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Original Article

Phytochemical evaluation of *Aegle marmelos* (L.) Correa (Bel) young plant parts for medicinal use

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ABSTRACT

Aegle marmelos commonly known as Bilva or Bel is an important medicinal tree being used in several herbal formulations. Bel root is an important ingredient of a very important Ayurvedic formulation " Dashmoola " (ten roots). The demand of the Bel roots is very high and increasing day by day. Moreover, there is a huge gap between demand and supply. In the present study, phytochemical evaluation of young Bel plants (roots and shoots) collected from central India was carried out with the objective to assess the suitability as raw drug. The suitability of alternative plant parts in place of root bark was also evaluated. This is the first attempt to assess the suitability of young roots obtained from high density Bel plantations with respect to their phytochemical constituents. The result revealed that the root bark of *A. marmelos* possess maximum number of phenolic compounds and alkaloid content in comparison to other plant parts. However, stem bark also contains almost similar number of chemical constituents and may substitute root bark.

Keywords: *Aegle marmelos*, Dashmoola, Plant parts, Chemical analysis, Substitute.

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INTRODUCTION

Aegle marmelos (L.) Correa ex Roxb. commonly known as Bilwa or Bel, belonging to the family Rutaceae, is used in various system of medicine, especially Ayurveda, Unani and Homeopathy. Every part of the tree has medicinal properties. In India it grows throughout the outer Himalayan regions, deciduous forests and south Indian plateau with altitude ranging from 210 to 1260 m [1]. *A. marmelos* is medium sized tree, up to 12 to 15 m tall with short trunk, thick, soft, flaking bark and spreading spiny branches, the lower ones drooping [2]. Leaf, fruit, root and decoction of the bark have been used in traditional medicine system for the treatment of various diseases [3].

A. marmelos is a slow growing species and takes many years to grow a matured tree. To obtain root a plant has to be uprooted entirely, thus destroying the plant forever. In addition, Red Data List of Indian Plants puts *A. marmelos* in vulnerable threat status [4]. There are many possible approaches to solve this problem like establishment of high-density plantations, utilization of roots of younger plants and use of alternative parts of the same plant such as aerial parts in place of underground parts. Possibilities of substitution of underground parts with aerial parts as a strategy for conservation of medicinal plants has been studied by researchers in *Eucomis autumnalis, Siphonochilus aethiopicus, Ocotea bullata, Warburgia salutaris, pelargonium sidoides* [5,6]. There is a greater need to discover suitable substitutes for roots. Furthermore, there is urgent need to establish high density Bel plantations for commercial production of raw material sustainably and to assess the suitability of young roots as potential raw drug.

A. marmelos roots are sweet, astringent, bitter and antipyretic. They are useful in dyspepsia, dysentery, diarrhoea, especially for patients having diarrhoea alternating with spells of constipation, stomachalgia, cardiopalmus, uropathy, gastric irritability in infants, vomiting, intermittent fever, vitiated conditions of vata, seminal weakness, and swellings [7-9]. The decoction of the root and root bark is useful in intermittent fever, hypo-chondriasis, and palpitation of the heart [7]. Anti-inflammatory activity of bel roots was evaluated by Benni et al., 2011[10]. The leaves of Bel fruit are astringent, febrifuge, laxative and expectorant, and are useful in inflammations, ophthalmia, deafness, catarrh, diabetes and asthmatic complaints [11]. The leaves and bark have been used in medicated enema. The unripe Bel fruits are bitter, acrid, sour, astringent, digestive and stomachic, and are useful in diarrhoea, dysentery and stomachalgia and helps in improving appetite and digestion [12,13]. Pitre and Srivastava (1987) demonstrated the antifungal activity of ethanolic root extract against *Aspergillus fumiganus* and Trichphyton *mentagrophytes*[14]. The ripe Bael fruits are astringent, sweet, aromatic, cooling, febrifuge, laxative and tonic, and are good for the heart and brain and in dyspepsia. Sweet drinks (Sharbats) prepared from the pulp of the fruits are useful as soothing agents for intestines of patients who have just recovered from bacillary dysentery.

