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Antioxidant and Anti-urease Activity of Various Solvent Extracts of "*Terminalia arjuna*" Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Author NA conducted the research activities in collaboration with co-authors and wrote the first draft of the manuscript. Author MA has supervised the designed research work and critically evaluated the results. Author KB managed the conduction of the research work, evaluation of results and guided about protocols of thesis writing. Author MKA gathered the literature review data. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Terminalia arjuna,* a popular cardio curative plant. Present work emphasizes on the evaluation of antioxidant and anti-urease activity of different fractions extracts of *Terminalia arjuna* seeds.
Methods: Six alternate fractions including methanol, ethanol, *n*- butanol, ethyl acetate, dichloromethane and distilled water were separately used for extraction of Arjun seeds. Antioxidant activity was evaluated through free radical scavenging (DPPH) assay method, using 1, 1-diphenyl-2- picryl hydrazyl (DPPH) as a standard free radical. Anti- urease activity was also assessed through anti-urease absorbance assay method.
Results: Methanol and ethanol extracts showed highly significant results (88±1.52 and 87±0.057 % inhibition respectively) than other fractions extracts but less significant than the standard ascorbic acid (93.73 ±1.12). All various fractions extracts of Arjun seed exhibited anti-urease activity up to some extent, but ethyl acetate extract exhibited highest results than other extracts as 70.4±0.15%

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inhibition of urease followed by *n*-butanol, ethanol, dichloromethane, methanol and aqueous extract respectively.
Conclusion: Present work is a sort of novel work as these activities of Arjun seed have not been reported till now. Evidence of the current study have provided a new way to utilize anti-urease and antioxidant potential of Arjun seeds for therapeutic purpose in current of various diseases.

Keywords: Terminalia arjuna; antifreeze activity; anti-oxidant activity; DPPH assay.

1. INTRODUCTION

Free radicals are basically oxygen-containing harmful molecules which release during the several degradation processes occurring inside the body. These free radicals can damage the normal functioning cells of the human body and can result in the development of several disorders like ulceration, heart diseases, skin wrinkles, freckles, aging, etc. [1].

Antioxidants are the molecules that can simply destroy the free radicals, to reduce the damage caused by them. Antioxidants molecules either could be naturally produced inside the body, e.g. enzymes, bilirubin, albumin, ferritin, myoglobin, glutathione, NADPH, uric acid, etc. or they could be intake exogenously as a part of dietary substances e.g. Vitamin C, flavonoids, β-carotene, Vitamin E, polyphenols, etc. [2]. Antioxidants are thus undeniably essential for preserving prime human health. Stem and bark of *Terminalia arjuna* a popular cardio active medicinal plant has been reported to possess highly significant antioxidant activity [3]. However, seeds portion of Arjun plant was still not evaluated for antioxidant activity. The present study is a novel work in a sense that for the first time antioxidant activity of Arjun seeds has been reported here using six different fractions of extraction. Urease is an enzyme that induces production of ammonia and carbon dioxide through hydrolysis of urea. This ammonia produces alkaline environment making it feasible for bacterial growth and manifestation of the disease. Many pathological conditions can produce through urease activity as including peptic ulceration, hepatic encephalopathy, infection stones, gastritis, etc. Another emphasis of present study is to evaluate the anti-urease activity of various fractions extracts of *Terminalia arjuna* seeds, which have not been ever reported in previous literature.

2. MATERIALS AND METHODS

2.1 Chemicals and Instruments

Satisfactory laboratory practices were implemented during the whole experimental work. All required chemicals were procured from Merck Chemicals (Germany). The used glassware was of Pyrex Japan. The instruments used in the experiments included Digital Rotary evaporator apparatus (Heidolph Laboratory Germany), Recirculating pump (Velp Scientifica Europe), Sonicator (Elmasonic, Germany), FTC 90 Refrigerated incubator (Velp Scientifica), Digital electronic balance (AUW 220D Japan) and 96-wells plate reader (Biotek Synergy HT, USA).

