RESEARCH ARTICLE

A Study on *In-vitro* Anti-Inflammatory and Antioxidant Activity of Aqueous Extract of *Terminalia chebula*

Azmathullah R¹, Dhanalakshmi P^{2*}, Gokul D³, Sudhakar V³

¹Medical Genetics and Molecular Diagnostics, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India.

²Department of Pharmacy Practice, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research

Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India.

³Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India.

Received: 19th May, 2024; Revised: 02nd June, 2024; Accepted: 10th June, 2024; Available Online: 25th June, 2024

ABSTRACT

During ancient times, naturally occurring herbs were a remedy for various ailments in the human body. The deciduous trees of *Terminalia* species are native to south Asia to southeast China, known as black-chebulic myrobalan. Parts of *Terminalia chebula* are comprehensively used as medicinal properties. Antioxidants play a noteworthy role by hindering the oxidation reaction, which prompts neurodegenerative disorders in humans. Antioxidant and anti-inflammatory qualities originate from plants. *T. chebula* has lavish phytochemical properties such as tannins, flavones, essential oils, and phenolic compounds, which give rise to countless possessions like anticancer, antibacterial, anti-inflammatory, and antioxidant activity. The antioxidative capacity of *T. chebula* fruit aqueous extracts have been assessed using the DPPH scrutiny. The purpose of the study is to get an aqueous extract of *T. chebula* that contains anti-inflammatory and antioxidant compounds. The concentrations were measured by using absorbance at wavelength 517 nm. The aqueous extracts of *T. chebula* fruits were considered with a 10 to 50 mL range (5 concentrations). Using %inhibition, 55% at the lowest concentration (10 μ L) and 84% at high concentration (50 μ L) shows antioxidant activity. The active extracts of *T. chebula* fruits demonstrate the anti-inflammatory effects of using BSA as a reagent. This assay measured the activity using 5 concentrations (10–50 mL). 75% at 10 μ L and 90% at 50 μ L concentration attained the anti-inflammatory activity. The study exhibits that the higher the concentration, the higher the activity. The extraction of these active compounds has active principles and novel research activities on anti-inflammatory and antioxidant properties.

Keywords: Properties, BSA, Antioxidant, DPPH, Terminalia chebula, Aqueous extract.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.57

How to cite this article: Azmathullah R, Dhanalakshmi P, Gokul D, Sudhakar V. A Study on *In-vitro* Anti-Inflammatory and Antioxidant Activity of Aqueous Extract of *Terminalia chebula*. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):907-910.

Source of support: Nil. Conflict of interest: None

INTRODUCTION

Terminalia chebula is used in conventional drugs and originates from the family Combretaceae, genus *Terminalia*, which is extensively grown in Taiwan. *T. chebula*, commonly known as Haritaki. The wilted developed fruit of *T. chebula* is a significant condiment with laxative, diuretic, homeostatic, antitussive, and cardiotonic capabilities that are commonly employed in the ancient Indian medical practice called as ayurveda.¹ According to one report, *T. chebula* has a tannin concentration of 32%.² Ellagic acid, gallic acid, mannitol, ethyl gallate, corilagin, tannic acid, chebulagic acid, ascorbic acid and other compounds in *T. chebula* aqueous extract.³ Most crucial element in extracting antioxidants is the extracting

