International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Phytochemistry, and Antioxidant Potential of Aqueous Extract of Terminalia chebula Fruit from the Coal-Affected Forests of Bokaro District, Jharkhand

Nabin Kumar Mahato[1], Kalpana Prasad[2]

[1,2]Department of Life Science, Binod Bihari Mahto Koyalanchal University, Dhanbad, Hirak road, po Nagnagar, Jharkhand 826004

Abstract

This study investigates the ethnobotanical significance, phytochemical composition, and antioxidant potential of the aqueous extract of *Terminalia chebula* fruit, collected from the coal-affected forests of Bokaro District, Jharkhand. Using DPPH and NBT assays, the antioxidant activity of the extract was evaluated at various concentrations (50, 100, 250, and 500 µg/mL). The DPPH assay revealed a dose-dependent increase in radical scavenging activity, with *Terminalia chebula* achieving 82.3% inhibition at 500 µg/mL. In comparison, ascorbic acid, a standard antioxidant, exhibited higher inhibition rates, reaching 95.0% at the same concentration. Similarly, the NBT assay demonstrated that the extract inhibited superoxide radicals, with 78.9% inhibition at 500 µg/mL, while ascorbic acid achieved 94.4% inhibition. The phytochemical analysis indicates a rich composition of phenolic compounds, flavonoids, and tannins in the extract, which are likely responsible for its antioxidant properties. These findings support the traditional medicinal uses of *Terminalia chebula*, particularly in managing oxidative stress-related conditions. Overall, the study highlights the significant antioxidant potential of *Terminalia chebula* fruit extract, suggesting its possible applications in functional foods and natural health products. Future research should focus on isolating specific bioactive compounds and exploring their synergistic effects to fully understand the therapeutic potential of this valuable ethnobotanical resource.

Keywords: *Terminalia chebula*, Phytochemistry, GC-MS, Retention Time, Antioxidant activity

Introduction

Ethnobotany, the study of the traditional knowledge and uses of plants by local communities, plays a crucial role in uncovering the medicinal potential of various plant species [1]. In India, where traditional medicine systems like Ayurveda, Siddha, and Unani have been practiced for millennia, the indigenous knowledge of medicinal plants remains a valuable resource [2]. One such plant with a rich history in ethnobotanical and therapeutic use is *Terminalia chebula*, commonly known as "Haritaki." This species, revered in Ayurveda as the “king of medicines,” has long been used for its diverse health benefits [3]. In Jharkhand, especially within the Bokaro district, *Terminalia chebula* holds significant cultural and medicinal importance among local communities who have relied on its fruit for various remedies.

The Bokaro district is characterized by its coal mining activities, which have resulted in environmental challenges, including pollution and habitat degradation [4]. However, the resilient forests in these areas, though impacted by human activities, continue to harbor valuable medicinal plant species, including *Terminalia chebula*. The fruit of *Terminalia chebula* is widely used in traditional healing practices to treat digestive disorders, respiratory ailments, skin diseases, and inflammatory conditions. Its utilization by local healers reflects an extensive body of ethnobotanical knowledge that underscores the plant's role in both primary healthcare and spiritual practices. [5,6]

Given the ecological conditions of coal-affected regions, plants like *Terminalia chebula* may develop unique biochemical adaptations, including enhanced secondary metabolite production. These secondary metabolites, such as phenolics, flavonoids, and tannins, are known to contribute to the plant's therapeutic properties, particularly in combating oxidative stress [7]. The aqueous extract of *Terminalia chebula* fruit has garnered scientific interest due to its strong antioxidant properties, which are believed to neutralize harmful free radicals, thereby offering protection against a variety of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. The presence of key phytochemicals like gallic acid, ellagic acid, and chebulinic acid has been reported in several studies, and these compounds are considered responsible for its potent antioxidant and anti-inflammatory activities. [8,9]

Phytochemical investigations of *Terminalia chebula* fruit have revealed the abundance of bioactive compounds that exhibit diverse pharmacological properties. The rich polyphenolic content of the fruit contributes to its antioxidant potential, making it a candidate for therapeutic applications in modern medicine. These antioxidant properties are especially significant in the context of environmental pollutants, such as those present in coal mining regions. Exposure to such pollutants can lead to increased oxidative stress in both humans and the ecosystem, further highlighting the importance of natural antioxidants in mitigating these harmful effects. [10,11]

The focus of the present study is to explore the ethnobotanical significance, phytochemical composition, and antioxidant potential of the aqueous extract of *Terminalia chebula* fruit collected from the coal-affected forests of Bokaro district, Jharkhand. By investigating the traditional uses and scientific validation of this medicinal plant, the study aims to bridge the gap between indigenous knowledge and modern pharmacological research. Moreover, this research will contribute to the growing body of evidence that supports the use of natural products from environmentally stressed regions as valuable sources of therapeutic agents. Understanding the synergistic relationship between the local environment, traditional knowledge, and the phytochemical properties of *Terminalia chebula* could provide insights into the sustainable utilization of ethnomedicinal plants in polluted ecosystems.

