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PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF APPLE BER, A HYBRID VARIETY OF ZIZIPHUS MAURITIANA

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ABSTRACT

Indian zuzube (*Ziziphus mauritiana*) or commonly called as Ber is a tropical fruit with lots of medicinal properties. A hybrid variety called *Apple Ber* has now hit the Indian market and is rapidly gaining popularity because of bigger size, crispy and sweet taste and higher shelf life. However, no study has been taken up to analyze if the hybrid fruit contains antioxidative properties. The fruit of *Apple Ber* extracted with different solvents showed the presence of flavonoids in aqueous and methanolic extract, while tannins were present in methanol and ethyl acetate extracts, as were triterpenes. Highest antioxidant activity was found with methanolic extract, followed by ethyl acetate extract. The methanolic extract, equivalent to 600 µg equivalent dry weight was able to produce the same effect as was with ascorbic acid with 1 mg ml^{-1} concentration. The present study shows that these hybrid fruits have enough quantity of antioxidants.

KEYWORDS: *Ziziphus mauritiana*, hybrid fruit, phytochemicals, antioxidant activity, peroxide radical, DPPH.

INTRODUCTION

The hybrid ber called *Apple Ber* developed in Thailand is making a significant place in Indian markets for last few years. *Apple Ber*, a new variety of horticultural fruit Indian jujube (*Ziziphus mauritiana* Lam.), is bigger fruit and is sweet, crispy and juicy as compared to its native varieties, which are smaller, less crispy and often sour in taste. *Apple Ber* appears to be like green Apple, the reason behind its name as Apple Plum or *Apple Ber*.

The fruit is eaten raw, pickled or used in beverages. The Indian jujube (ber) is quite nutritious and rich in vitamin C and is second only to guava and much higher than citrus or apples. Because of the presence of ascorbic acid, the fruit contains good antioxidant properties, and hence are used in a variety of traditional medicines (Heuzé et al., 2007). However, the hybridized fruit, or *Apple Ber*, is a newer version of the original fruit and not much information on the phytochemicals and antioxidant activity is available. The present study is an attempt to investigate the phytochemicals and the antioxidant activities of the *Apple Ber*, being sold in India.

MATERIALS AND METHODS

Sample collection and preparation

The fruits of *Apple Ber* were collected from the local market in Jabalpur, India during the January-February 2018. This is the high time of these fruits being sold in the markets. The fresh fruits were chipped and dried under shade for 10 days. The dried fruits were then ground in a mixer grinder to obtain the powder to pass the 100 µM sieve. The powder was stored in an airtight jar until used.

Extraction of phytochemicals

The dried fruit powder (10 g) was first extracted with water using cold percolation for 48 h. During the extraction, the suspension was kept refrigerated to avoid fungal colonization. The suspension was filtered to get aqueous extract. The residue was dried and was sequentially extracted with methanol, ethyl acetate and petroleum ether. All the four extracts were concentrated in vacuo to obtain 10 ml final volume (1 g ml^{-1} concentration).

Phytochemical screening

The secondary metabolites from *Apple Ber* were qualitatively screened for the presence of important phytochemicals as per Harborne (1998). The presence and absence of the phytochemical were noted.

Antioxidant activity

The antioxidant activity was measured as the plant's extracts' ability to scavenge 2,2, diphenyl-1-picrylhydrazyl (DPPH) free radical modified method of Kumar et al. (2007). For this, 2.366 mg of 2,2-diphenyl-1-picrylhydrazyl (Sigma, USA) was dissolved in 100 ml of absolute ethanol to obtain 60 µM DPPH. All four plants extract was dried, and 25 mg of dried extract was dissolved in 25 ml of absolute ethanol and then was further diluted to obtain 200, 400, 600, 800 and 1000 µg equivalent dry matter. The sample solution of each tested material (500 µl) was mixed with the same volume of DPPH solution and allowed to stand for 1 h at room temperature in dark. The absorbance was then measured at 517 nm using a spectrophotometer against blank that received 500 µl of absolute alcohol instead of plant sample. The ascorbic acid in 1 mg ml^{-1} concentration was used as positive control. The percentage scavenging effect of ascorbic acid was also measured against the blank. The % antioxidant activity was calculated as -

$$\% \text{ Antioxidant activity} = \frac{A - Ax}{A} * 100$$

Where

A- Absorbance of DPPH solution with ethanol,

A_x- Absorbance of DPPH solution with test solution

RESULTS

The fruit of *Apple Ber* extracted with different solvents showed the presence of important phytochemicals, especially the phenolic group compounds. Flavonoids were detected in aqueous and methanolic extract, while tannins were present in methanol and ethyl acetate extracts, as were triterpenes. Apart from these phenolic group compounds, presence of alkaloids, anthraquinones, saponins, and sterols were also detected (Table 1).

Table 1: Phytochemical profile of fruit of *Apple Ber* (hybrid *Ziziphus mauritiana*), when extracted with different solvents.

