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Research Article

Phytochemical extraction and comparative analysis of antioxidant activities of *Areca catechu* L. nut extracts

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Abstract

FRAP assay proved all the extracts of *Areca catechu* L. nut have antioxidant properties because IC_{50} values of all the extracts of the same were less than that of ascorbic acid. Remaining antioxidant assays like DPPH radical scavenging assay, H_2O_2 scavenging assay, and Fe^{2+} chelating assay showed more antioxidant properties in ethyl acetate extract and nonpolar solvent extracts like n- hexane, and chloroform respectively. Antioxidant properties of *Areca catechu* L. nut varied depending upon the different solvent extract.

Keywords

Areca catechu L. nut, Antioxidant activity, Metal chelating, scavenging activity, Reducing power

Introduction

Plants are a good source for the discovery of various products of medicinal value for drug development. Nowadays several chemicals obtained from plants are important drugs used in different countries in the world (Amudhan et al. 2012). *Areca catechu* L. (Arecaceae), widely found in South and Southeast Asia, and it can be chewed to reduce accumulated fluid in the abdominal cavity and kill worms (Gallo et al. 2008). It is useful for the treatment of innumerable diseases (Penj et al. 2015). Ancient medicines like Ayurveda and Siddha used areca nut as one of the ingredients in oils for healing wounds by burning (Verma et al. 2012). It is one of the most commonly used drugs in the world that have anthelmintic, antibacterial, antifungal, anti-inflammatory, and antioxidant activities (Wang et al. 2001). The WHO has pointed out nearly 25 useful effects of *Areca catechu* and also reported that all the alkaloids present in it showed medicinal properties (Bhat et al. 2017).

Higher plants and their constituents provided a rich source of natural antioxidants (Shahidi et al. 2015). Antioxidants, which can inhibit the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very useful for the prevention of many diseases (Zhang et al. 2009). Measuring antioxidant properties of compounds required a suitable method that addresses the mechanism of antioxidants (Amorati et al. 2019). The present study aims to compare the antioxidant properties of different solvent extracts of *Areca catechu* L. nut (methanol, ethyl acetate, chloroform, toluene, and n-hexane) using FRAP assay, DPPH radical scavenging antioxidant assay, Hydrogen peroxide scavenging antioxidant assay, and Metal (Fe^{2+}) chelating antioxidant assay.

Materials and methods

Chemicals

Methanol, ethanol, ethyl acetate, chloroform, toluene, n- hexane, hydrogen peroxide, sodium phosphate, acetic acid, potassium dichromate, glacial acetic acid, DPPH, ascorbic acid, ferric chloride, ferrozine, EDTA, sodium acetate, TPTZ, ferrous sulphate, etc. are purchased from Eswarr Scientific and Co., Karumandapam, Trichy- 620 001.

Collection and authentification of plant materials

Healthy unripened Areca catechu L. nuts were collected from Kollam district of Kerala, India. It was dehusked and dried for three weeks. The dried seeds were powdered. The plant Areca catechu and *Areca catechu* L. nut were authenticated by JNTBGRI, Thiruvananthapuram, Pin 695 562, Kerala, India, and voucher specimens (Specimen Numbers TBGT/95955 & TBGT/95956) are deposited at the herbaria of the same research institute.

Method of extraction

Soxhlet extraction is a very useful method for extraction purposes especially in plant materials (Abubakar et al 2020). Soxhletation is a process of extraction conducted in a device called soxhlet (Syintia et al. 2019). The powdered *Areca catechu* L. nut was subjected to extraction using the soxhlet apparatus (Anajwala et al. 2010). Repeat the same with various solvents based on polarity for about 16–24 hours (Kalaivani et al. 2019). The Soxhlet extractor is placed onto a flask containing the extraction solvents (Hirondart et al. 2020). The Soxhlet is then equipped with a condenser was used to get the extracts used in this study (Gopalasatheeskumar 2018). The powdered *Areca catechu* L. nut (25 g) was made as a roll with quality tissue paper and placed in the thimble (Maria et al. 2012). The solvent was heated to reflux (Abdul et al. 2012). The solvents used in the extraction are methanol, n- hexane, ethyl acetate, toluene, and chloroform. The process was continued up to when the solution became colorless in the thimble (Naczk et al.2006). The flask was removed from the Soxhlet extractor and poured into 250 ml beaker and evaporated to get the different solvent extracts of *Areca catechu* L. nut (Naveen et al. 2010).

