

Antioxidant and cytotoxic effect of Terminalia arjuna - an in-vitro study

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ABSTRACT

Introduction: Terminalia arjuna is one of the important herbal plants with cardioprotective, antihyperlipidemic activity and many more applications. In this present investigation, we have prepared aqueous and ethanolic extracts of Terminalia arjuna.

Objective: To comparatively evaluate the antioxidant and cytotoxic effect of aqueous and ethanolic extracts of Terminalia arjuna.

Methodology: We have studied antioxidant activity of the plant extract by DPPH assay. The cytotoxic effect of prepared Terminalia arjuna plant extract was analyzed using Brine shrimp lethality assay.

Results And Conclusion: Regarding the antioxidant activity, both the extracts showed very good activity. However, aqueous extract was more effective at all concentrations. Regarding the cytotoxic effect, only in 2 µL concentration, 80% of nauplii were alive for ethanolic extract and 90% of nauplii were alive for aqueous extract. In other concentrations, the results were similar. This proves the less toxic nature of aqueous and ethanolic extracts. Based on the results, we are planning to use aqueous extract of Terminalia arjuna as a local drug delivery agent in the treatment of chronic periodontitis.

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INTRODUCTION

Insufficient and inefficient oral hygiene practices are considered to be the root cause of gingival inflammation as the later is due to plaque accumulation, and it needs mechanical methods for removal. Toothbrushes, dental floss, oral irrigators, conventional interdental brushes, and wooden interdental aids are few among others that are regularly used for plaque removal. However, these tools are found to be ineffective in the complete removal of plaque[1]. Hence, modern medicine has come up with a variety of mechanical apparatus to clean the plaque. Despite the agreed process of mechanical cleaning, an user may not have access to all surfaces of teeth, such as beneath and in-between the teeth with any of the tools mentioned earlier. It is a hindrance to the manual rupture of plaque among people who are unwilling to do so.

Earlier studies have supported the use of plant extracts in improving periodontal health by decreasing the bacterial load. India is a long-known practitioner of alternative medicines such as Ayurveda, Siddha, homeopathy, and naturopathy in treating several ailments. Most of these gained their popularity with the notion of having less to nil side effects among the users. Traditional practitioners and researchers have explored several medicinal plants, which are currently in use. However, there are many more whose activity has not been investigated[2]. With the advent of globalization, medicinal plants have also gained popularity among the western world, and recent years have witnessed natural-product biosynthesis. One such plant is Terminalia arjuna, whose antioxidant potency is measured in the present study.

KEYWORDS:

Terminalia arjuna,
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T. arjuna is known to decrease cholesterol, reduce hypertension, reduce blood clots, and prevent coagulation of blood. It was also found to be effective against several bacteria and fungi. Phytoconstituents such as triterpenoids were found to effectively treat cardiovascular diseases, while tannins and flavonoids were found to have anticancer properties[3].

Its leaves, bark, and fruits are used in extracting active compounds such as ethyl gallate, gallic acid, arjunolic acid, ellagic acid, and flavones, which are investigated for their antibacterial properties and hence can be used against oral bacteria. The bark of the tree Terminalia arjuna (Roxb) is a herb of the Combretaceae family, which contains hypolipidemic compounds and flavonoids with high antioxidative properties. Indeed, animal studies have demonstrated the antiatherogenic, antioxidant and hypoglycemic properties of T. arjun [4].

Although it is widely used in Ayurvedic medicine for various CVDs, the antioxidant and hypocholesterolemic effects of T. arjuna tree bark have been reported in a randomized placebo-controlled trial with 105 patients with CHD. T. arjuna tree bark powder exerts antioxidant action comparable with vitamin E, as well as exhibiting a hypocholesterolemic effect. Although these are preliminary data, further work is needed to verify these observations[5].

MATERIALS AND METHODS

The study was approved by Institutional Ethical Committee of Meenakshi Ammal Dental College & Hospital (MADC/IEC-1/03/2022).