solvent used.⁴ In Ayurvedic medicine, Haritaki is considered one of the three fruits in the famous triphala formulation, known for its rejuvenating and balancing properties. *T. chebula* extract has boosted PC12 cell proliferation.⁵ Antioxidant supplements are advised to offer cellular protection because they are crucial for human health.⁶ Strong anticancer, antiviral, antimicrobial, and anti-inflammatory properties are exhibited by phenolic substances such as the high quantities of corilagin, gallic acid, and ellagic acid in *T. chebula* fruit extract.⁷ The pathophysiology of myocardial infarction has been linked to reactivity of oxygen species (ROS) generation, a proportionate decrease in the heart's natural antioxidant state, and elevated oxidative stress.⁸ A majority of *Terminalia* species offer a range of biologically pharmaceutical, and medicinal applications.⁹⁻¹⁷ T. chebula fruits are extensively utilised in homoeopathic, Unani, and herbal medicine. Historically, individuals made use of the dried ripe fruit of T. chebula to cure bladder problems, heart conditions, gout, piles, vomiting, hiccups, pneumonia, and throat irritation. It is shown to have analgesic, anti-inflammatory, and wound-healing mechanism.¹⁸ Numerous conditions, including cavities, gum recession, ulcerated oral stools, hemorrhoids, memory loss, paralysis, neuropathy, depression, epilepsy, tumors, intermittent fever, etc.¹⁹ Due to the emergence of possible adverse reaction such as drowsiness, decreased breathing, as well as adverse skin reactions, the most often prescribed medications for the therapeutic management of analgesia have been restricted.²⁰ To give scientific evidence for the activity, the current study examined the anti-inflammatory and antioxidant capability of

T. chebula fruit extracts.

MATERIALS AND METHODS

Collection of Plant and Sample Preparation

T. chebula dried fruit powder was purchased from Poonamallee, Tiruvallur district, Chennai, India. The prior procedure was followed in the process of making of the aqueous extract.

Preparation of Fruit Aqueous Extract

A quantity of 5 g of *T. chebula* dry fruit powder was weighed and dissolved in 100 cc of water distilled in a round-bottom flask and heated at 50°C in a heating mantle. Cooled at room temperature and by using Whatman No. 1 filter paper, refrigerated and airtight container for later research.

Evaluation of Anti-inflammatory Activity

In this experiment, bovine serum albumin (BSA) is the reagent utilized. Because BSA accounts for over 60% of all proteins in animal serum, it is frequently utilized in cell culture. Different quantities of *T. chebula* aqueous extract such as 10 to 50 μ L combined with 2 mL of 1% bovine albumin fraction. And 1N HCL was used to bring the pH down to 6.8. After that, the reaction mixture was heated for 10 minutes at 37°C in a water bath using a heating mantle. After cooling the mixture, the absorbance value at 660 nm was measured. For control, an equivalent volume of plant extract was swapped out for DMSO. Analysis was done on anti-inflammatory activity.

Evaluation of Antioxidant Activity

The assay for DPPH (1,1-diphenyl-2-picrylhydrazil), using radical scavenging, the extract's antioxidant potential was assessed. DPPH is regarded as a stable lipophilic free radical with purple-colored nitrogen in its core. The DPPH radical receives an electron from the antioxidant, causing a gradual change in color to pale yellow at a wavelength of 517 nm. By adding 50% of the methanol solution to an equal volume of DPPH solution at different doses (0.1 mM), 2 mL of the synthesized *T. chebula* aqueous extract in the 10 to 50 mL (5 different concentrations) was obtained. After the contrary, the mixture was allowed to sit at room temperature for 30 minutes in the dark. It was measured with an absorbance 517 nm. The

ascorbic acid was used as the reference, and the methanol solution combined with 0.1 mM DPPH solution served as the control in this instance. The following formula was used to determine the IC_{50} value and percentage of inhibition:

%inhibition = Absorbance of control - Absorbance of test sample x 10

Absorbance of control

RESULTS

The fruit extract's aqueous of antioxidant activity of *T. chebula* is shown in Figure 1. The concentration (10 μ L) attained 55% efficiency, as shown above, and with time, the concentration (50 μ L) reached 84%, which is nearly identical to the standard. It thus has possible antioxidant properties.

Anti-inflammatory characteristics of *T. chebula* aqueous extract of fruits are shown in Figure 2. As previously mentioned, the maximum concentration, 50 μ L, had a 90% more potent effect than usual, while the lowest dosage, 10 μ L, only achieved 75%. It appears to have some anti-inflammatory properties.

Statistical Analysis

Microsoft Excel (Microsoft Corporation in Redmond, WA), was utilized for the tabulation of data. This data was then transmitted to IBM Corp.'s SPSS version 22.0 software, located in Armonk, NY, for statistical analysis. The control and experimental extracts were assessed using an independent t-test at doses ranging from 10 to 50 μ L, with a *p*-value of less than 0.05 considered to be significant.