Many promising Ayurvedic formulations e.g., Dashmoolarishta, Dassmulakwath, Bilvadileha, Bilvadi Yoga, Brihat Panchamoola consist Bel roots as one of the ingredient [15,16]. Dashamoolarishta is popularly regarded as immune modulator and general restorative tonic in geriatrics and for women having problem with conception and pregnancy [17].

Extensive investigations have been carried out on *A. marmelos* and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, flavanoids, fatty acids and aminoacids have been isolated from its different parts [16,18]. The available literature on the chemical constituents of *A. marmelos* appears to be very exciting. However, very less information is available on the chemical constituents of roots. It is one of important ingredient of very important Ayurvedic preparation Dashmoola. Despite wide spread uses of *A. marmelos* in folklore and classical formulations in India, the literature contain sparse information on its chemical constituents in root and shoot parts.

Considering the above facts, phytochemical evaluation of the roots and shoots of *A. marmelos* collected from high density plantation established in central India was carried out with the objective to assess the suitability of young roots as raw drug in herbal formulations. Different plant parts like root bark, root wood, stem bark and stem wood were also analysed with the aim to find out alternative plant parts in place of roots.

MATERIALS AND METHODS

Whole plants of *A. marmelos* were uprooted from the plantations established in Nasik, Maharashtra, India. The uprooted plants (three to four years old) were washed with water to remove soil, shade dried and powdered in a homogenizer. Before making powder shoot and root portions were separated. Growth attributes of uprooted plants like height, collar diameter was measured in field. Fresh and dry weights of different plant parts were taken to determine the moisture content. The powdered material was taken for chemical analysis.

Total phenols, flavonoids, tannins, cardiac glycosides and alkaloid contents were estimated from different plant parts of *A. marmelos.* Total phenol was estimated by Folin-Ciocalteau method [19], tannin by Folin-Denis method [20], cardiac glycosides by gravimetric method [21], alkaloids by 1, 10 phenanthrolene method and flavonoids by aluminium chloride colorimetric method [22].

Estimation of total phenols: The samples were extracted by grinding 0.5 g of powdered material in 80% ethanol and the homogenate was centrifuged. The supernatant was evaporated to dryness and residue was dissolved in a known volume of distilled water. The extract (0.2ml) and catechol (standard phenolic compound) were taken; volume made upto 3 ml, mixed with Folin Ciocalteau reagent 0.5 ml and left for 3 minutes. 3 ml of 20% Na_2CO_3 was then added to the mixture. The mixtures were kept in water bath for exactly 1 minute and the total phenols were determined by measuring the absorbance at 650 nm against blank. The standard curve was prepared using range of 0 - 2 ml of 100µg /ml solutions of catechol in distilled water. The concentration of phenols (g/100g) present in the sample was then calculated from the standard curve.

Estimation of flavonoids: 1 g sample was taken in 25 ml of 95% ethanol and left for 24 hrs at 37˚C, filtered and filtrate was adjusted to 25 ml with 80% ethanol. 0.5 ml of extract was separately mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by using quercetin. The concentration of flavonoids (g/100g) present in the sample was then calculated using the standard curve.

Estimation of tannins: 0.5 g of the powdered plant material was taken in a 250 ml conical flask and 75 ml distilled water was added in it. The solution was boiled for 30 minutes, cooled, centrifuged at 2000 rpm for 20 minutes and filtered. The supernatant was collected and volume was made up to 100 ml. 1 ml of sample extract was transferred to 100 ml volumetric flask containing 75 ml of distilled water. To this 5 ml of Folin-Denis reagent, 10 ml of 35% sodium carbonate solution was added and made to 100 ml with distilled water. The solution was shaken well and the absorbance was read at 700 nm after 30 minutes. Blank was prepared with water. The tannin content of the samples was calculated from the standard graph and represented as tannic acid equivalent.