Preparation of plant extract (Fig 1a)

Bark of disease-free plants was carefully selected for the study. It was cut into small pieces, shade dried for 10-15 days. Upon air drying at room temperature, it was grounded into powder under sanitized conditions.

Preparation of aqueous extract (Fig 1b,1c)

About 2gm air-dried powder of Terminalia arjuna powder was mixed with 100 ml sterile distilled water and boiled for 15 min. It was filtered with tea filter and allowed to cool. This extract was filtered using Whatman No. 1 filter paper and boiled again to get concentrated extract. It was reduced to get concentrated 5ml aqueous extract. It was refrigerated at 4°C for future use.

Preparation of ethanolic extract (Fig 1b,1c)

About 2gm air-dried powder of Terminalia arjuna powder was mixed with 100 ml sterile distilled water and boiled for 15 min. It was filtered with tea filter and allowed to cool. This extract was filtered using Whatman No. 1 filter paper and boiled again to get concentrated extract. It was reduced to get concentrated 5ml ethanolic extract.

Antioxidant activity[6] (Fig 2a,2b)

The DPPH assay was used to test the antioxidant activity of T.arjuna aqueous and ethanolic extracts. Diverse concentrations (2-10 µg/mL) of T.arjuna extract were mixed

with 1 mL of 0.1 mM DPPH in methanol and 450 µL of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed depending on the absorbance at 517 nm. The percentage of inhibition was determined from the following equation:

$$\% \text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control × 100

The antioxidant activity of prepared Terminalia arjuna plant extract was analyzed using DPPH assay[7]. Different concentrations of aqueous and ethanolic extracts of T.arjuna were used (10µL,20µL, 30µL, 40µL & 50µL). Ascorbic acid was used as the standard.

At minimum concentration (10µL), T.arjuna showed 42% inhibition for aqueous extract and 37% inhibition for ethanolic extract. At 30µL concentration, T.arjuna showed 65% inhibition for aqueous extract and 37% inhibition for ethanolic extract. At higher concentration (50 µL), higher % of free radical scavenging activity was observed for T.arjuna aqueous extract (90%) and ethanolic extract(76%). Regarding the antioxidant activity, both the extracts showed very good activity. However, aqueous extract was more effective at all concentrations (Fig 3).

Cytotoxic effect

The cytotoxicity of T.arjuna aqueous and ethanolic extracts was assessed using Brine shrimp lethality assay.

BRINE SHRIMP LETHALITY ASSAY[8]: (Fig 4a&4b)

Preparation of tank set-up

Day 1 - Sea water was prepared and Shrimp eggs were added; aeration was provided

Day 2 - Sterilized 6 well ELISA plates were filled with 10-12 ml of saline water (2g of iodone free salt was weighed and dissolved in 200 ml distilled water); live active 10 naupliis were added in each well. 5 different concentrations of T.arjuna extract (5, 10, 20, 30, 50 µl) were prepared and added in the wells and incubated for 24 h.

Day 3 - After 24 h, the total number of live and dead nauplii were counted and the mortality rate was checked using the following formula.

Number of dead nauplii

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of live nauplii} + \text{Number of dead nauplii}} \times 100$$

Different concentrations of T.arjuna were used to check cytotoxicity (2,4,8,16,32 & 64 µL). Only in 2 µL concentration, 80% of nauplii were alive for ethanolic extract and 90% of nauplii were alive for aqueous extract. In other concentrations, the results were similar. This proves the less toxic nature of aqueous and ethanolic extracts (Fig 5a, 5b).

DISCUSSION

Antioxidant activity of aqueous extract of T.arjuna

The medicinal plants are an important health resource in all the regions of India and particularly among the primitive

communities. With the current trend on increasing awareness in traditional medicine, the plant-derived agents have been attracting much interest as natural alternatives to synthetic compounds because microbes slowly develop resistance against antibiotics. Scientists are trying to tap the pharmaceutical and food values of these many unidentified plants. It is believed that plants will be a major source of new chemicals and raw materials for the pharmaceutical industry.

