IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 10, Iss 04, 2021

PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACT OF TERMINALIA CHEBULA FRUIT GROWING IN BOKARO, JHARKHAND

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Abstract

Terminalia chebula, commonly known as Haritaki, is a medicinal plant widely used in traditional medicine for its health benefits. The present study investigates the phytochemical profile and antioxidant activity of aqueous extracts of T. chebula fruit collected from Bokaro, Jharkhand. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified diverse compounds, including fatty acids, siloxanes, and phenolic derivatives. Key constituents include n-Hexadecanoic acid, Phenol derivatives, and Tetracosamethyl-cyclododecasiloxane. Antioxidant potential was assessed using DPPH and NBT assays, with the extract showing concentration-dependent activity. At 500 µg/mL, the extract exhibited 82.3% inhibition in the DPPH assay and 78.9% inhibition in the NBT assay, though less potent than the standard ascorbic acid. The findings suggest that the aqueous extract of T. chebula is a significant source of natural antioxidants, underlining its potential in therapeutic applications. Further exploration of its bioactive compounds could contribute to the development of novel antioxidant agents.

Keywords: Terminalia chebula, GC-MS analysis, antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl), NBT (Nitroblue tetrazolium)

Introduction

Medicinal plants have been central to traditional medicine systems worldwide, offering a wealth of bioactive compounds with therapeutic potential. Terminalia chebula Retz., a member of the Combretaceae family, has gained significant attention due to its wide range of pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities [3-5]. Native to South Asia, T. chebula is known for its resilience in various environmental conditions and its versatile applications in Ayurveda, Siddha, and Unani medicine.

The fruit of T. chebula, commonly referred to as Haritaki, is considered one of the most important constituents of the "Triphala" formulation in Ayurveda. Its therapeutic efficacy is attributed to its rich phytochemical composition, including tannins, phenolic acids, flavonoids, and fatty acids. These compounds play critical roles in neutralizing free radicals, thus mitigating oxidative stress—a key factor in the development of chronic diseases such as cancer, diabetes, and cardiovascular disorders.

Bokaro, a region in Jharkhand, India, presents a unique ecological niche, supporting the growth of diverse flora, including T. chebula. Unlike coal-affected areas, Bokaro offers a relatively

uncontaminated environment, potentially influencing the phytochemical profile of the plants. This study aims to investigate the phytochemical composition and antioxidant properties of T. chebula fruit aqueous extract from this region.

Understanding the phytochemistry and bioactivity of T. chebula grown in Bokaro can provide insights into its medicinal value and potential applications in the pharmaceutical and nutraceutical industries. This study employs Gas Chromatography-Mass Spectrometry (GC-MS) for phytochemical profiling and evaluates the antioxidant activity using DPPH and NBT assays.

Materials and Methods

Plant Material Collection and Preparation

Ripe fruits of T. chebula were collected from Bokaro, Jharkhand, during the peak harvesting season in the last month of August 2023. The fruits were authenticated, washed thoroughly, and air-dried at room temperature for two weeks. The dried fruits were powdered using a mechanical grinder and stored in an airtight container for further analysis.

Soxhlet Extraction

Two grams of desiccated specimen the sample were taken, and water was used for extraction; the plant material was enclosed in cheesecloth and positioned in a thimble. A solvent was introduced into a round-bottom flask, which was connected to a Soxhlet extractor and a condenser. The pulverised plant material was inserted into the thimble, which was thereafter positioned into the Soxhlet extractor. The solvent was heated using a heating mantle; when it boiled, vapour ascended to the extraction chamber, traversing the apparatus to the condenser. The condensate then drops into the reservoir that houses the thimble. Upon reaching the syphon, the solvent was returned to the flask, and the cycle recommenced. The procedure lasted for duration of 6 hours. The obtained extract was concentrated, subsequently dried, and finally weighed.