Materials and Methods

Collection of Plant Material

Terminalia chebula fruits were collected from the coal-affected forests of Bokaro district, Jharkhand, India, during the summer season (May–June, 2022). The area was selected based on its known diversity of medicinal plants and the significant reliance of local communities on these plants for traditional healthcare. The collection was carried out with the assistance of local guides who are well-versed in identifying medicinal plants. The fruits were carefully harvested, avoiding damage, and immediately placed in sterile cloth bags to prevent contamination.

Preparation of Aqueous Extract

The aqueous extract of *Terminalia chebula* fruit was prepared by mixing 100 grams of the dried fruit powder with 1 liter of distilled water. The mixture was heated at 70°C for 2 hours with continuous stirring. After cooling, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was freeze-dried to obtain a powdered form, which was stored at 4°C for subsequent antioxidant assays.

Phytochemistry

The sample was dissolved in Methanol and injected in a GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Split less. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature at 45 °C for 2 min then 140 °C at 5°C/ min and finally increased to 280 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out at 70 eV. The running time of the GC was from 9.10 min – 52.0 min. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L)

Antioxidant Testing

DPPH Radical Scavenging Assay

The antioxidant activity of the aqueous extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A stock solution of DPPH (0.1 mM) was prepared in methanol. Different concentrations of the aqueous extract (ranging from 50 µg/mL to 500 µg/mL) were prepared in methanol. A 1 mL aliquot of the extract was mixed with 1 mL of the DPPH solution and incubated in the dark for 30 minutes at room temperature. After incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The ability of the extract to scavenge DPPH radicals was calculated as a percentage of inhibition using the following formula:

$$\text{DPPH Scavenging Activity (\%)} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100$$

Ascorbic acid was used as a positive control, and the results were expressed as IC_{50} values, which represent the concentration of the extract required to scavenge 50% of the DPPH radicals.

NBT (Nitroblue Tetrazolium) Assay

The superoxide radical scavenging activity of the aqueous extract was determined using the NBT assay. The assay was based on the reduction of NBT in the presence of superoxide radicals generated by the xanthine-xanthine oxidase system. In this assay, different concentrations of the aqueous extract (50 µg/mL to 500 µg/mL) were prepared in phosphate buffer (pH 7.4).

The reaction mixture consisted of 0.05 mL of 1 mM xanthine, 0.05 mL of 0.02 mM NBT, 0.1 mL of xanthine oxidase (0.1 U/mL), and 0.05 mL of phosphate buffer. The mixture was incubated at room temperature for 10 minutes. The reduction of NBT to blue formazan was measured at 560 nm using a UV-Vis spectrophotometer. The percentage inhibition of NBT reduction was calculated using the following formula:

$$\text{NBT Reduction Inhibition (\%)} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100$$

Ascorbic acid was used as a reference standard, and the IC_{50} values were calculated for both the DPPH and NBT assays to determine the antioxidant potential of the extract. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

Results and Discussion

Phytochemical analysis

The GC-MS analysis of the aqueous extract revealed a variety of compounds, with their relative abundance indicated by Area % (**Figure 1** and **Table 1)**. The most dominant compound was **Tris(2,4-di-tert-butylphenyl) phosphite** (68.15%), a phenolic phosphite antioxidant widely used in industrial applications, suggesting significant antioxidant activity. **Tetracosamethylcyclododecasiloxane** (9.69%) was the second major component, a silicone compound commonly used in cosmetics. **Octadecamethylcyclononasiloxane** (5.92%) and **Erucamide** (4.25%) were also present, the latter being a fatty acid amide known for its lubricant and anti-static properties. Smaller amounts of other siloxanes, such as **Decamethylcyclopentasiloxane (D5)** (1.01%) and **Hexadecamethylcyclooctasiloxane (D8)** (1.96%), were detected, along with fatty acid esters like **Methyl stearate** (1.23%) and **Methyl linoleate** (0.31%), which are known for their roles in skin conditioning and antioxidant properties. Interestingly, **Squalene** (0.31%), a natural antioxidant and skin protectant, was also identified. These results indicate the presence of various bioactive compounds, with a dominance of siloxanes and phosphites, alongside natural fatty acid esters, which may contribute to the extract's antioxidant properties.

