

Original article

***In vitro* antioxidant activity and quantitative elemental analysis of *Adansonia digitata* L. fruit using inductively coupled plasma optical emission spectroscopy**

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Abstract

Adansonia digitata L.(AD) belongs to the family Malvaceae and genus *Adansonia*, native to the African continent. Various parts of this tree are used for health benefits as well as for food supplements. The dried fruits of AD were taken for the present study. Preliminary phytochemical screening of the different extracts of the fruits showed the presence of various secondary metabolites such as tannins, terpenoids, alkaloids, cardiac glycosides, phenolics, saponins, flavonoids and steroids. Since the fruits are rich source of vitamin C, the antioxidant screening of ethyl acetate and methanol extract were done by three different methods. 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), radical scavenging assays showed excellent antioxidant activity for the fruit of AD. The elemental analysis of the fruits was done by using ICP-OES and the results revealed the presence of various macronutrients (calcium, potassium, magnesium, phosphorus)and micronutrients (iron, sodium, manganese, zinc) in very good amount. The present study reveals that AD fruit can be used as a good source of antioxidant and in mineral deficiency ailments.

Key words: *Adansonia digitata* L.(AD), phytochemical screening, secondary metabolites, antioxidant, ICP-OES, micronutrients

1. Introduction

Adansonia digitata L. trees (family-Malvaceae, genus-*Adansonia*), generally known as "baobab" are indigenous to African countries. AD trees are deciduous, huge, royal trees up to 25 m high, which has a life expectancy of several years (Baum, 1995).The branches of this tree are thick and wide spreading, and the trunk is little firm. This tree has different types of stems.The young tree has a pointed stem whereas the matured tree has cylindrical or bottle shaped stem (Sidibe *et al.*, 1996). The bark is fibrous, flat and brownish to grey in color. The leaves appear alternative at branches or on the trunk. The blossoms are pendulous and delivered during both wet and dry seasons. Baobab fruits are found to have different shapes often, globose or ovoid (Sidibe and Williams, 2002).

The baobab contains a fleshy pulp and big seeds enclosed inside the shell. The natives make soup with the leaves and the pulp for the drinks and for the food preparation (Yazzie *et al.*, 1994; Obizoba and Amaechi, 1993). The baobab fruit is otherwise known as "wonder fruit" due to the high nutritional values, enriched with

vitamins, fatty acid and minerals (Gruenwald, 2009). High amount of ascorbic acid and dietary fiber are present in the baobab products (Vertuani, 2002). The fruits contain seven to ten times more vitamin C (280-300 mg/100 g) than the oranges (51 mg/100 g) (Manfredini *et al.*, 2002; Täufel *et al.*, 1993). Some studies reveal that the intake of 40 g of AD fruits meets the optional daily requirements of ascorbic acid in the expecting women (19-30 years) (Chadare *et al.*, 2009; Diop *et al.*, 1998; Eromosele *et al.*, 1991; Gebauer *et al.*, 2002).

The AD seeds are identical and entrenched in the pulp. The color of the seeds varies from dark brown to reddish black. The fresh, dry or crushed kernels are eaten as such by the locals and they used in cooking also. The kernels are high energy giving food when compared to leaves (Chadare *et al.*, 2010). Many minerals such as phosphorus, calcium and magnesium are present in the seeds. Broiled seeds can be used instead of peanut as side dish and fermented baobab seeds can be used in flavoring soups (Addy and Eteshola, 1984). The oil which is produced from the seeds by pressing it is an important sources of nutritional oils and has been used in pharmaceutical industry (Nzikou *et al.*, 2010).

AD tree contains different classes of secondary metabolites. These secondary metabolites are identified from various parts of the tree such as leaves, fruit pulp, seeds and from the roots. Terpenoids, lipids, steroids, amino acids, carbohydrates, vitamins and flavonoids are some of the classes of secondary metabolites which has been isolated from various parts of this tree. The seed oil contains

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tocopherol, campesterol, cholesterol, stigmasterol and isofucosterol. The flavonoid epicatechin also has been isolated from baobab fruit. It is known to possess antioxidant activity (Chauhan *et al.*, 1984; Chauhan *et al.*, 1987; Shukla *et al.*, 2001). Some aromatic compounds such as isopropyl myristate and nonanal are also has been identified using GC-MS from the fruit pulp (Cisse *et al.*, 2009). Various reports show that the fruit pulp contains various amino acids (Glew *et al.*, 1997).