DISCUSSION

T. chebula is a highly adaptable plant possessing an extensive array of pharmacological and therapeutic characteristics. This versatile medicinal plant offers a single plant that produces many compounds with a broad variety of chemical structures.¹³ In this study phytochemical component of the fruit extract in aqueous form from *T. chebula* was analyzed for the anti-inflammatory and antioxidant activity results shown in Figures 1 and 2. It possesses strong anti-inflammatory and antioxidant properties. Analysis of antioxidants in *T. chebula* aqueous extract was evaluated using DPPH assay. Briefly, 5 mL of

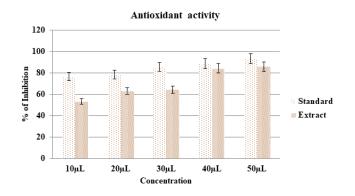


Figure 1: Antioxidant activity of aqueous fruit extract T. chebula

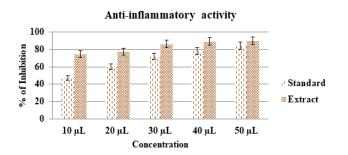


Figure 2: Anti-inflammatory activity of aqueous fruit extract T. chebula

extract with 10 to 50 μ L/mL concentrations were prepared. Antioxidant, free radical scavenging activities of chebulinic acid.²¹ In pathophysiological circumstances, higher reactive oxygen species (ROS) production is an indicator of oxidative stress and could play a major role in heart failure.²² Because free iron raises blood hematocrit and hemoglobin concentration, it raises the risk of myocardial infarction.²³ This causes the blood to become morse viscous and has a thrombogenic effect.²⁴ Rat paw edema caused by carrageenan is the most commonly used main test for anti-inflammatory drug screening *in-vivo*.²⁵ Pain and fever are brought on by prostacyclin, histamine, serotonin, leukotrienes, and bradykinins.²⁶

This evaluation demonstrates the *T. chebula* fruit's aqueous extract has strong antioxidant activity, which may have scavenged superoxide and hydroxyl radicals.

CONCLUSION

The results of this investigation demonstrated the effectiveness of *T. chebula* aqueous fruit extract as an anti-inflammatory and antioxidant. Thus, it can be applied in a variety of biomedical applications.

AUTHOR'S CONTRIBUTION

I sincerely thank Saveetha Dental College and Chettinad hospital and Research Institute, Chettinad Academy of Research and Education. After discussing the methodology, all authors approved the manuscript's final draft.

REFERENCES

- 1. Barthakur NN, Arnold NP. Nutritive value of the chebulic myrobalan (Terminalia chebula Retz.) and its potential as a food source. Food chemistry. 1991 Jan 1;40(2):213-9.
- 2. Chattopadhyay RR, Bhattacharyya SK. Terminalia chebula: An update. Pharmacognosy Reviews. 2007;1(1).
- 3. Grover IS, Bala SA. Antimutagenic activity of Terminalia chebula (myroblan) in Salmonella typhimurium. Indian Journal of Experimental Biology. 1992 Apr 1;30(4):339-41.
- 4. Hinneburg I, Neubert RH. Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (Fagopyrum esculentum) herb. Journal of agricultural and food chemistry. 2005 Jan 12;53(1):3-7.
- Chang CL, Lin CS, Lai GH, Chen YH, Tuan WC, Hsu CM. Influence of Terminalia chebula extracts on the effect of PC12 cell growth. J Trad Med. 2010;21(1):23-30.
- 6. Yanai N, Shiotani S, Hagiwara S, Nabetani H, Nakajima M. Antioxidant combination inhibits reactive oxygen species

mediated damage. Bioscience, biotechnology, and biochemistry. 2008 Dec 23;72(12):3100-6.