In oxidation process, highly reactive and harmful chain reactions of oxygen species are generated, which cause damage to living organism. The oxygen centered free radicals and other reactive oxygen species (ROS), which are continuously produced, result in cell death. This oxidative damage caused by free radical is related to pathogenesis of many chronic diseases including chronic periodontitis. ROS which includes superoxide free radical, hydrogen peroxide, hydroxyl free radical and singlet oxygen play a key role in the oxidative damage of these diseases. Antioxidant is a molecule, which removes free radical intermediates and inhibit other oxidation reactions by being oxidised themselves[9].

Many plant based compounds such as ascorbic acid, polyphenols, vitamin E, carotenes, xanthophylls and tannins had been investigated for their antioxidant potential. T.arjuna is a plant which is a very rich source of antioxidants. The name *Terminalia* is derived from latin 'Teminalis' due to terminal crowding of the leaves in many species of the genus *Terminalia*[10].

The active components of T.arjuna are tannins, triterpenoid saponins (arjunic acid, arjunolic acid, arjungenin and arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, Oligomeric Proanthocyanidines (OPCs), phytosterols, calcium, magnesium, zinc and copper[11&12]. Among these, arjungenin & its glucoside bring free radical scavenging action on superoxide release from PMN cells[13]. Triterpenoids, phenolics and flavonoids are considered to be responsible for their beneficial antioxidant and vascular amplification activity[14].

Similar study by Dwivedi et al showed better antioxidant activity of aqueous extract of Terminalia arjuna[4]. Also, it was found that T. arjuna helps in maintaining the cholesterol level at the normal rate, as it contains the antioxidant properties similar to vitamin E[15].

Comparative study of the antioxidant potential of T. arjuna bark and leaves ethanolic extract and its different solvent fractions was carried out by Kumar et al[7]. The study demonstrated that the antioxidant properties were due to presence of flavonoids, tannins and oligomeric proanthocyanidins. It was observed that arjunic acid and aglycone isolated from the fruit were strong antioxidants or free radical scavenger and more potent than ascorbic acid

CYTOTOXICITY [16-18]

Terminalia arjuna is known by its various classical names, such as Arjuna, Dhavala, Kaubha, Nadsaraja, Veeravrikskha, Partha and Indradru[19&20]. Side effects of the plant-derived agent can be tested relative to direct toxicity upon animal cells because of their close association with human tissues or cells.

The Brine Shrimp Lethality Assay (BSLA) has been used routinely in the primary screening of the extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties

of the test materials. Brine shrimp nauplii have been previously utilized in various bioassay systems.

The results of our study depict that percent mortality of nauplii increased with increase in concentration. However at low concentration(2µl), percent mortality was less for aqueous extract (90%) compared to ethanolic extract (80%). In order to avoid any toxic side effects, we are planning to use 2µl concentration of aqueous extract in future studies.

Cytotoxicity results depict the general trend among plants of genus Terminalia, which are known to contain cytotoxic compounds such as hydrolysable tannins[21].

Our study revealed similar results to the study by Alam et al, and he attributed the results to flavonoids and tannins in T.a [23].

The high toxicity exerted by the extracts of T.arjuna in brine shrimp lethality bioassay suggests bioactive principles in the plant[22].

The findings of this study help towards the use of this Terminalia arjuna as a traditional remedy and help to find out the dose of the Terminalia arjuna

CONCLUSION

Thus, it can be concluded that the antioxidant effect of T.a could be explored further not only for systemic illness associated with inflammation but also inflammatory conditions of the oral cavity. A local drug delivery system with the extracts could be formulated and its use for the management of chronic inflammatory conditions such as periodontitis. It is noteworthy that several antimicrobial agents such as tetracycline, chlorhexidine, and doxycycline have been tried as local drug delivery formulations for periodontitis management. In this connection T.a extracts could also be tried as gels, ointments, and mouthwashes and in addition, they could be modified for intrapocket delivery in the form of microspheres, in situ gels, and nanoformulations. If fruitful clinical results are obtained, T.a could be included in the list of nature-derived host modulatory agents for periodontitis management.