Adansonin is an alkaloid which is reported to be found in the bark. It has strophanthus like action. Due to this, the bark has been used as an antidote to strophanthus poisoning (Watt and Breyer-Brandwijk, 1962). The local people use the seeds for diarrhoea and hiccoughs. The seed oil has been used to treat swollen gums, and also used to relieve toothache and skin problems. The leaves have varied usages such as diaphoretic, expectorant, prophylactic against fever and astringent. It is reported that the leaves possess anti-asthmatic, and antihistamine properties. The leaves are also used for the treatment of inflammations, general fatigue, diarrhoea, kidney and urinary bladder diseases, worm infestations and insect bites (Bosch *et al.*, 2004; Wickens, 1982; Burkil, 1985).

The AD seed oil has protecting, moisturizing and soothing action (Wren and Stucki, 2003). It is reported that the leaves of this tree has anti-inflammatory, insecticidal and antipyretic activities (Kamatou *et al.*, 2011).

Compounds which can delay or prevent the oxidation process are called antioxidants. These compounds can repair the defense mechanism against the attack of the free radicals. The present study involves the antioxidant screening of AD fruits since it is reported that the fruits are rich with vitamin C (Diarra, 2006).

Some fruits are marketed as super fruits due to the presence of wide variety of nutrients. And because of this, they will have very good health effects. Baobab fruit is also called as a super fruit since it contains many nutrients (Sena, 1998). In this regard, the present study involves the elemental analysis of the AD fruits to evaluate the micronutrients present in it. Inductively coupled plasma-optical emission spectrometry (ICP-OES Model-Spectrogenesis) is used for the elemental analysis in this study. The excitation temperature of argon in ICP is very high, *i.e.*; 5000 to 7000 K, whereas the excitation temperature of air-acetylene flame in atomic absorption spectrometers is 2000 to 3000 K. Due to the high temperature, many elements gets excited and can be analyzed. It has high speed also when compared to atomic absorption spectroscopy (AAS).

So the present work is aimed to assess the antioxidant and ICP-OES analysis of the fruits of AD.

2. Materials and Methods

The AD fruits were collected from Sudan during the Autumn season (Kordofan, Sudan). The fruits were identified and authenticated by the Municipality of Al Abyad, Sudan. The dried fruits were powdered along with the seed and pulp. 50 g of the powdered fruit material was extracted by ultra-sonication method, using different solvents with increasing polarity such as ethyl acetate and methanol. The ethyl acetate and methanol extracts were collected (2 g each) and evaluated further.

2.1 Preliminary phytochemical evaluation

The two different extracts of the fruits were evaluated for the presence of tannins, saponins, cardiac glycosides, terpenoids, flavonoids, alkaloids and steroids using standard protocol (Sofowora, 1993; Chirag *et al.*, 2018).

2.1.1 Presence of alkaloids (Mayer's test)

1 ml of various fruit extracts were heated along with 1% aqueous hydrochloric acid (5 ml) and then filtered. To the Mayer's reagent, this filtrate was added. Production of cream colored precipitate indicates a positive result.

2.1.2 Presence of tannins

All the solvent free fruit extracts were treated with 1 ml of 5% FeCl₃ solution. Development of bluish black precipitate indicates a positive result for tannins.

2.1.3 Presence of cardiac glycosides (Keller - Killani test)

1 ml of FeCl₃ solution was added to two milliliters of 99% acetic acid. This has been added to various fruit extracts. 1 ml of concentrated sulphuric acid (98%) was added through the sides without disturbing the reaction mixture. A brown ring formed between the two layers of liquid indicates a positive result.

2.1.4 Presence of saponins (Froth test)

Dilute the fruit extracts with 20 ml of distilled water and shaken it for 15 min. If the froth produced is stable for few min, it indicates the presence of saponins.

2.1.5 Presence of flavonoids

3 ml of 1% aluminum chloride solution was added to 5 ml of various fruit extracts. Development of yellow colored solution indicates the occurrence of flavonoids. To this mixture, 5 ml of dilute ammonia solution and 3 ml of concentrated sulphuric acid (98%) were added. If the yellow color disappears in few minutes indicates a positive result.

2.1.6 Presence of terpenoids (Salkowski test)

5 ml of various fruit extracts were treated with 2 ml of chloroform. 3 ml of sulphuric acid (98 %) was added slowly without disturbing the solution. A positive result shows the reddish brown coloration between the two layers.