2.2 Collection and Identification of Plant Material

Plant material was collected from the localized flora of Bahawalpur and identification of Arjun seeds (specimen voucher No. 2206/L-S) was done by Department of Life Sciences, the Islamia University of Bahawalpur.

2.2.1 Preparation of plant extract

Arjun seeds were cleaned thoroughly by washing them and then dried under shade for 2-3 weeks, in front of constant air blow of electric fan. Seeds are very rigid to grind them intact, so prior to making the coarse powder of seeds it is better to cut them into small pieces. Then 200g of seed powder was separately macerated in 750 ml of each fraction (ethanol, methanol, ethyl acetate, *n*-butanol, dichloromethane and water). Filtration of each extract was done after 72 hours of standing. Filtration residues were re-macerated in 400 ml of same fractions. This method of sequential extraction was repeated for the 3rd time in same way. Rotary evaporator apparatus was used for extract drying. After drying extracts were stored in small glass bottles at 4ºC [4].

2.3 DPPH Free Radical Scavenging Assay

2.3.1 Preparation of test solution

Stock solution of 5mg/ml of all different fractions extracts of "*Terminalia arjuna*" seeds was prepared in methanol (99.8%).

2.3.2 Antioxidant activity

The antioxidant activity of six different extracts of *"Terminalia arjuna"* seeds was determined through the DPPH (2, 2-diphenyl-1-picryl-hydrazyl) method as described by [1,5] with a little bit modifications. DPPH is a stable free radicle frequently used to evaluate the antioxidant activity of various medicinal plants and other synthetic formulations. Protocol of this assay included the preparation of a stock solution of 100 µM DPPH in methanol. Then a 96 well plate was sterilized with alcohol swab and filled with a test volume of 100 µl, comprising of 10 micro litre of sample to be examined and 90 micro litre of DPPH stock solution. Test sample was mixed by slightly shaking well plate against a smooth glass surface and then allowed to settle for 30 minutes in an incubator at 37ºC. The free radical scavenging activity was determined through HT Biotech® USA micro plate reader at the wavelength of 517 nm. Ascorbic acid was taken as standard antioxidant in the assay. The test samples were arranged in triplicate sequence on the 96 well plates. For the estimation of IC_{50}, serial dilutions of the extracts were prepared and tested again, the values were calculated by using the Amherst USA software, Ez-fit5 Perrella Scientific Inc. Reduction in absorbance value is specified with high free radical scavenging activity which was resolved by the following formula:

$$\%\, I = \left(\frac{Abs.of\ Control - Abs.of\ Test\ Solution}{Abs.of\ Control}\right) x\ 100$$

Where,

The absorbance (abs.) of Control = Total radical activity without inhibitor.

The absorbance (abs.) of Test = Activity in presence of test compound [1].

2.4 Anti-urease Assay

Analysis of the anti-urease activity of different fractions extracts of Arjun seeds was done following the method of [6] with minute alterations. Dilution of 500 µg/ml of each extract was used as a test sample. Each test well of the sterilized 96-well plate was filled with a total volume of 200 µl, initial 85 µl of which consist of 15 µl of 1 M phosphate buffer, 15 µl of urease enzyme 0.25 mg/ml and 15 µl of the test sample. After 15 minutes of incubation at 37℃ temperature wells were added with 40 µl of substrate and then again incubated at previous conditions. A pre-read of absorbance was taken at 630 nm followed by addition of 70 µl of alkaline reagent and 45 µl of phenol catalyst (1% w/v phenol and 0.005% w/v sodium nitroprusside). Final read was taken at 630 nm after 50 minutes of incubation. Thiourea was taken as positive control and buffer was taken as negative control. Serial dilution of each fractions extract was done in order to calculate $IC_{50,}$ using Enzyme Inhibition Kinetics Software (EZ-Fit™ Perrella Scientific). Percent inhibition (%I) was calculated through following formula:

$$\%\, I = [100 - \left(\frac{Abs.of\ Test\ Solution}{Abs.of\ Control}\right) x\ 100$$