Antioxidant activity determination by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging

DPPH assay method is very simple and used to find the overall antioxidant capacity of the sample (Moniruzzaman et al. 2012). For the DPPH reagent, the concentration used was 2.5 mg/ml. One ml of ethanol solution was added to the test tubes followed by the addition of *Areca catechu* L. nut extracts, 0.1 ml of 25–100 µg /ml (1 mg/ml stock). The DPPH reagent was added to each tube in a dark environment. Then, it was wrapped with aluminum foil and incubated on the shaker at room temperature for 15 minutes. The absorbance was recorded at 517 nm. Ascorbic acid was used as the standard at the same concentration (Athavale et al. 2012).

$$\text{DPPH scavenging effect (\%)} = \left(1 - \frac{Ac}{As}\right) \cdot 100$$

Where A_c is the absorbance of the control which contains DPPH solution and A_S is the absorbance presence of different solvent extracts of *Areca catechu* L. nut (Gulcin et al. 2010). The percentage (%) inhibition of DPPH activity is used to find the change in absorbance between the sample and control. Ascorbic acid in ethanol was taken as a positive control and the assays were repeated for getting a triplicate of each sample concentration (Tuekaew et al. 2014).

Antioxidant activity determination by hydrogen peroxide scavenging

The ability of plant extract to scavenge hydrogen peroxide is determined by using the reaction mixture containing 0.5ml of H_2O_2 (1ml of 30% of H_2O_2 was made up to 45 ml with distilled water), 1 ml of sodium phosphate buffer (pH 7.4), and 0.4 ml water. 0.1ml of the sample (25–100 µg/ml), was added to start the reaction. 2 ml dichromate acetic acid reagent (Dichromate acetic acid -5% potassium dichromate with glacial acetic acid in ratio 1:3) was added after 1 min to stop the reaction. The tubes were heated for 10 minutes, cool and the green color appeared was read at 240 nm using a spectrophotometer. Extracts (25–100 µg/ml) in distilled water is mixed to H_2O_2 and absorbance at 340 nm is noted after 10 min against a blank solution missed with phosphate buffer without hydrogen peroxide (Alam et al. 2013).

$$H_2O_2 \text{ scavenging effect (\%)} = \left(1 - \frac{Ac}{As}\right) \cdot 100$$

Where A_C is the absorbance of the control and As is the absorbance of different solvent extracts of *Areca catechu* L. nut (Gulcin et al. 2010).

Antioxidant activity determination by ferrous ions (Fe^{2+}) chelating activity

The reaction mixture contains 1.0 ml of various concentrations of the herbal extract (25–100 µg /ml) and 0.05 ml of 2 mm $FeCl_2$. The reaction was carried out by the addition of 0.2 ml of 5 mm ferrozine. The reaction mixture was prepared and kept for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A smaller absorbance of the reaction mixture showed a higher ferrous ion chelating ability. All the reagents except the sample contained in the control. EDTA was used as a standard for comparison (Shahat et al. 2013).

$$\text{Ferrous ion } (Fe^{2+}) \text{ chelating effect (\%)} = \left(1 - \frac{Ac}{As}\right) \cdot 100$$

Where A_C is the absorbance of the control and A_S is the absorbance of different solvent extracts of *Areca catechu* L. nut (Gulcin et al. 2010).