2.1.7 Presence of steroids

Various fruit extracts were treated with acetic anhydride. To this solution, sulphuric acid (98%) 2 ml was added. If a blue color develops from light purple color indicates the occurrence of steroids.

2.1.8 Presence of phenolics

2 ml of various fruit extracts were treated with 5 ml of neutral ferric chloride. Appearance of bluish green color shows the occurrence of phenolic compound.

2.2 *In vitro* antioxidant screening

The radical scavenging activity of the different fruit extracts were done by various spectrophotometric methods. When a radical reacts with an antioxidant molecule that is capable to give a hydrogen atom, those reactions can be tested by spectrophotometric methods. Ascorbic acid was used as the standard antioxidant.

2.3 Antioxidant screening by DPPH* assay

For the DPPH* assay, a modified method was used to evaluate the free radical scavenging activity of various extracts (Wali *et al.*, 2015). 0.1 mM DPPH solution was prepared in methanol and stored in dark at 4°C. Aliquots of different concentrations of the fruit extracts (20-100 µg/ml) were taken and 2.96 ml of DPPH solution was added. All the samples were mixed carefully and kept in the dark for 15-20 min at room temperature until the readings were taken. The absorbance of all the samples were taken at 517 nm, using the UV-Spectrophotometer (Shimadzu, UV-1800). 3 ml of DPPH solution was taken as the blank. All the readings were taken in multiple times (in triplicate). The radical scavenging activities of all the extracts of the AD fruits were tested and the results were expressed as percentage inhibition of DPPH*. The percentage of radical scavenging activity of the fruit extracts and the standard were calculated using the below formula:

$$\text{Percentage radical scavenging effect of the sample} = \left\{ \frac{AC - AS}{AC} \right\} \times 100$$

where AC is the absorbance of DPPH* without sample and AS is the absorbance of the DPPH* with the extracts/standard.

2.4 Antioxidant screening by ABTS Assay

The reaction based on the reduction of absorbance of ABTS* radical cation with the sample was applied to antioxidant determination of AD fruit extracts (Lee *et al.*, 2010). This was measured spectrophotometrically. 7 mM ABTS solution and 2.45 mM potassium persulphate solution were prepared in distilled water. These solutions were mixed in 1:1 ratio. When ABTS reacts with potassium persulfate, ABTS⁺ cation radical is produced. This mixture was stored in dark for 12 h at room temperature before use. Methanol was used to adjust the absorbance of ABTS⁺ solution to 0.700 at 734 nm. Various concentrations of the fruit extracts (20-100 µg/ml) were mixed with 3.995 ml of diluted ABTS⁺ solution and kept for 20 min before taking the reading. The absorbance was measured at 734 nm. 3 ml of the dilute ABTS⁺ solution was used as the blank. All the readings were taken in multiple times (in triplicates).

The percent ABTS⁺ scavenging effect of the extracts at 734 nm was calculated using the formula given below:

$$\text{The percentage ABTS}^+ \text{ scavenging activity} = \left\{ \frac{AC - AS}{AC} \right\} \times 100$$

AC is the absorbance of ABTS without sample and AS is the absorbance of the ABTS with the extracts/standard.

2.5 Antioxidant screening by hydrogen peroxide assay

The radical scavenging activity of AD fruit extracts towards hydrogen peroxide radicals were determined using a modified method (Mathew, 2006; Kuntal Das *et al.*, 2017). 40 mM hydrogen peroxide solution was used for screening the antioxidant activity of the fruit extracts. Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer with a pH of 7.4 and stored until the use. 0.6 ml hydrogen peroxide solution was added to different concentrations of the fruit extracts (20-100 µg/ml) and to the standard. The absorbance of the extracts and the standard were measured at 230

nm. Phosphate buffer (pH 7.4) without hydrogen peroxide was used as the blank. All the measurements were taken in triplicates.

The percentage radical scavenging activity of AD fruit extracts and standard compounds towards hydrogen peroxide radicals were calculated using the below formula:

$$\text{The \% scavenging activity of hydrogen peroxide} = \left[\frac{AC - AS}{AC} \right] \times 100$$

AC is the absorbance of the phosphate buffer (blank) and AS is the absorbance in the presence of the fruit extracts or standards.

3. Statistical analysis

The experimental results were performed in triplicates and all data were expressed in mean \pm S.E.