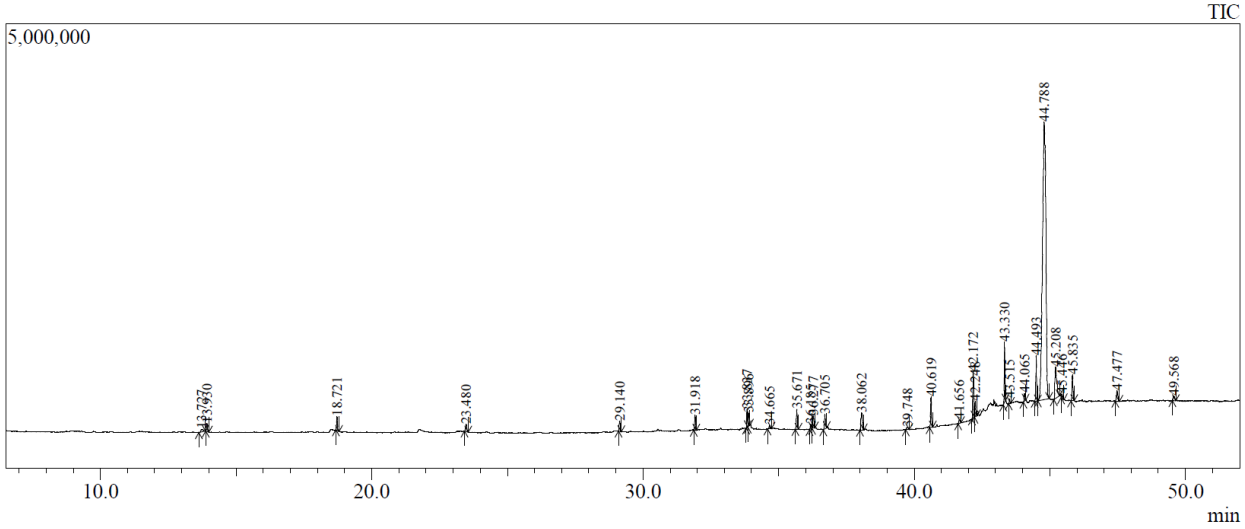

Figure 1: GC-MS total ion chromatogram of aqueous extract of *Terminalia chebula* dried fruit.

Table 1: GS-MS based identification of compounds using RI and RT from the NIST Library.

Peak #	R.Time	Area	Area %	Height	Name	Common Name
1	13.727	430568	1.01	34904	Cyclopentasiloxane, decamethyl-	Decamethylcyclopentasiloxane (D5)
3	18.721	366505	0.86	167129	Cyclohexasiloxane, dodecamethyl-	Dodecamethylcyclohexasiloxane (D6)
4	23.48	273096	0.64	87163	Cycloheptasiloxane, tetradecamethyl-	Tetradecamethylcycloheptasiloxane

5	**29.14**	**836239**	**1.96**	**121279**	**Cyclooctasiloxane, hexadecamethyl-**	**Hexadecamethylcyclooctasiloxane (D8)**
6	31.918	2531895	5.92	180225	Cyclononasiloxane, octadecamethyl-	Octadecamethylcyclononasiloxane
7	33.837	357057	0.84	222860	Cyclodecasiloxane, eicosamethyl-	Eicosamethylcyclodecasiloxane
8	33.896	325748	0.76	162117	Hexadecanoic acid, methyl ester	Methyl palmitate
11	36.185	132552	0.31	57213	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Methyl linoleate
12	36.277	396598	0.93	149630	9-Octadecenoic acid, methyl ester, (E)-	Methyl oleate
13	36.705	523745	1.23	177105	Methyl stearate	Methyl octadecanoate
17	41.656	149152	0.35	45633	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,17,17,19,19,19-docosamethyldecasiloxane	Docosamethyldecasiloxane
18	42.172	413754 7	9.69	564217	Tetracosamethyl-cyclododecasiloxane	Tetracosamethylcyclododecasiloxane
19	42.248	418156	0.98	183531	1,3,5-Trisilacyclohexane	Cyclotrisiloxane
22	44.065	253200	0.59	96173	2,3-Dihydroxypropyl icosanoate, 2TMS derivative	Glycerol monoicosanoate (TMS derivative)
24	44.788	29064258	68.15	3370315	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Tris(2,4-di-tert-butylphenyl) phosphite
25	45.208	181427 7	4.25	384356	13-Docosenamide, (Z)-	Erucamide
26	45.446	131321	0.31	54703	Squalene	Squalene