Antioxidant activity determination by FRAP assay

By mixing 300 micromoles sodium acetate buffer (pH 3.6), 10.0 micromoles TPTZ (tripyridyltriazine) solution, and 20.0 micromoles $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume for making FRAP reagent. Samples at different concentrations (25 – 100 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured and compared with known standard ascorbic acid (Amin et al. 2013). Freshly prepared $FeSO_4$ was used for calibration. The antioxidant property based on the ability to reduce Fe^{3+} irons of the sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per microgram of the sample. Using the linear regression (y = mx + c), half-maximal inhibitory concentration (IC_{50}) was calculated (Irshad et al.2012). It is also obtained from logistic regression model as

$$Y = \frac{\text{Max}}{1 + \left(\frac{x}{IC_{50}}\right) \cdot \text{Hill coefficient}}$$

Statistical analysis

All assays were repeated for getting a triplicate and statistical analysis was done by ANOVA. The data were interpreted as mean ± SD.

Result and discussion

Four different in vitro methods have been set up to assess antioxidant properties of different solvent extracts (methanol, toluene, ethyl acetate, chloroform, and n-hexane) of *Areca catechu* L. nut. The antioxidant properties of different extracts of *Areca catechu* L. nut vary based on the polarity of solvents (Baby et al. 2014) IC_{50} is the concentration required to result in a 50% antioxidant activity, compared with control (Kusmardiyani et al. 2016).

Half maximal inhibitory concentration (IC_{50}) of ethyl acetate and n- hexane extracts of *Areca catechu* L. nut is less than that of the IC_{50} of ascorbic acid in DPPH and H_2O_2 scavenging activity respectively. IC_{50} values of ethyl acetate and chloroform extracts of the same are less than of EDTA in ferrous ions (Fe^{2+}) chelating assay. IC_{50} values of all the extracts of *Areca catechu* L. nut are less than that of ascorbic acid in FRAP assay. A smaller IC_{50} means higher antioxidant activity (Chaouche et al. 2014).

Antioxidant properties of different solvent extracts follows the order in DPPH radical scavenging assay as ethyl acetate > toluene > chloroform > n- hexane >

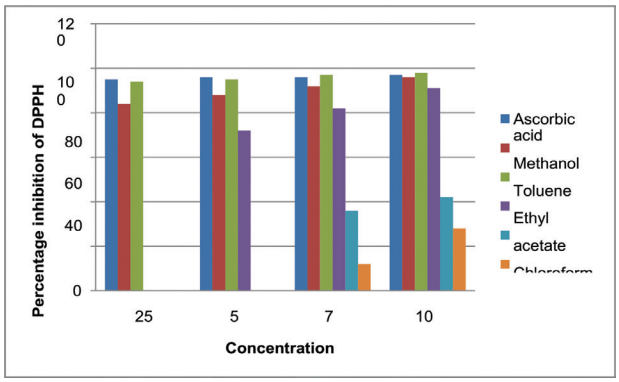

Figure 1. The graphical representation of DPPH radical scavenging activity of methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut (Standard – Ascorbic acid).

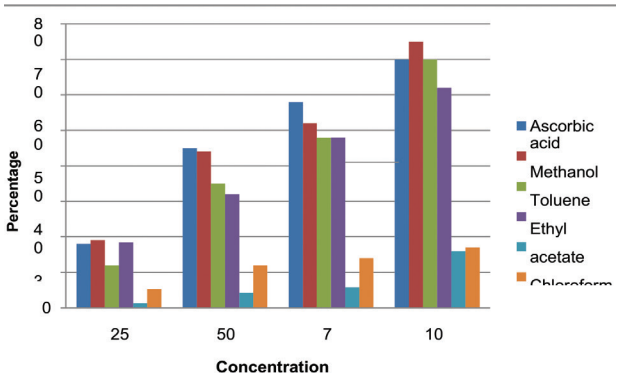

Figure 2. The graphical representation of H_2O_2 scavenging activity of methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut (Standard – Ascorbic acid).

methanol and in H_2O_2 scavenging assay the order follows as n-hexane > ethyl acetate > chloroform toluene > methanol. Methanol extract has the least antioxidant properties from both DPPH radical scavenging and H_2O_2 scavenging assay. The antioxidant properties from metal chelating assay, the order follows as chloroform > ethyl acetate > toluene > methanol > n-hexane and it is in FRAP assay as ethyl acetate > toluene > n-hexane methanol > chloroform.