3.1 Elemental analysis of the fruits using ICP-OES

The elemental analysis of the fruits of AD were carried out using ICP-OES. This is the ideal method for the elemental analysis. This method uses plasma and a spectrometer to analyze the sample for the presence of elements. The plasma is generated when the argon gas gets ionized. The sample solution which was in an atomized stage was introduced in to the plasma using the torch tube. When the plasma energy is applied to the sample for analysis, the elements or atoms gets excited. Certain rays are emitted, when the excited atoms return to the lower energy level. These rays are corresponding to the photon wavelength which were measured. Depending on the position of the photon rays, the type of the element can be determined. The intensity of the rays gives the idea about the content of the element.

The ICP-OES operating parameters were as follows: The polychromatic-thermally stabilized to +30°C \pm 0.5°C, wavelength range 175-177 nm. The detector was fitted in continuous order and was 2048 pixels per array. The UV system was optimized for low pure rates and argon flow rate was 0-1.01/min. 27.12 MHz was the RF-generator frequency. The power output used was 0.7 to 1.7 kW. The dimensions were, spectrometer 870 x 1165 x 748 mm (34.3 x 45.0 x 29.5 inches). The weight of spectrometer was approximately 145 kg. Environmental conditions: Room temperature was 5-35°C, relative humidity was 5-35°C non-condensing. The argon supply of the instrument was grade \geq 4.6 (99.996%). The pressure was 7.5 bar (109 psi).

3.2 Digestion procedure

2 g of powdered fruit sample was taken in a glass beaker. To it, 3 ml concentrated nitric acid was added and heated on a hot plate to digest the organic matter. Cooled the solution when it was clear. 1 ml of hydrochloric acid was added to the cooled solution. It was then heated on a hot plate, cooled and filtered to a volumetric flask and made up to the volume with distilled water.

3.3 Method

The dried fruits of AD were analyzed for macro and micronutrients by using ICP-OES. Appropriate dilution was made with respect to the calibration range used. The solutions were introduced in to the ICP by free aspiration. By comparing emission intensities of elements in the test specimen with the prepared standards, the concentrations of the elements were calculated and reported.

4. Results

4.1 Preliminary phytochemical evaluation

The results of the preliminary phytochemical evaluation of the different fruit extracts of AD showed in Table 1. Various secondary metabolites are present in the fruit of AD. Tannins, terpenoids, alkaloids, cardiac glycosides, phenolics, saponins, flavonoids and steroids were present in the methanol extract of the fruit. Terpenoids, flavonoids, alkaloids, cardiac glycosides, phenolics and steroids were present in the ethyl acetate extract of the fruit. The presence of these secondary metabolites make the fruit biologically active for various ailments. The antioxidant activities of this fruit is due to the presence of phenolics and flavonoids that are evident in the preliminary screening.

Table 1: Phytochemical screening of various extracts of *A. digitata*

Phytochemical screening tests	Methanol	Ethyl acetate
Tannins	+	-
Terpenoids	+	+
Flavonoids	+	+
Alkaloids	+	+
Cardiac glycosides	+	+
Phenolics	+	+
Saponins	+	-
Steroids	+	+

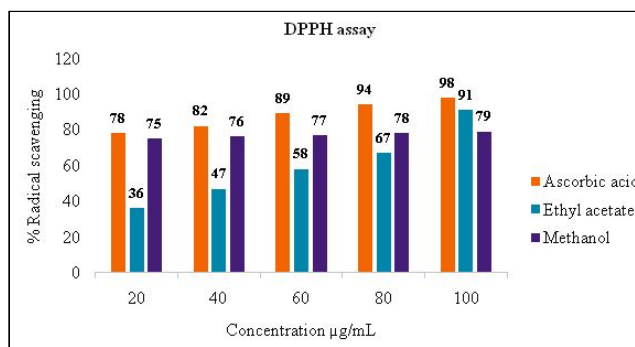
(+) indicates present and (-) indicates absent

4.2 In vitro antioxidant screening of the fruit extracts

Many techniques have been established for the screening of the anti-oxidant activity of plant extracts. In this study, the antioxidant activity of ethyl acetate and methanolic extracts of the fruits of AD were compared to ascorbic acid, a known anti-oxidant. The antioxidant activity of the different fruit extracts and ascorbic acid were evaluated by 3 types of *in vitro* tests such as DPPH* free radical scavenging, ABTS*+ radical scavenging and hydrogen peroxide scavenging assays.

