Original Research Article

Evaluation of in vitro Antioxidant Potential of hydromethanolic bark extract of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. Bark Extract

ABSTRACT

The qualitative phytochemical analysis and *in vitro* antioxidant activity of the hydromethanolic bark extract of *Terminalia arjuna* (TAE) was investigated to understand its pharmacological and medicinal importance using various *in vitro* antioxidant assays, including total antioxidant capacity and reducing power. The bark of *Terminalia arjuna* was collected from the Medicinal Plants Research and Development Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, India and authenticated. Qualitative biochemical analysis showed the presence of numerous phytochemicals in TAE. Antioxidant activities were evaluated using standard antioxidants like ascorbic acid for comparison. The extract consistently exhibited strong antioxidant activity in a concentration-dependent manner. The hydromethanolic bark extract of *Terminalia arjuna* showed notable total antioxidant capacity of 133.04 mg ascorbic acid equivalents per g of extract. The reducing power increased progressively with concentration, ranging from 0.045 at 20 µg/mL to 0.355 at 200 µg/mL. These findings support the potential of bioactive compounds for developing safe and cost-effective herbal treatments and thus could prevent many radical-related ailments. Further investigation may also promote environmentally sustainable practices.

***Keywords: Terminalia arjuna*, Total antioxidant activity, Reducing power, Bark extract**

1. INTRODUCTION

Since ancient times, natural herbs, plants and herbal products have been used to promote human health. The biological activities of these herbal materials are associated with their role in maintaining plant cell integrity under stressful and abnormal conditions (Venkatachalam *et al*., 2020). The Indian subcontinent holds a huge collection of medicinal plants that are employed in a variety of traditional therapeutic methods (Thakur *et al*., 2021). In India, medicinal plants have been used to treat a variety of ailments since the Rig Veda (4500-1600 BC), Atharva Veda, Charaka Samhita, and Susruta Samhita. According to the Botanical Survey of India (BSI), at least 30,000 (or two-thirds) of the 45,000 identified plant species are of high interest due to their therapeutic properties (Kumar, *et al*., 2023). The estimated total amount of plants implemented in various Indian medicinal systems is 2000 in Ayurveda, 1300 in Siddha, 1000 in Unani, 800 in Homoeopathy, 500 in Tibetan, 200 in Modern, and 4500 in folk. Traditional folk medicine in India makes use of around 25,000 effective herbal mixtures (Mukherjee et al., 2014; Sudha 2018). Secondary metabolites, particularly phenolic and flavonoid compounds, play a crucial role in protecting plant cells under adverse circumstances (Rocchetti *et al*., 2019). The antioxidant and free radical scavenging properties of plants are largely attributed to the presence of flavonoids, anthocyanins, and flavones (Pandey and Ambwani, 2022). Medicinal plants are considered rich sources of bioactive compounds and have gained significant attention for their ethnomedicinal applications in humans (Priya *et al*., 2019).

Terminalia species are renowned worldwide for their antioxidant and biochemical properties in traditional medicine (Dwivedi & Chopra 2014). *Terminalia arjuna*, a member of the Combretaceae family, is commonly known by various names such as Arjuna, Dhavala, Kaubha, Nadisaraja, Veeravriksha, Partha and Indradru. It is widely distributed across India, including regions like the Indian subcontinent, the Himalayan tract of Uttar Pradesh, Chota Nagpur, Odisha, West Bengal, Punjab, Deccan and Konkan (Amalraj and Gopi 2017). The bark of *Terminalia arjuna* has been used as a cardiotonic in heart failure, ischemia, cardiomyopathy, atherosclerosis and myocardial necrosis, as well as for the treatment of many human ailments such as blood disorders, anaemia, venereal and viral disease, and to maintain optimum health. It is used to treat fractures, ulcers and hepatic diseases, and it has hypo-cholesterolemic, antibacterial, antimicrobial, antitumoral, antioxidant, antiallergic, antifeedant, antifertility and anti-HIV properties (Kumar *et al*., 2013). It has been claimed that *Terminalia arjuna* has considerable hypolipidemic characteristics. It is believed that the saponin glycosides in *Terminalia arjuna* are responsible for its inotropic effects, while the flavonoids/ phenolics provide antioxidant activity as well as vascular amplification action, hence validating the plant's various activities for cardio-protective function (Maulik and Talwar, 2012). Numerous bioactive compounds such as arjunolic acid (Verma *et al*., 2012), arjunic acid (Khatkar & Ansari 2017), arjungenin (Rane et al., 2016), gallic acid (Suganthy et al., 2018) and terminoic acid (Ahmad et al., 1983) were isolated and characterized from the *Terminalia arjuna* bark. Bark of *Terminalia arjuna* was

also used in the food industry (Kumar et al., 2021; Bishnoi *et al.*, 2021) and as a natural colorant (Adeel *et al.*, 2022).