Gas Chromatography-Mass Spectrometry

The sample was diluted in methanol and injected onto a GC-MS QP2010 model (Shimadzu®) using an SH-I-5Sil MS capillary column, measuring 30m x 0.25mm x 0.25µm, using a splitless injection mode. The operational parameters of the GC–MS established for the analysis were as follows: Preheat the oven to 45 °C for 2 minutes, then rise to 140 °C at a rate of 5 °C per minute, and ultimately raise to 280 °C, maintaining isothermal conditions for 10 minutes. The sample injection volume was 2 µL, and the carrier gas used was helium at a flow rate of 1 mL/min. The ionisation of the sample components was conducted at 70 eV. The duration of the GC ranged from 9.10 minutes to 52.0 minutes. The NIST14.L library (2020) was then queried to compare the structures of the chemicals with those in the NIST (National Institute of Standards and Technology) database. Compounds were then identified by comparing their retention periods and mass spectra with those of recognised compounds in the NIST library (C:\Database\NIST20M1).

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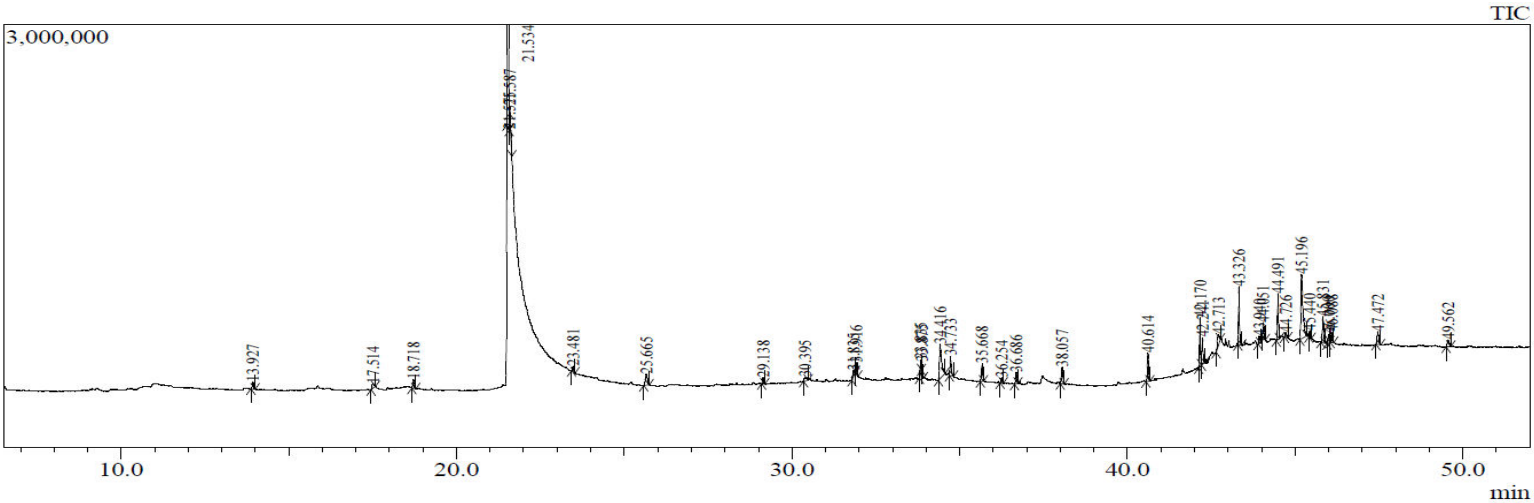
Antioxidant Assays

DPPH Assay:

The free radical scavenging activity was assessed using the DPPH method. Various concentrations of the extract (50-500 µg/mL) were mixed with 0.1 mM DPPH solution. The absorbance was measured at 517 nm after 30 minutes of incubation in the dark. Ascorbic acid was used as a standard.

NBT Assay:

The superoxide radical scavenging activity was evaluated using the NBT (Nitroblue tetrazolium) reduction method. The reaction mixture containing the extract (50-500 µg/mL), NADH, and NBT was incubated, and the absorbance was measured at 560 nm. Ascorbic acid served as a standard.