The study is compliant with Ethical Standards statement.

The study was approved by Institutional Ethical Committee of Meenakshi Ammal Dental College & Hospital (MADC/IEC-1/03/2022).

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Conflict of interest

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Authorship contributions

Vijayalakshmi - Drafting the manuscript

Ambalavanan - Review of manuscript

Rajeshkumar - Analysis of results

REFERENCES

- Ng E, Lim LP. An overview of different interdental cleaning aids and their effectiveness. *Dent J* 2019;7(2):56. doi: 10.3390/dj7020056. PMID: 31159354; PMCID: PMC6630384.
- Rudra S, Kalra A, Kumar A, Joe W. Utilization of alternative systems of medicine as health care services in India: Evidence on AYUSH care from NSS 2014. *PLoS One*. 2017;4:12(5):e0176916. doi: 10.1371/journal.pone.0176916. PMID: 28472197; PMCID: PMC5417584.
- Amalraj A, Gopi S. Medicinal properties of Terminalia arjuna (Roxb.) Wight & Arn.: A review. *J Tradit Complement Med* 2016;7(1):65-78. doi: 10.1016/j.jtcme.2016.02.003. PMID: 28053890; PMCID: PMC5198828.
- Dwivedi S, Chopra D. Revisiting Terminalia arjuna - An ancient cardiovascular drug. *J Tradit Complement Med* 2014;4(4):224-31. doi: 10.4103/2225-4110.139103. PMID: 25379463; PMCID: PMC4220499.
- Gupta R, Singhal S, Goyle A, Sharma VN. Antioxidant and hypocholesterolaemic effects of Terminalia arjuna tree-bark powder: a randomised placebo-controlled trial. *J Assoc Physicians India* 2001 ;49:231-5. PMID: 11225136.
- Venkat kumar and S. Rajeshkumar Anti-inflammatory, antioxidant, antibacterial effect and phytochemical analysis of Mucuna pruriens seed extract. *International Journal of Pharmatech Research* 2017;10(1):273-283.
- Kumar JS, Gajendran PL, Arjunker R & Rajeshkumar S. Antioxidant and antimicrobial activity of lycopene extract against oral pathogens. *Plant Cell Biotechnology and Molecular Biology* 2020;21(27-28), 78-88.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 1982;45(5):31-4. doi:10.1055/s-2007-971236. PMID: 17396775.
- Nauma Hafeez, Lakshmi Thangavelu, Anitha Roy, Rajeshkumar S, Raghunandhakumar S. and Geetha RV. Assessment of oxidative stress and antioxidant levels in chronic periodontitis patients. *Alinteri Journal of Agriculture Sciences*, 35(2): 151-155
- Parker R N. "Common Indian Trees and How to Know Them", Vishhkar A.A. Publ., Jaipur. 1999; p. 67.
- Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Tech* 2002;37:153-161
- Biswas M, Kar B, Bhattacharya S, Kumar RBS, Ghosh AK et al. Antihyperglycemic activity and antioxidant role of T.arjuna leaf in streptozocin-induced diabetic rats. *Pharmaceutical Bio* 2011;49:335-340.
- Pawar RS, Bhutani KK. Effect of oleanane triterpenoids from Terminalia arjuna - a cardioprotective drug on the process of respiratory oxyburst. *Phytomedicine* 2005 May;12(5):391-3. doi: 10.1016/j.phymed.2003.11.007. PMID: 15957375.