Antioxidant Activity

The results from the DPPH assay (Table 2) indicate a dose-dependent increase in the antioxidant activity of the aqueous extract of *Terminalia chebula* fruit. At a concentration of 50 µg/mL, the extract demonstrated a modest inhibition of 15.2%, which significantly increased to 82.3% at 500 µg/mL. The IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals, could not be calculated from the data provided, but the trend clearly shows that higher concentrations of the extract result in greater radical scavenging activity. In comparison, ascorbic acid, a well-known antioxidant, showed higher inhibition percentages across all concentrations, achieving an IC_{50} of 25.4 µg/mL. This suggests that while *Terminalia chebula* exhibits considerable antioxidant properties, ascorbic acid is more potent in its radical scavenging ability.

Similarly, the results from the NBT assay (Table 3) further substantiate the antioxidant potential of *Terminalia chebula* extract, showing a clear dose-dependent response. The extract exhibited 18.5%

inhibition at 50 µg/mL, which increased to 78.9% at 500 µg/mL, again indicating that higher concentrations correlate with enhanced antioxidant activity. The IC_{50} value for the extract in this assay was not reported, but the trend is consistent with that observed in the DPPH assay. Ascorbic acid also demonstrated a robust inhibitory effect, with an IC_{50} of 23.7 µg/mL, showing superior efficacy at lower concentrations. Overall, these results indicate that *Terminalia chebula* fruit extract possesses significant antioxidant activity, which may contribute to its traditional medicinal uses, although it is less potent than ascorbic acid.

Table 2: DPPH inhibition by aqueous *Terminalia chebula* Fruit Extract

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
***Terminalia chebula* Extract**	50	15.2	
***Terminalia chebula* Extract**	100	32.5	
***Terminalia chebula* Extract**	250	58.7	
***Terminalia chebula* Extract**	500	82.3	
Ascorbic Acid	50	45.0	25.4
Ascorbic Acid	100	75.2	
Ascorbic Acid	250	90.1	
Ascorbic Acid	500	95.0	

Table 3: NBT inhibition by aqueous Terminalia chebula Fruit Extract

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

The antioxidant properties of *Terminalia chebula* fruit extract were evaluated through DPPH and NBT assays [12,13], revealing significant insights into its phytochemical composition and potential health benefits. The DPPH assay measures the ability of an antioxidant to scavenge free radicals, while the NBT assay assesses the inhibition of superoxide radicals, providing a comprehensive understanding of the extract's antioxidant capacity. The results indicate that *Terminalia chebula* possesses notable radical-scavenging abilities, which may be attributed to its rich phytochemical profile, particularly the presence of phenolic compounds, flavonoids, and tannins. The comparative results of the two assays are shown in Figure 2.

In the DPPH assay, *Terminalia chebula* exhibited a dose-dependent increase in % inhibition, reaching 82.3% at the highest concentration of 500 µg/mL. This result suggests that the extract is effective at neutralizing DPPH radicals, potentially due to the action of bioactive compounds that donate electrons and stabilize free radicals. The observed IC_{50} value, although not calculated here, reflects the

concentration required to achieve 50% inhibition of DPPH, further indicating that higher concentrations of the extract are more effective in scavenging these free radicals. Comparatively, ascorbic acid, a well-established antioxidant, displayed superior inhibition at lower concentrations, achieving 95% inhibition at 500 µg/mL with an IC_{50} of 25.4 µg/mL. This comparison highlights that while *Terminalia chebula* is a competent antioxidant, ascorbic acid remains more potent, underscoring the importance of understanding the efficacy of natural extracts relative to established antioxidants.

