypes	Mean extraction yield (mg/g DW) at different harvesting stages (days after fruit set - DAFS)		
	20 DAFS	30 DAFS	40 DAFS
CZCM-2011	7.65	6.90	4.80
CZCM-2012	7.55	7.05	4.96
CZCM-2021	6.95	6.03	4.48
CZCM-2025	7.26	7.20	5.50
Mean	7.35	6.79	4.93
CD (p = 0.05)	0.52		
SEm±	0.18		

Preparation of standard curve and calculation of total phenolic content

Total phenolic content in fruit extracts was determined by Folin-Ciocalteu method using gallic acid as the standard. The absorbance values obtained at different concentrations of gallic acid were used for the preparation of standard curve (Fig. 2). In this method, electron is transferred from phenolic groups to phosphomolybdic phosphotungstic acid complex. This results in appearance of blue colour which is measured colorimetrically at 760 nm. A regression equation ($Y = 0.0113x - 0.0055$; $R^2 = 0.9913$) was obtained from the standard curve and the total phenolic content was calculated as mg gallic acid equivalents (GAE) per gram of extract. The phenolic compounds present in fruits or any other plant parts might represent antioxidant activity. Therefore, total phenol content in fruit extracts were determined and given in Table 4. The data indicated that the total phenol content did not vary significantly between different genotypes but it differed significantly when compared between the data of 30 and 40 days after fruit set irrespective of genotypes. Though, the highest content of total phenol were found in the fruits harvested at 20 days after fruit set in all the genotypes but the differences were non-significant between phenol content at 20 and 30 days after fruit set. The total phenol content decreased significantly in fruits harvested after 40 days of fruit set irrespective of genotypes. From these data it appears that total phenol content is more in less matured fruits, but it was almost same up to 30 days after fruit set when the fruits also attain optimum size. Therefore, it will be more economical if the fruits are harvested after about 30 days of fruit set when they attain maximum total phenol along with higher fruit yield due to increased fruit size. The presence of polyphenols in *C. myxa* fruits was also reported by Aberoumand (2011) during phytochemical screening which indicated the presence of alkaloids, saponins, polyphenols and steroids. The presence of phenolic compounds such as phenols, tannins and saponins were also reported positive

in fruit extract of different accessions of *Cordia dichotoma* (Nandekar and Mulani 2013) which is synonymous of *C. myxa*. In general, the total phenol content at different maturity stages followed similar trend as observed in case of extraction yield of phenolic substances. The trends of phenolic content according to fruit maturity stages, however, cannot be generalized as it may depend upon several other factors such as species besides growing environment. In *Eugenia jambolana*, for example, the total phenol content decreased from mature green stage to half ripened fruits but it increased again in fully ripened fruits (Balamurugan,2014).

Table 4
Total phenol content in fruit extracts (1000 ppm) of *Cordia myxa* genotypes (GAE mg g^{-1}) at different maturity stages

Genotypes	20 DAFS	30 DAFS	40 DAFS
CZCM-2011	49.22	48.43	32.96
CZCM-2012	48.22	50.46	36.89
CZCM-2021	49.95	50.53	42.61
CZCM-2025	51.73	52.02	40.07
CD (p = 0.05)	3.76		
SEm±	11.05		

Free radical scavenging activity

Several methods and modifications have been used to determine anti-oxidant activity/free radical scavenging activity. DPPH assay is the preferred colorimetric method for the determination of free radical scavenging activity of plant extracts because of its simple and rapid reaction[15]. In this method, the DPPH radicals react readily with the antioxidant compounds in the extract resulting in the disappearance of blue colour.

The present results suggest that the extracts of *C. myxa* fruits are apparently good free radical scavengers both at 100 and 500 ppm concentration as shown in Fig. 3. The free radical scavenging activity was found maximum in fruits harvested at 20 days (mean values of 41.49 and 82.56 % at 100 and 500 ppm respectively) after fruit set irrespective of genotypes and concentration of the extract and it declined with delay of harvesting stage with the lowest value in the fruits harvested after 40 days of fruit set. This indicated that the presence of total phenolics and its free radical scavenging activity depends more on fruit maturity stages rather than genotypes. The value of free radical scavenging activity of synthetic antioxidant (BHT) at 200 ppm was 80.0% ±1.72. It is worthwhile to mention here that *C. myxa* fruit extract (500 ppm) exhibited free radical scavenging activity at par with 200 ppm of synthetic antioxidant. Similar trend (34–58%) of free radical scavenging activity in in fruit extract of different accessions of *Cordia dichotoma* was also reported (Concalves et al. 2005). Fukumoto and Mazza (2000) stated that the ability of the antioxidant compounds to lose hydrogen ion as well as their conformation