IC_{50} values of different solvent extracts using DPPH radical scavenging, H_2O_2 scavenging, metal chelating, and FRAP assay are shown in Table 1.

The percentage inhibition of DPPH radical scavenging, H_2O_2 scavenging and metal chelating activity are shown in Figs 1–3 respectively. The absorbance obtained from FRAP assay are shown in Fig. 4.

GC-MS analysis of methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut were recorded (Private communications).

Conclusion

FRAP antioxidant assay proved all the extracts of *Areca catechu* L. nut have antioxidant properties. Remaining antioxidant assays like DPPH radical scavenging activity,

H_2O_2 scavenging activity, and Fe^{2+} chelating activity showed more antioxidant properties in ethyl acetate extract and nonpolar solvent extracts like n- hexane, and chloroform extracts respectively. Variation of antioxidant properties due to the difference in polarity of the solvents. The separation of compounds from different solvent extracts will lead to more studies related to the medicinal properties of *Areca catechu* L. nut.

Table 1. IC_{50} values of ascorbic acid (standard), EDTA (standard), methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut.

Extracts of *Areca catechu* L. nut and Standard solutions	DPPH Inhibition (%)	H_2O_2 Inhibition (%)	Metal Chelating Inhibition (%)	FRAP Assay (%)
Ascorbic acid (Standard)	61.75±3.33	34.00±1.00	–	72.00±0.05
EDTA (Standard)	–	–	48.31±4.35	–
Methanol	159.15 ±1.15	401.00±1.75	79.68±3.32	51.00±1.50
Toluene	64.69±2.30	336.00±1.25	50.00±1.50	23.00±2.30
Ethyl acetate	38.77±1.50	96.00±2.30	46.91±2.25	15.00±0.02
Chloroform	69.06±2.50	219.00±3.15	7.40±3.50	66.00±1.25
n-Hexane	76.25±1.75	23.00±1.50	81.36±1.75	50.00±0.50

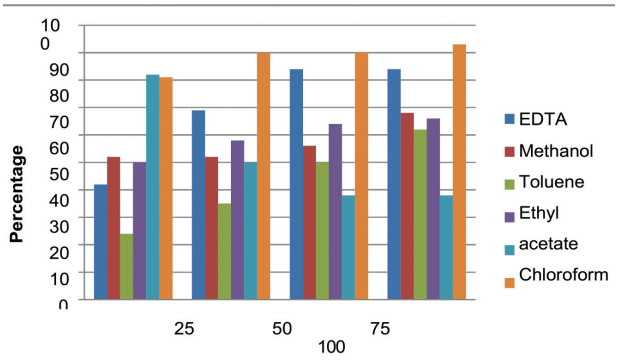

Figure 3. The graphical representation of metal chelating activates of methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut. (Standard – EDTA).

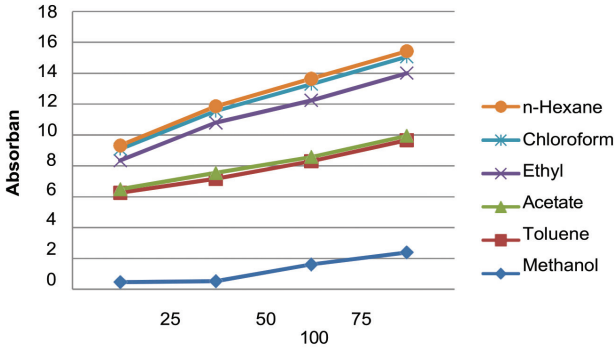

Figure 4. Total ferric reducing power (FRAP) of methanol, toluene, ethyl acetate, chloroform and n-hexane extracts of *Areca catechu* L. nut. (Standard – Ascorbic acid).

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