4.3 Antioxidant screening by DPPH* assay

DPPH or 2,2-diphenyl-1-picrylhydrazyl is a nitrogen-based free radical which is stable and it is in powder form. DPPH has a violet color in its stable form. It changes the color to yellow when reduced by the process of electron-transfer or hydrogen-transfer. The compounds which are able to get reduced by this reaction are commonly called as radical scavengers. The maximum wavelength at which DPPH* free radical get reduced is at 517 nm. This free radical converts to a stable diamagnetic molecule by receiving an electron or hydrogen from antioxidant molecules. Those plants having antioxidant activity react with DPPH and get reduced to DPPH-H that results in the diminutions of absorbance. The radical scavenging activity of the fruit extracts or compounds is indicated by the degree of discoloration in terms of hydrogen donating ability (Soares *et al.*, 1997; Gireesha and Raju, 2016). The antioxidant activity of different concentrations of ethyl acetate and methanol extracts of AD fruits and ascorbic acid are demonstrated in Figure 1. Maximum radical scavenging was 79% shown by the methanol extract with 100 µg/ml concentration. Both ethyl acetate and methanol extracts showed good radical scavenging effect when compared with the standard which was ascorbic acid.

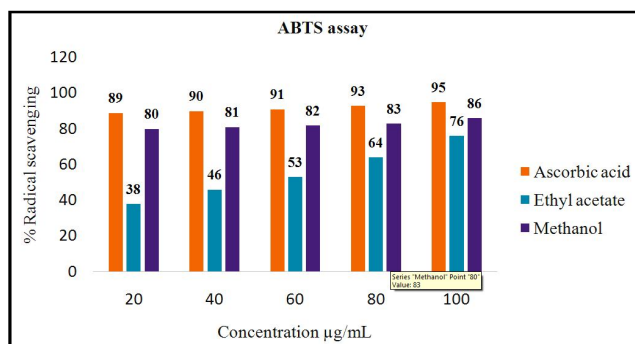


Values are the means of three independent readings

Figure 1: DPPH assay of various extracts of *A. digitata*.

4.4 Antioxidant screening by ABTS assay

ABTS or 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) can act as an electron donor for the reduction. The nitrogen atom of ABTS loses an electron to form ABTS radical cation (ABTS*+) (Marc *et al.*, 2004; Naveen *et al.*, 2017). Potassium persulphate can oxidize ABTS and as a result ABTS cation radical is formed which is bluish green in color. The ABTS assay method depends on the ability of antioxidants to reduce the ABTS radical cation and to decolorize it. It was measured spectrophotometrically at 734 nm. The same procedure was repeated for the standard also. As in Figure 2, the two extracts of the fruits of AD shows an effective ABTS*+ radical scavenging activity when compared with that of ascorbic acid which was the standard. Methanol extract showed maximum of 86% radical scavenging activity with 100 µg/ml concentration.

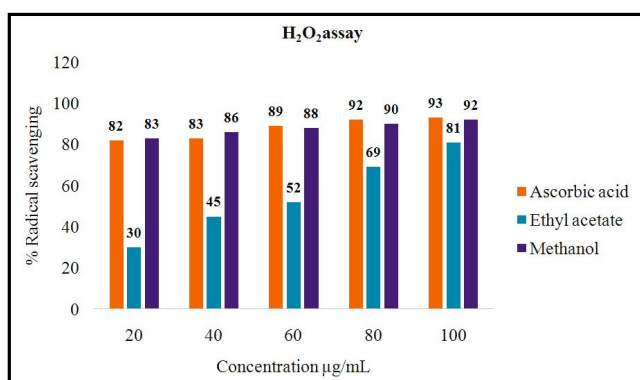


Values are the means of three independent readings

Figure 2: ABTS assay of various extracts of *A. digitata*.

4.5 Antioxidant screening by hydrogen peroxide assay

Hydrogen peroxide H_2O_2 is non-reactive but can be lethal to living cells sometimes. In the living cells, it can be converted to hydroxyl radical ($\bullet OH$) which is a free radical. Hydrogen peroxide reacts with biomolecules and can cause total tissue damage and finally cell death. A good antioxidant can remove the hydrogen peroxide and helps in the defense systems in cell or body systems (Halliwell, 1991). The antioxidant activity of the extracts of AD fruit on hydrogen peroxide and its comparison with ascorbic acid is presented in Figure 3. Ethyl acetate and methanolic extracts showed effective scavenging ability in a concentration dependent order. The methanol extract showed the maximum radical scavenging activity of 92% with 100 µg/ml concentration.