determine their free radical scavenging activity. DPPH solution is blue colored and gives maximum absorbance at 517 nm. It readily receives electrons from antioxidant compounds and get converted into stable diphenyl hydrazines having light yellow colour (Soares et al. 1997). The discoloration of blue colour of DPPH solution depends upon the free radical scavenging ability of the extract (Molyneux 2004). Phenolic compounds present in plant extracts make stable phenoxy radicals by reacting with free radicals. The fruits harvested at 20 days after fruit set also had higher extract yield and total phenol content. Therefore, the correlation coefficient between total phenol content (1000 ppm) and free radical scavenging activity of fruit extract of *C. myxa* (100 ppm) was calculated which showed positive and significant correlation with R square value of 0.9667. Earlier studies have also reported the association of phenolic compounds with antioxidant activities (Hantano et al. 1989, Liu et al. 2009). This trend was further strengthened by recent findings by Hadakar et al. (2020) where in positive correlation was recorded in mango fruits between the total phenol and antioxidant activity both in peel and pulp.

Present study established the fact that stage of harvesting of fruit is very important along with genotypes for harnessing highest quantity of phytochemicals and mineral content. It is very much clear from the study that the harvesting of fruits at about 30 days after fruit set is ideal stage for obtaining highest content of nutrients and thus maximum antioxidant activity and free radical scavenging activity of this important species. The presence of the secondary metabolites i.e. polyphenols, antioxidants have contributed to its medicinal value as well. Many of these effects have been linked to their known functions as strong antioxidant, free radical scavenger. Comparing the fruits nutritional, antioxidants and mineral contents with recommended dietary allowances, *Cordia myxa* fruits in general and selected genotypes in particular may prove as a good supplement for human health of local inhabitants, if harvested at appropriate stage of maturity.

Declarations

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Conflict of Interest

The authors have no conflict of interest to declare that are relevant to the content of this article.