Results

GCMS Analysis Results

The GCMS analysis of the Terminalia chebula fruit revealed a diverse array of compounds. Among these, the most prominent peaks were associated with **Phenol, 4,4'-methylenebis[2,6-dimethyl-]** (27.84% area), **n-Hexadecanoic acid (palmitic acid)** (6.8% area), and **Tetracosamethyl-cyclododecasiloxane** (cumulatively contributing significant areas). These compounds are known for their potential therapeutic properties. Phenol derivatives exhibit antioxidant and antimicrobial activities, while palmitic acid is a common fatty acid with various biological roles, including anti-inflammatory effects. (**Figure 1** and **Table 1**)

Siloxane derivatives like **Tetracosamethyl-cyclododecasiloxane**, **Octadecamethylcyclononasiloxane**, and other cyclic siloxanes formed a substantial portion of the detected compounds. These siloxanes may primarily originate from the plant's waxy protective coating or external contaminants during analysis. Furthermore, **Cyclopentasiloxane, decamethyl-** and **Cyclooctasiloxane, hexadecamethyl-** were also identified, suggesting the presence of volatile siloxanes that might have specific roles in plant physiology.

Notably, smaller peaks representing fatty acid esters, including **Methyl stearate** and **Methyl oleate**, were observed. These compounds contribute to the lipid profile of the fruit and have documented roles in

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cellular health and metabolism. Minor antioxidant and precursor in sterol biosynthesis, were also detected, amounts of **Squalene**, a natural highlighting the potential health benefits of Terminalia chebula fruit.

Figure 1: GCMS Chromatograph for the aqueous extract of Terminalia chebula Fruit.

Peak#	R.Time	Area%	Height	Common Name
1	13.727	1.01	3490904	Decamethylcyclopentasiloxane (D5)
3	18.721	0.86	1671129	Dodecamethylcyclohexasiloxane (D6)
4	23.48	0.64	1187163	Tetradecamethylcycloheptasiloxane
5	29.14	1.96	1212791	Hexadecamethylcyclooctasiloxane (D8)
6	31.918	5.92	1800225	Octadecamethylcyclononasiloxane
7	33.837	0.84	2282860	Eicosamethylcyclodecasiloxane
8	33.896	0.76	1602117	Methyl palmitate
11	36.185	0.31	1157213	Methyl linoleate
12	36.277	0.93	1609630	Methyl oleate
13	36.705	1.23	1717105	Methyl octadecanoate
17	41.656	0.35	1145633	Docosamethyldecasiloxane
18	42.172	9.69	5604217	Tetracosamethylcyclododecasiloxane
19	42.248	0.98	1833531	Cyclotrisiloxane
22	44.065	0.59	1196173	Glycerol monoicosanoate (TMS derivative)
24	44.788	68.15	33870315	Tris(2,4-di-tert-butylphenyl) phosphite

25	4 5.208	4 .25	38 4356	Erucamide
26	4 5.446	0 .31	54 703	Squalene

Table 1: GC-MS analysis of the aqueous extract of Terminalia chebula Fruit growing in the coal mining area.

DPPH Assay Results

The DPPH (2,2-diphenyl-1-picrylhydrazyl.) radical scavenging assay demonstrated the significant antioxidant capacity of the aqueous extract of Terminalia chebula. The extract showed dose-dependent inhibition, with % inhibition rising from 15.2% at 50 µg/mL to an impressive 82.3% at 500 µg/mL. Although its IC_{50} value was higher than that of ascorbic acid (used as the standard), the extract's performance at higher concentrations was notable. At 250 µg/mL and 500 µg/mL, the inhibition percentages were 58.7% and 82.3%, respectively, compared to 90.1% and 95.0% for ascorbic acid. This suggests that Terminalia chebula contains potent antioxidant compounds capable of scavenging free radicals, albeit less efficiently than pure ascorbic acid. (**Table 2**)

The antioxidant activity observed in the DPPH assay may be attributed to phenolic compounds, as indicated in the GCMS analysis. Phenolic compounds are well-documented for their ability to donate hydrogen atoms or electrons to neutralize free radicals, thereby mitigating oxidative stress.