Estimation of total alkaloids: 100 mg of sample was dissolved in 20 ml 80 % ethanol and was kept overnight. Then sample was centrifuged at 1000 rpm for 20 min. 2 ml of 0.025 M ferric chloride ($FeCl_3$) and 2 ml of 0.05 M of 1,10 phenanthrolene was added in 2 ml of resultant supernatant solution. Then the mixture was kept on water bath at 70°C for 30 minutes. The absorbance of red colour complex was measured at 510 nm against reagent blank. Alkaloid contents were measured and calculated with the help of standard curve of colchicine.

Estimation of cardiac glycosides: 5 gm of sample was taken in 100 ml distilled water. 10 ml conc. H_2SO_4 (prediluted with 10 ml H_2O) was added. It was then refluxed for 6-8 hours. Cooled and extracted with chloroform (2x25ml). The chloroform layer was then washed with distill water till it is acid free. Transferred to a pre weighed beaker and dried in an oven to a constant weight. Percentage of cardiac glycoside was calculated from the following formula:

$$\text{Percentage of cardiac glycoside} = \frac{(B-A) \times 100 \times 2}{\text{Weight of sample}}$$

Where,
B = weight of beaker with content
A = weight of empty beaker

Statistical analysis: All the samples were analyzed in triplicates. Data are expressed as means ± SD. Data were subjected to statistical analysis using SPSS (Version 14.0) analytical software.

RESULTS AND DISCUSSION

Data on morphological traits were recorded from the harvested *A. marmelos* plants and presented in Table 1. The shoot length of the harvested plants ranged from 46- 83 cm. The collar diameter of shoot ranged from 6-10 cm. Root length of harvested plants ranged from 26-43 cm and collar diameter of roots varied from 5 – 9 cm. Fresh shoot biomass varied from 40.5 g to 208.0 g. However, fresh root biomass ranged from 46.0 g to 266. 50 g. Variation in root and shoot biomass indicates variation in the growth of plants. Shoot moisture content ranged from 14.18 to 38.27 percent. However, root moisture content varied from 58.70 to 61.29 %. There is not much difference in the moisture content of different diameter class roots of young *A. marmelos* plants.

The results of phytochemical analysis of *A. marmelos* are depicted in Table 2. The study revealed that the concentration of phenols (0.85%), flavonoids (0.09%), tannins (0.36%), alkaloids (0.34%) and cardiac glycosides (1.94%) was higher

in root bark samples of *A. marmelos*. However, in root wood samples total phenol (0.82 %), total flavanoids (0.03%), tannins (0.19%), alkaloid (0.18%) and cardiac glycosides (1.56%) content were lower in comparison to root bark. Further, concentration of total phenol (0.62%), total flavanoids (0.09%), tannins (0.02%), alkaloid (0.30%) and cardiac glycosides (1.39%) in stem bark samples were almost at par with the root bark. Findings of the study revealed that Bel shoot bark can be used as a substitute for Bel roots in certain conditions.

Root and shoot bark samples of *A. marmelos* possess higher amount of analyzed phytoconstituents e.g., phenols, flavanoids, tannins, cardiac glycosides and alkaloid in comparison to other plant parts like root wood and stem wood. On the basis of phytochemical contents, stem bark can be used as substitute of root bark. Our findings are corroborated by Sulaiman and Balachandran (2013). They suggested that there are possibilities for substitution of *A. marmelos* roots with other plant parts on the basis of phytochemical analysis [23]. They suggested that roots can be substituted by stem.