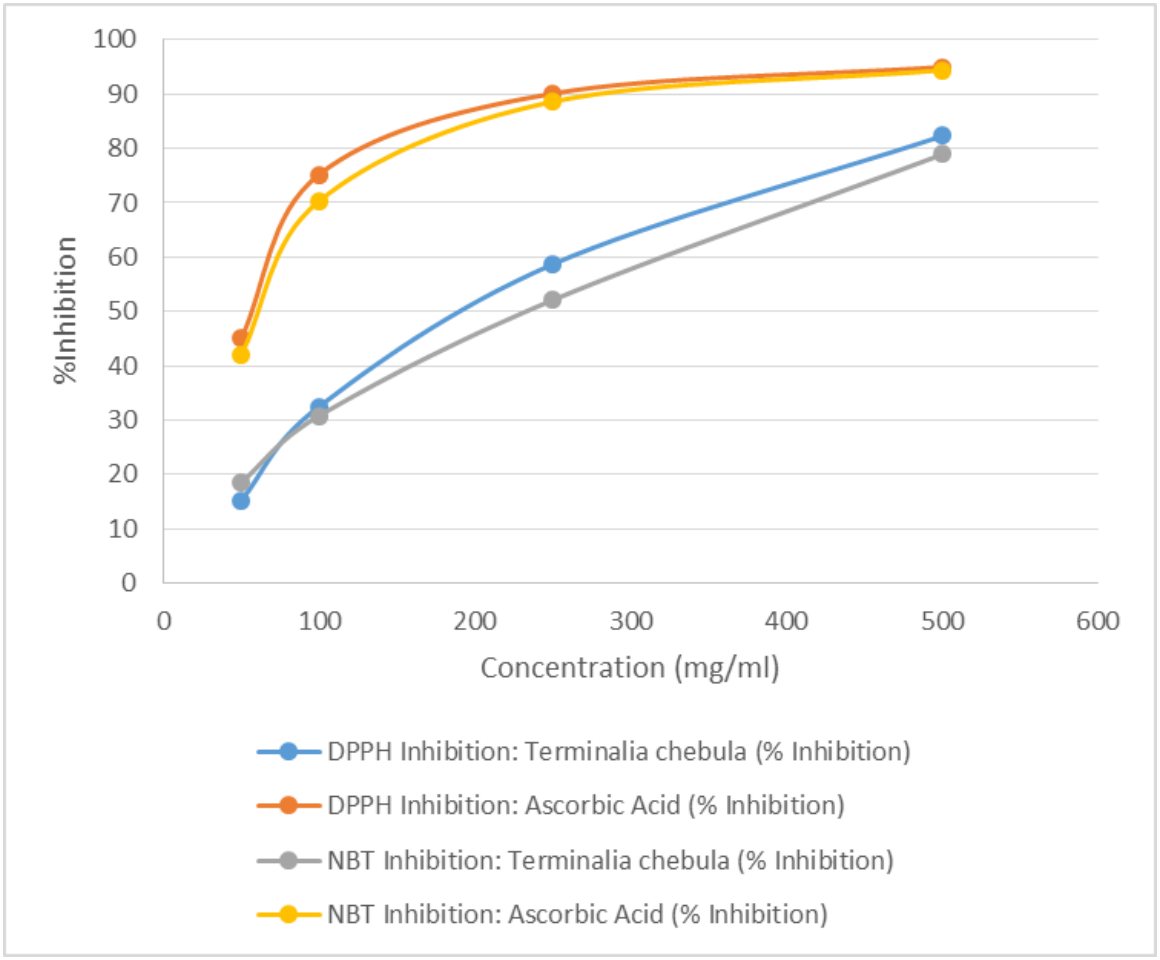

Figure 2: Comparison of the antioxidant activity of the extract using DPPH and NBT assays.

The NBT assay results echoed the findings from the DPPH assay, with *Terminalia chebula* extract showing an increase in % inhibition of superoxide radicals from 18.5% at 50 µg/mL to 78.9% at 500 µg/mL. This trend suggests that the extract can effectively neutralize superoxide radicals, which are often implicated in oxidative stress and related diseases. The inhibition of superoxide radicals is crucial, as these radicals can lead to the formation of more harmful reactive oxygen species (ROS) through various biological pathways. Again, ascorbic acid demonstrated higher % inhibition values across the concentrations tested, achieving 94.4% at 500 µg/mL with an IC_{50} of 23.7 µg/mL. This consistent performance of ascorbic acid in both assays reinforces its status as a benchmark for antioxidant activity.
The phytochemical analysis of *Terminalia chebula* reveals a diverse range of compounds contributing to its antioxidant potential. The presence of phenolic compounds, flavonoids, and tannins is well-documented in the literature, correlating with enhanced radical scavenging activities. These compounds

exhibit strong electron-donating abilities, allowing them to mitigate oxidative damage effectively. Additionally, the high concentration of tannins can enhance the extract's ability to chelate metal ions, reducing the generation of free radicals through Fenton-like reactions.