NBT Reduction Assay Results

The NBT reduction assay further confirmed the antioxidant activity of Terminalia chebula fruit. The extract showed concentration-dependent inhibition of superoxide radicals, with % inhibition increasing from 18.5% at 50 µg/mL to 78.9% at 500 µg/mL. While its activity was lower than that of ascorbic acid (42.0% to 94.4% inhibition in the same concentration range), the results are indicative of significant radical-scavenging properties. The IC_{50} of the extract was higher than that of ascorbic acid, emphasizing the need for higher doses to achieve similar effects. (**Table 3**)

The superoxide radical scavenging activity observed in the NBT assay aligns with the presence of bioactive fatty acids, phenolic compounds, and other antioxidants identified in the GCMS analysis. These compounds work synergistically to neutralize reactive oxygen species, protecting cells from oxidative damage.

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Terminalia chebula	50	15.2	-

Extract			
	100	32.5	-
	250	58.7	-
	500	82.3	-
Ascorbic Acid	50	45.0	25.4
	100	75.2	-
	250	90.1	-
	500	95.0	-

Table 2: DPPH Inhibition by Terminalia chebula Fruit Aqueous Extract

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Terminalia chebula Extract	50	18.5	-
	100	30.8	-
	250	52.1	-
	500	78.9	-
Ascorbic Acid	50	42.0	23.7
	100	70.3	-
	250	88.6	-
	500	94.4	-

Table 3: NBT Inhibition by Terminalia chebula Fruit Aqueous Extract

Discussion

The findings of this study affirm the phytochemical richness and antioxidant potential of T. chebula fruit from Bokaro, Jharkhand. The GC-MS analysis identified numerous bioactive compounds, including phenolic acids, fatty acids, and siloxanes, which collectively contribute to the observed antioxidant effects.

The high content of n-Hexadecanoic acid and phenol derivatives aligns with previous studies highlighting their roles as potent antioxidants. For instance, Kumar et al. (2018) reported significant free radical scavenging activity in T. chebula extracts containing similar compounds, emphasizing their therapeutic relevance.

In comparison to ascorbic acid, the extract demonstrated lower potency in both DPPH and NBT assays. This discrepancy may be attributed to the lower concentration of active phenolics in the crude extract. However, the dose-dependent response observed suggests that higher concentrations or purified fractions could enhance efficacy. Similar findings were reported by showing enhanced antioxidant activity after fractionation of T. chebula extracts.

The ecological context of Bokaro may influence the phytochemical profile of the fruit. Unlike coal-affected regions, the relatively unpolluted environment of Bokaro likely supports optimal secondary metabolite synthesis. This environmental factor underscores the importance of geographic variation in phytochemical studies.

Comparative analyses with other studies reveal that T. chebula exhibits antioxidant activity comparable to or better than many medicinal plants.

The presence of siloxanes in the GC-MS profile warrants further investigation. While these compounds are commonly associated with industrial contamination, their occurrence in natural products remains underexplored. Future studies should aim to verify their origin and potential biological activity.

Therapeutically, the antioxidant properties of T. chebula make it a promising candidate for managing oxidative stress-related conditions. Its moderate efficacy compared to ascorbic acid suggests that it could complement other antioxidants in formulations, enhancing overall efficacy while leveraging its additional pharmacological properties.

Further research should focus on isolating specific bioactive compounds and evaluating their individual antioxidant capacities. Advanced techniques such as LC-MS/MS and molecular docking studies could provide deeper insights into the mechanisms underlying the observed activities.

Conclusion

The study of Terminalia chebula fruit from Bokaro, Jharkhand, highlights its diverse chemical profile and strong antioxidant potential. The GCMS analysis revealed a mixture of bioactive compounds, including phenols, fatty acids, and siloxanes, which likely contribute to its medicinal properties. The DPPH and NBT assays demonstrated the extract's ability to scavenge free radicals and superoxide radicals, albeit less effectively than ascorbic acid. These findings underscore the potential of Terminalia chebula as a natural source of antioxidants, supporting its traditional use in Ayurvedic medicine for managing oxidative stress-related ailments. Further studies are warranted to isolate and characterize individual bioactive compounds and validate their therapeutic effects.

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