- Rangsriwong P, Rangkadilok N, Satayavivad J, Goto M, Shotipruk A. Subcritical water extraction of polyphenolic compounds from Terminalia chebula Retz. fruits. Separation and Purification Technology. 2009 Apr 7;66(1):51-6.
- 8. Rathore N, Kale M, John S, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat erythrocytes. Indian journal of physiology and pharmacology. 2000 Apr 1;44(2):161-6.
- Manosroi A, Jantrawut P, Ogihara E, Yamamoto A, Fukatsu M, Yasukawa K, Tokuda H, Suzuki N, Manosroi J, Akihisa T. Biological activities of phenolic compounds and triterpenoids from the galls of Terminalia chebula. Chemistry & Biodiversity. 2013 Aug;10(8):1448-63.
- 10. Khan ZMH, Faruquee HMD, Shaik MDM. Medicinal Plant Research. 2013;3:70.
- 11. Gupta PC. Int J Pharm Pharm Sci. 2012;4:62
- SuryaPrakash DV, Sree Satya N, Avanigadda S, Vangalapati MM. Antioxidant activity of methanolic extract of Phyllanthus amarus. Int J Res Pharm Biomed Sci. 2012;3:679.
- Bag A, Bhattacharyya SK, Chattopadhyay RR. The development of Terminalia chebula Retz.(Combretaceae) in clinical research. Asian Pacific journal of tropical biomedicine. 2013 Mar 1;3(3):244-52.
- Walia H, Arora S. Terminalia chebula—a pharmacognistic account. Journal of Medicinal Plants Research. 2013 May;7(20):1351-61.
- Arya A, Nyamathulla S, Noordin MI, Mohd MA. Antioxidant and hypoglycemic activities of leaf extracts of three popular Terminalia species. Journal of Chemistry. 2012;9(2):883-92.
- Lee D, Boo KH, Woo JK, Duan F, Lee KH, Kwon TK, Lee HY, Riu KZ, Lee DS. Anti-bacterial and anti-viral activities of extracts from Terminalia chebula barks. Journal of the Korean Society for Applied Biological Chemistry. 2011 Apr;54:295-8.
- Prabhu S, Narayan S, Devi CS. Mechanism of protective action of mangiferin on suppression of inflammatory response and lysosomal instability in rat model of myocardial infarction. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2009 Jun;23(6):756-60.
- 18. RAI AK, Joshi R. Evaluation of antimicrobial properties of fruit extracts of Terminalia chebula against dental caries pathogens.
- Shaik HA, Eswaraih MC, Lahari M, Rao BM, Ali SF, Shaik HA. Evaluation of analgesic and anti-pyretic activities of ethanolic extract of Terminalia pallida (EETP) stem in experimental animals. SJAMS. 2013 Oct 2;1:5-8.
- 20. Sudhan, Janakiraman, Ahmad SF, Wani A, Ahmed SS. Phytochemicals from Piper betle (L.) as Putative Modulators of a Novel Network-Derived Drug Target for Coronary Artery Disease: An In Silico Study. Processes. 2023 Oct 25;11(11):3064.
- 21. Goodman LS. Goodman and Gilman's the pharmacological basis of therapeutics. New York: McGraw-Hill; 1996.
- 22. Michel T, Feron O. Nitric oxide synthases: which, where, how, and why?. The Journal of clinical investigation. 1997 Nov 1;100(9):2146-52.
- 23. Chittrarasan S, Radhakrishnan A, Lella T, Rao K. L. N, Sugumar P, Ravi S, Elango A. Assessment of Efficacy and Safety of Methanolic Leaf Extract of Prosopis Juliflora in Lipopolysaccharide Induced Systemic Inflammatory Response

Syndrome (SIRS) in Wistar Albino Rats. Biomed Pharmacol J 2023;16(3).

- 24. Sharma SB, Dwivedi S, Kumar N, Prabhu KM, Madan N. Studies on oxidative stress, serum iron and iron binding capacity in subjects prone to the risk of coronary artery disease. Indian Heart Journal. 2000 Sep 1;52(5):583-6.
- 25. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema

in hind paw of the rat as an assay for antiinflammatory drugs. Proceedings of the society for experimental biology and medicine. 1962 Dec;111(3):544-7.

 Asongalem EA, Foyet HS, Ngogang J, Folefoc GN, Dimo TH, Kamtchouing PI. Analgesic and antiinflammatory activities of Erigeron floribundus. Journal of ethnopharmacology. 2004 Apr 1;91(2-3):301-8.