The substantial presence of these phytochemicals in *Terminalia chebula* supports the traditional uses of this plant in folk medicine, particularly for ailments related to oxidative stress, such as inflammation, cardiovascular diseases, and certain cancers [14,15]. Furthermore, the varying degrees of inhibition observed between the DPPH and NBT assays can be attributed to the different mechanisms of action and types of free radicals being targeted. While DPPH primarily assesses the ability to neutralize stable free radicals, NBT evaluates the inhibition of superoxide anions, which may react differently with various antioxidant compounds.

Conclusion

Overall, the results suggest that while *Terminalia chebula* fruit extract demonstrates significant antioxidant activity, its efficacy is context-dependent, varying with the type of assay used and the specific concentrations tested. The extract's potential as a natural antioxidant agent is promising, particularly in the context of developing functional foods or supplements aimed at mitigating oxidative stress. However, further investigations are warranted to isolate and characterize the specific phytochemicals responsible for the observed activities, as well as to assess their synergistic effects when combined with other natural antioxidants. This research could pave the way for innovative applications of *Terminalia chebula* in both traditional and modern healthcare practices, emphasizing the need to integrate ethnobotanical knowledge with contemporary scientific inquiry.

References

1. Rupani R, Chavez A. Medicinal plants with traditional use: Ethnobotany in the Indian subcontinent. Clinics in dermatology. 2018 May 1;36(3):306-9.
2. Guerrini A, Sacchetti G. From ethnobotany towards modern botanicals as paradigm of the medicinal plants research: the case of ayurveda. MEDICINAL & AROMATIC PLANTS. 2013;1(8):e136-1.
3. Upadhyay A, Agrahari P, Singh DK. A review on the pharmacological aspects of Terminalia chebula. Int. J. Pharmacol. 2014 Aug 15;10(6):289-98.
4. Prasad K. Current Status of Medicinal Plants in the Bokaro District of Jharkhand. International Journal of Scientific and Research Publications. 2015 Oct.
5. Kumari P, Tiwari SK, Choudhary AK. Current status of medicinal plants: A case study from the palamu tiger reserve & betla national park, Jharkhand, India. International Journal of Herbal Medicine. 2017;5(6):09-13.
6. Mahato NK, Prasad K, Mahato AK. Phytochemical Profiling of Aqueous Methanol Extract of Terminalia bellirica from Bokaro District of Jharkhand, India. European Journal of Medicinal Plants. 2024 Apr 12;35(2):11-28.
7. Mahesh R, Bhuvana S, Hazeena Begum VM. Effect of Terminalia chebula aqueous extract on oxidative stress and antioxidant status in the liver and kidney of young and aged rats. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease. 2009 Aug;27(6):358-63.
8. Sultan MT, Anwar MJ, Imran M, Khalil I, Saeed F, Neelum S, Alsagaby SA, Al Abdulmonem W, Abdelgawad MA, Hussain M, El-Ghorab AH. Phytochemical profile and pro-healthy properties of

Terminalia chebula: A comprehensive review. International Journal of Food Properties. 2023 Sep 22;26(1):526-51.

9. Vemuri PK, Dronavalli L, Nayakudugari P, Kunta A, Challagulla R. Phytochemical analysis and biochemical characterization f terminalia chebula extracts for its medicinal use. Biomedical and Pharmacology Journal. 2019 Sep 25;12(3):1525-9.
10. Rathinamoorthy R, Thilagavathi G. Terminalia chebula-review on pharmacological and biochemical studies. International Journal of PharmTech Research. 2014 Jan;6(1):97-116.
11. Walia H, Arora S. Terminalia chebula—a pharmacognistic account. Journal of Medicinal Plants Research. 2013 May;7(20):1351-61.
12. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food chemistry. 2012 Feb 15;130(4):1036-43.
13. Sim Choi H, Woo Kim J, Cha YN, Kim C. A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells. Journal of Immunoassay and Immunochemistry. 2006 Jan 1;27(1):31-44.