3. RESULTS

Methanol extract showed highest antioxidant activity 88±0.046 while ethanol extract also showed antioxidant activity 87±0.034. Ethyl acetate, n-butanol, dichloromethane and aqueous extract found not active as compared to ascorbic acid 98±0.01 which were given as standard. Ethyl acetate extract showed highest anti-urease activity 69.4±0.15. n-butanol, ethanol, dichloromethane, methanol and aqueous extract also showed anti-urease activity. The detailed results of antioxidant and anti-urease activity of various fractions extracts of *Terminalia arjuna* seeds are shown in Table 1 and Table 2, respectively.

3.1 Statistical Analysis

All the values are expressed as Mean of % inhibition ± SEM. One way ANOVA test was applied for data analysis followed by LSD multiple comparison tests. A level of $P < 0.05$ was taken as statistically significant.

4. DISCUSSION

Oxidative stress is considered as a prospective source of illness in human beings. Recent studies have explored that plant antioxidants can effectively reduce the oxidative stress of vital organs including heart, liver, and kidney, etc. [7,8].

Previous studies regarding the evaluation of the antioxidant potential of *Terminalia arjuna* leaves and bark have been reported, which suggest that *Terminalia arjuna* plant possess potential antioxidant activity [1,3,5,9]. However, seeds

Table 1. Antioxidant activity of various fractions extracts of *Terminalia arjuna* seeds

S. no	Fractions	Concentration (µg/ml)	% Inhibition (5 mg/ml)	IC_{50} (µg/ml)
1	Ethanol	-	87±0.034	2416
2	Methanol	-	88±0.046	1415
3	Ethyl acetate	-	21±0.02	-
4	*n*- Butanol.	-	18±0.05	-
5	Dichloromethane	-	09±0.07	-
6	Aqueous	-	22±0.09	-
7	Ascorbic acid	0.5 (mmol/ml)	98±0.01	

Values are expressed as Mean %inhibition ±SEM (n=3). Superscripts having different letters are significantly different at 0.05 level (P < 0.05)

Table 2. The anti-urease activity of various fractions extracts of *Terminalia arjuna* seeds

S. no	Fractions	Concentration (µg/ml)	% Inhibition (500 µg/ml)	IC_{50} (µg/ml)
1	Ethanol	500	63.6±1.15	-
2	Methanol	-	58.1±0.57	-
3	Ethyl acetate	-	69.4±0.15	-
4	*n*-Butanol	-	67.1±2.08	-
5	Dichloromethane	-	60.7±1.54	-
6	Aqueous	-	57.9±0.57	-
7	Thiourea	0.375 mmol	93.74±0.12	-

Values are expressed as Mean %inhibition ±SEM (n=3). Superscripts having different letters are significantly different at 0.05 level (P < 0.05)

portion was still untouched in this respect. Present study has verified that seeds of Arjun plant also possess highly significant antioxidant potential and can be applied therapeutically for management of various oxidative stresses in the human body. However, toxicity evaluations are further required.

Urease enzyme converts urea to ammonia making the internal environment alkaline which is favorable for bacterial growth. This is the leading pathophysiology of various infectious diseases including infectious stone, peptic ulcer, gastritis, hepatic encephalopathy, etc. [10,11].

Urease blocking agents are currently under consideration for securement of gastric ulcer [12] and other infections which are produced by urease releasing bacteria, etc. [13]. Therefore, new treatment methodologies are established focusing on inhibition of urease activity for the curement of infectious diseases caused by urease secreting bacteria. The present study has explored that *Terminalia arjuna* seeds possess significant anti-urease activity and thus can be utilized in this aspect for securement of urease relating diseases.

5. CONCLUSION

The present study concludes that methanol and ethanol extracts of Arjun seeds possess high antioxidant potential (87.0±1.15 and 88.3 respectively). Arjun seeds also possess considerable anti-urease activity, these properties of Arjun seeds can be utilized therapeutically for securement of oxidative stress and for reducing the morbid effects of urease activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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