Values are the means of three independent readings

Figure 3: H₂O₂ assay of various extracts of *A. digitata*.

4.6 Elemental analysis of the fruits of AD using ICP-OES

All the plants and trees contain various macronutrients and micronutrients. These nutrients are responsible for the health benefits or the biological activities of these plants or trees. The micronutrients include essential and non-essential elements which are present in the tree. The dried fruit samples of AD were analyzed by ICP-OES. The relative standard deviation for the different samples were determined for the precision of the procedure. AD fruit contains various macro and micro nutrients. Calcium (8865 mg/kg) and potassium (11119 mg/kg) are the two main nutrients which are present in very good amount. The results of the macro and micro elemental analysis were shown in Table 2.

5. Discussion

From results obtained for the preliminary phytochemical screening (Figure 1), it was observed that the ethyl acetate and methanolic extracts of the fruits of AD contains phenolics and flavonoids. This portrays that the antioxidant activity of the fruit may be due to the presence of the secondary metabolites phenolics and flavonoids which has to be investigated further. It also showed the presence of terpenoids, steroids, alkaloids and cardiac glycosides and the effects has to be investigated further. Polar solvents such as methanol or ethanol can extract out maximum amount of phenolic content from the plant materials (Mohsen and Ammar, 2008). Phenolics and flavonoids are very important plant secondary metabolites which shows high radical scavenging activity due to the presence of hydroxyl group in their structure (Kuntal Das *et al.*, 2017; Gireesha and Raju, 2016). Flavonoids are the other important secondary metabolites which can facilitate the radical scavenging activity. This is due to the structure and substitution pattern of the hydroxyl group which is present in it (Chirag Modi *et al.*, 2018).

The antioxidant screening of both ethyl acetate and methanol extracts were carried out in three different methods. ABTS⁺ and DPPH* radicals are often used for the determination of the radical scavenging activity of the plant extracts because of their stability (Soares *et al.*, 1997; Gireesha and Raju, 2016). Hydrogen peroxide is not a very reactive compound as such, but due to the ability to produce free hydroxyl radicals in the tissues, it is considered as

toxic (Halliwell, 1991; Mathew, 2006). Therefore, removing these toxic materials from the tissue is very essential. Ascorbic acid was used as the standard scavenging agent. Ascorbic acid acts as an excellent antioxidant which damages the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine (Beyer, 1994).

The methanol extract of the fruits of AD showed very potential antioxidant activity when compared with the standard. (Figures 1, 2, 3). As shown in the figures the methanolic extract showed significant reducing power with increase in concentration. Methanol is more polar when compare to ethyl acetate. This can be the reason for the difference in the antioxidant activity of both the extracts.

Finding out the composition of the fruits are important for nutritional and toxicological studies (Dolan and Capar, 2002). The results of the elemental analysis by ICP-OES (Table 2) showed the presence of various micro and macro nutrients in the fruits of AD. The fruit is found to be very rich in calcium and potassium which is very essential for the health.

Table 2: Elemental analyses of *A. digitata* fruits by ICP-OES

Macro elements		
S. No.	Elements	Amount (mg/kg)
1	Calcium	8865
2	Chromium	4.0
3	Potassium	11119
4	Magnesium	2585
5	Phosphorus	5887.6
Micro elements		
	Elements	Amount (mg/kg)
1	Boron	15.2
2	Cobalt	<0.1
3	Copper	9.2
4	Iron	70.3
5	Manganese	8.1
6	Molybdenum	0.4
7	Sodium	402.1
8	Nickel	2.5
9	Vanadium	1.5
10	Zinc	17.5
11	Aluminum	56.3
Other elements		
1	Silver	0.5
2	Arsenic	<0.1
3	Barium	7.3
4	Beryllium	<0.1
5	Cadmium	0.3
6	Chromium	4.0
7	Lead	<0.1
8	Tin	<0.1
9	Strontium	29.5

6. Conclusion

In the present study, it is found that AD fruits contain various secondary metabolites including phenolics and flavonoids. These compounds are responsible for the antioxidant activity of the fruits of AD. All the results of antioxidant activity were compared with that of the ascorbic acid. The radical scavenging activity of these fruits were found to be almost similar to that of the standard. So this fruit can be used as an excellent antioxidant. The health benefits of the fruits are due to the presence of various macro and micronutrients which are present in the fruits in good quantity. This was estimated using ICP-OES. The fruit contains very high amount of potassium. This fruit can be used as a good source for potassium and calcium.

Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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