#	Phytochemical	Extraction solvents			
		Aq	Me	EA	PE
1.	Alkaloids	+	+	-	-
2.	Anthraquinone	+	-	-	-
3.	Cardiac glycosides	-	-	-	-
4.	Coumerins	-	-	-	-
5.	Flavonoids	+	+	-	-
6.	Resins	-	-	-	-
7.	Saponins	+	-	-	-

8.	Sterols	-	-	+	+
9.	Tannins	-	+	+	-
10.	Triterpenes	-	+	+	-

Aq-Aqueous, Me-Methanol, EA-Ethyl acetate, PE-Petroleum ether

+ positive, - negative

Table 2 shows the percent of antioxidant activity of different extract of *Apple Ber* as the capability to scavenge DPPH free radical, as compared to the control, ascorbic acid (1 mg ml^{-1}). The table shows that the antioxidant activity increases in a dose dependent manner. Highest antioxidant activity was found with methanolic extract, followed by ethyl acetate extract. Aqueous and petroleum ether extracts showed marginal antioxidant activities. As shown in Fig 1, the methanolic extract, equivalent to 600 µg equivalent dry weight was able to produce the same effect as was with ascorbic acid with 1 mg ml^{-1} concentration. Higher amount of equivalent dry weight produced higher antioxidant activities against DPPH free radical.

Table 2: Antioxidant activity of fruit of *Apple Ber* (hybrid *Ziziphus mauritiana*), when extracted with different solvents, as the capability to scavenge DPPH free radical, as compared to the control, ascorbic acid (1 mg ml^{-1}).

#	Sample	% antioxidant activity in different extracts			
		Aq	Me	EA	PE
1.	200 µg dry matter	12.4	56.8	45.3	9.2
2.	400 µg dry matter	18.9	73.9	54.6	11.2
3.	600 µg dry matter	24.2	87.6	67.4	13.6
4.	800 µg dry matter	27.2	96.2	81.9	17.2
5.	1000 µg dry matter	28.5	97.2	88.0	18.9

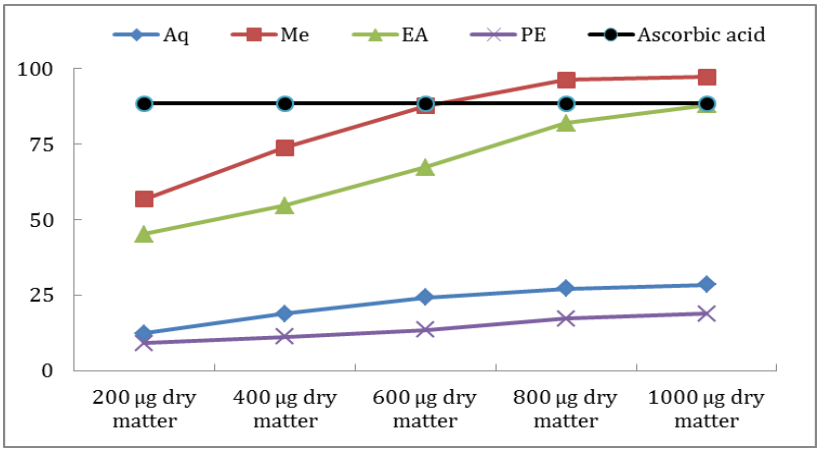

Fig 1: DPPH scavenging activity (%) of ascorbic acid and different concentrations of fruit of hybrid *Z. mauritiana* extracted with different solvents.

DISCUSSION

It is now well accepted that increasing individual fruit and vegetable consumption up to 600 g per day (the baseline of choice) can reduce the total worldwide burden of disease. In fruits, polyphenols constitute the main bioactive phytochemicals that have been proven effective in the prevention of certain chronic diseases such as coronary heart diseases, cancers and diabetes (Asami et al., 2003), because of their free radical-scavenging activities. Our study also shows the presence of polyphenolic phytochemicals, i.e. flavonoids, tannins and triterpenes. Consumption of fruit is known to provide a wide variety of flavonoids, which play a protective role by reducing the risk for cancer and cardiovascular diseases (Hollman et al., 1996). The present study showed the presence of flavonoids in aqueous and methanolic extract of hybrid *Ziziphus mauritiana.* The antioxidant properties of *Ziziphus maauritiana* wild type are well known (Lamien-Meda et al., 2008).

The native fruit of *Ziziphus mauritiana* contains good amount of sugars, organic acids and polyphenols (Muchuweti et al., 2005), and is used for various ailments traditionally. A hybrid fruit is created when plant breeders intentionally cross-pollinate two different varieties of a plant, aiming to produce an offspring, or hybrid, that contains the best traits of each of the parents. These are legitimate, interesting varietals that taste good and offer beneficial dietary nutrients. *Apple Ber* is a hybrid *Ziziphus mauritiana* fruit, which was aimed to improve taste, and the shelf life of the fruit. Since, no data on the phytochemical properties and hence the medicinal use of these fruits is available, it is necessary to evaluate these parameters, as these fruits are making their market rapidly, and soon the native variety of Indian zuzube (Ber) will be thrown out of the market. The present study shows that these hybrid fruits have enough quantity of antioxidants and will be able to counter many diseases related to reactive oxygen species.

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