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Figures

Figure 1

Fruits of four genotypes of Cordia myxa at 30 days after fruit set

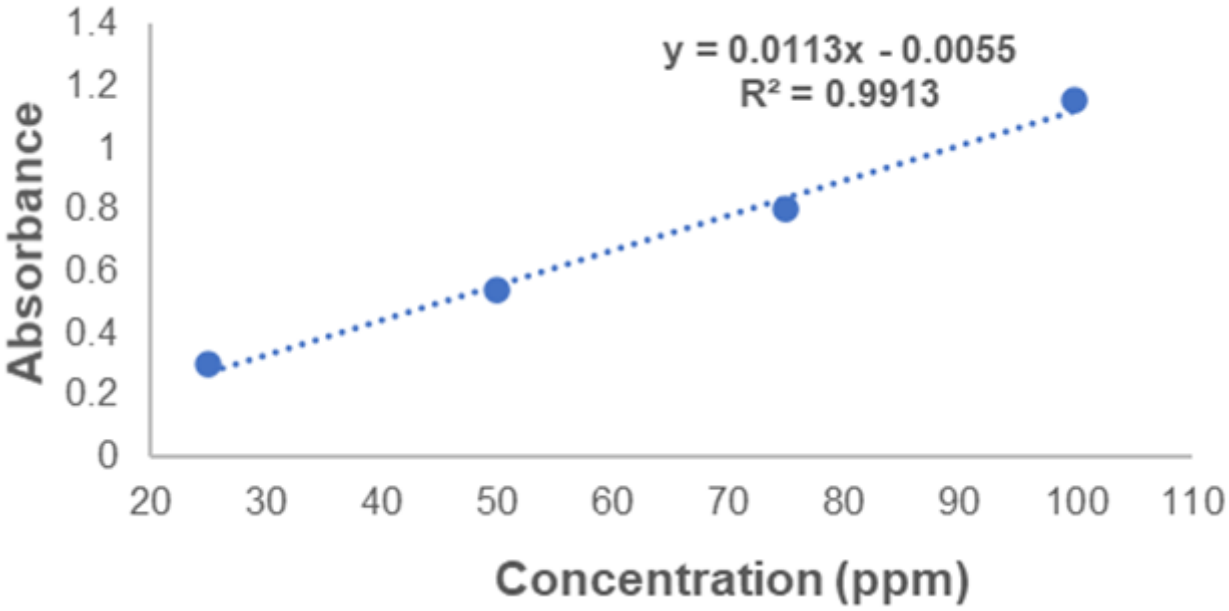

Figure 2

Standard curve for gallic acid

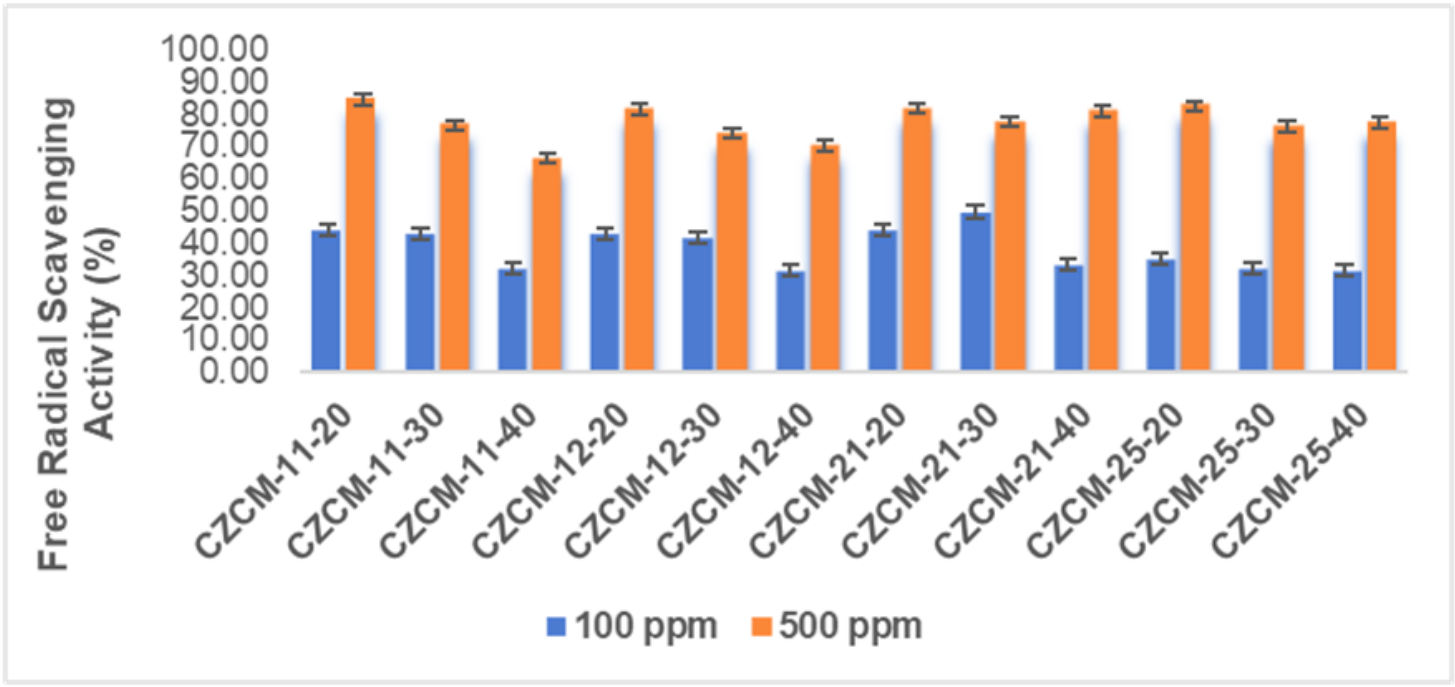

Figure 3

Free radical scavenging activity of fruits of different genotypes of Cordia myxa (CZCM-11 CZCM-12 CZCM-21 CZCM-25) at different maturity stages i.e. 20, 30 and 40 days after fruit set)