- Nauma Hafeez, Lakshmi Thangavelu, Anitha Roy, Rajeshkumar S, Raghunandhakumar S. and Geetha RV. Assessment of oxidative stress and antioxidant levels in chronic periodontitis patients. *Alinteri Journal of Agriculture Sciences*, 35(2): 151-155
- Parker R N. "Common Indian Trees and How to Know Them", Vishhkar A.A. Publ., Jaipur. 1999; p. 67.
- Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. *Int J Food Sci Tech* 2002;37:153-161
- Biswas M, Kar B, Bhattacharya S, Kumar RBS, Ghosh AK et al. Antihyperglycemic activity and antioxidant role of T.arjuna leaf in streptozocin-induced diabetic rats. *Pharmaceutical Bio* 2011;49:335-340.
- Pawar RS, Bhutani KK. Effect of oleanane triterpenoids from Terminalia arjuna - a cardioprotective drug on the process of respiratory oxyburst. *Phytomedicine* 2005 May;12(5):391-3. doi: 10.1016/j.phymed.2003.11.007. PMID: 15957375.
- Selvapriya, S., Priyadarshini, R., Rajeshkumar, S. and Sinduja, P. Anti-Inflammatory and Cytotoxic Effect of Stevia and Neem Based Herbal Formulation", *Journal of Pharmaceutical Research International*, 2021;33(59A):150-160. doi: 10.9734/jpri/2021/v33i59A34259.]
- Dwivedi, S., Chopra, D. Revisiting Terminalia arjuna - an ancient cardiovascular drug. *J Tradit. Complement. Med* 2014;4:224-231.
- Neelam Soni and Vinay Kumar Singh. Efficacy and advancement of Terminalia arjuna in Indian Herbal Drug Research: A Review. *Trends in Applied Sciences Research*, 2019;14:233-242.
- Sandra Sagar, Abilasha R, Pratibha Ramani, Rajeshkumar S. Cytotoxic and antimicrobial effects of herbal formulation (Ficus benghalensis, Azadirachta indica and Menthapiperita) based mouthwash. *Oral and Maxillofacial Pathology Journal* 2022;13(2)
- Rajkumar RJ, Rajasekar A & Rajeshkumar S. Antimicrobial and antioxidant activity of clove and cinnamon herbal formulation: An in-vitro study. *Plant Cell Biotechnology and Molecular Biology* 2020; 21(25-26):11-17.
- Selvapriya, S., Priyadarshini, R., Rajeshkumar, S. and Sinduja, P. Anti-Inflammatory and Cytotoxic Effect of Stevia and Neem Based Herbal Formulation", *Journal of Pharmaceutical Research International*, 2021;33(59A):150-160. doi: 10.9734/jpri/2021/v33i59A34259.]
- Sharma, P.C., M.B. Yelne and T.J. Dennis, 2000. Database on Medicinal Plants Used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi, India
- Chaitali H. Ved, Nikhil S. More, Sonali S. Bharate and Sandip B. Bharate Cytotoxicity screening of selected Indian medicinal plants using Brine-Shrimp Lethality Bioassay. *Advances in Natural and Applied Sciences* 2010;4(3): 389-395.
- Sadika Akhter, Md. Ismail Hossain, Md. Ismail Hossain, Mohammad Shahriar Mohiuddin Ahmed Bhuiyan. Phytochemical Screening, Antibacterial, Antioxidant and Cytotoxic Activity of the Bark Extract of Terminalia Arjuna. *European Journal of Scientific Research* 2012;86(4):543-552.
- M Alam Morshed, Azim Uddin, Akhlaqur Rahman, Tahrim Hasan, Saurov Roy, Abdullah Al Amin, Rajibul Ahsan, Rezuanul Islam. In-vitro antimicrobial and cytotoxicity screening of Terminalia arjuna ethanol extract. *International Journal of Biosciences (IJB)* ISSN: 2220-6655 (Print) Vol. 1, No. 2, p. 31-38, 2011