Parmar et al., (2014) reported that *A. marmelos* root sample showed higher diuretic activity in Albino rats in comparison to leaves. Since leaves also showed diuretic activity, hence leaves in higher concentration may replace roots in Dashamoola [24]. Although, it was based on the diuretic activity of Bel roots and leaves. They did not evaluate variation in chemical constituents between roots and leaves. However, it also supports use of alternative plant parts. Pandey and Mandal, (2012) developed not destructive harvesting practices of *Terminalia arjuna* and *Litsea glutinosa* bark and also studied utilization of alternative plant parts[25]. They reported that bark of main branches of *T. arjuna* can be utilized as a substitute of trunk bark as it possesses similar phytoconstituents [25].

In the present study, as all the samples were collected from the high density plantations of bel established at Nasik, Maharashtra and age of the collected bel plants was between three to four years. The amount of chemical constituents may increase with the age and maturity of the plant as it was studied by Pandey et al. (2011) [26]. Jena et al., 2017 have also suggested substitution of roots with other renewable plant parts of *Premna latifolia* (another Dasmoola species) may be a possible strategy for conservation of important species [27]. A careful look at the results obtained in the present investigations clearly indicates that the stem bark and root are comparable chemically and the root may be safely substituted or replaced with the stem bark on the basis of similarities in phyto constituents. However, detailed pharmacological studies are required to validate the findings. Further clinical studies have to be carried out to prove the above said claims.

CONCLUSION

A. marmelos root is one of the ingredients of a well known ayurvedic formulation, Dashmoola. The demand of its roots is very high in ayurvedic industries. However, the availability is less due to decrease in population. There is a huge gap between demand and supply of this important species. Considering the demand of the species, the high density plantations of this species were established in Nasik, Maharashtra for production of quality and genuine raw material on sustainable basis. Results of phytochemical analysis revealed the presence of important phyto constituents such as phenols, tannins, flavonoids, alkaloids and cardiac glycosides. Bel roots and shoots contain almost all analysed phyto-constituents but there is variation in their concentration. However, *A. marmelos* root bark contains highest quantity in comparison to other plant parts but stem bark contains almost similar amount of phytoconstituents. On the basis of findings of the study, young Bel roots may be used as raw drug for preparation of herbal formulations. Further, Bel stem bark may be substituted for Bel root individually as well as in Dashamoola preparation. Keeping this in mind, it is suggested to prefer *A. marmelos* young roots instead of roots obtained from matured trees. Further, utilization of shoot bark instead of Bel root or root bark will facilitate prevention of destruction of whole tree. The study suggests that utilization of young roots and substitution of root bark with other plant parts will lead to conservation of valuable resource vis-a-vis provide raw material on sustainable basis.

Conflict of interest: The authors declare that no conflict of interests existed in the organization, results, presentation and the finance of the article.

Table 1. Morphological attributes of different plant parts of Bel (*Aegle marmelos)*

S. No.	Shoot (cm)		Root (cm)		Fresh weight (g)		Dry weight (g)		Moisture (%)	
	Length	Collar dia	Length	Collar dia	Shoot	Root	Shoot	Root	Shoot	Root
1	83	10	43	9	208.0	266.5	178.5	108.5	14.18	59.28
2	46	7	26	5	40.5	46	25.0	19.0	38.27	58.70
3	66	6	41	6	60.5	62	38.0	24.0	37.19	61.29

Table 2. Phytochemical analysis of different plant parts of Bel (*Aegle marmelos)*

S. No.	Sample Name	Total Phenol %	Total Flavonoid %	Tannins %	Alkaloids %	Cardiac glycosides %
1.	Root Bark	0.85± 0.05	0.09± 0.003	0.36± 0.05	0.34± 0.03	1.94± 0.10
2.	Root wood	0.82± 0.09	0.03± 0.001	0.19± 0.03	0.18± 0.02	1.56± 0.08
3.	Stem bark	0.62± 0.04	0.09± 0.003	0.02± 0.002	0.30± 0.04	1.39± 0.09
4.	Stem wood	0.52± 0.04	0.06± 0.002	0.02± 0.002	0.21± 0.03	1.57± 0.09

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