The phytochemical profile and oxidative potential of *Terminalia arjuna* highlight its possible applications in managing metabolic disorders in humans (Beigi *et al*., 2018; Stokes *et al*., 2020). While the ethnomedicinal benefits of *Terminalia arjuna* have been extensively studied, its application as a feed ingredient or in medicated feed for livestock largely unexplored. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethoxyquin, are commonly used in the feed industry to maintain food and feed quality (Lourenço *et al*., 2019). However, prolonged use of these synthetic compounds at high concentrations has been linked to tumour-promoting effects and neurodegenerative diseases (Kumar *et al*., 2018).

There is a growing interest in discovering plant-based alternatives that are safer, more affordable, and physiologically compatible with animals. Natural antioxidants derived from plants, such as *Terminalia arjuna*, have shown great potential as a healthier substitute for synthetic antioxidants (Meena *et al*., 2021). Despite the wealth of ethnomedicinal and pharmacological knowledge on *Terminalia arjuna*, its potential nutraceutical application in livestock remains underutilized. This creates an opportunity for further research to explore its use in promoting animal health and reducing feed-related risks.

In light of these considerations, the present study focuses on the phytochemical analysis and antioxidant capacity of *Terminalia arjuna* extract *in vitro*. The findings may pave the way for future studies on its potential to enhance cognitive functions and cellular health while mitigating the risks associated with oxidative stress. This could ultimately contribute to the development of natural feed additives for livestock, promoting sustainable animal production and human health.

2. MATERIAL AND METHODS

2.1 Collection of the plant material

The bark of *Terminalia arjuna* was collected from the Medicinal Plants Research and Development Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, India in 2021. The plant sample was rinsed with running tap water, shade dried, pulverized using a grinder and kept in airtight container at 4°C until further use. The identification of *Terminalia arjuna* was confirmed by Dr. D.S. Rawat, Assistant Professor, Biological Science Department, C.B.S.H., G.B. Pant University of Agriculture and Technology, Pantnagar, India. The voucher specimen was also deposited to Biological Science Department for documentation. The herbarium accession no is GBPU-1024.

2.2 Extraction procedure

The extraction of hydromethanolic *Terminalia arjuna* bark extract (TAE) was performed as per the procedure described earlier (Singh *et al*., 2021; Chand *et al*., 2024; Ambwani *et al*., 2024). One hundred gm of shade-dried bark powder was added to 1000mL of a 1:1 mixture of double distilled

water and methanol. For 48 hours the mixture was homogenized in an incubator cum shaker at 37°C. It was filtered through muslin cloth and subsequently through Whatman filter paper No. 1. The filtrate was concentrated by rotary evaporator at 45°C to remove excess solvent before being freeze-dried followed by lyophilization. The lyophilized extract was weighed and kept at -20°C in a deep freezer until further usage. The percent yield was determined by dividing weight of plant extract obtained by 100gm of dry powder.

2.3 Phytochemical analysis

Qualitative phytochemical tests for the identification of phenols, flavonoids, tannins, steroids, saponins, alkaloids, carbohydrate were carried out for TAE by using standard protocol (Singh *et al*., 2021).

2.3.1 Test for phenols

To check the presence of phenols, 2mL of plant extract was mixed with 2 mL of ferric chloride solution. A solution with a blue-green tint indicates the presence of phenols.

2.3.2 Test for flavonoids

Two mL of plant extract was added to 2mL of 10% lead acetate to detect the presence of flavonoids. The yellowish green hue indicated the presence of flavonoids.

2.3.3 Test for tannins

Few drops of 1% lead acetate was added to 5mL of the plant extract. The presence of yellow precipitate indicates the presence of tannins.

2.3.4 Test for steroids

The colour changed from violet to blue-green after addition of 2mL of sulphuric acid through the test tube's sides to the mixture containing 0.5g plant extract and 2mL of acetic anhydride indicated the presence of steroids.

2.3.5 Test for saponins

To 5mL of extract, 10mL of distilled water was added and rapidly shaken for 30 seconds. The presence of saponins is indicated by the formation of foam.

2.3.6 Test for alkaloids

Five mL of extract is mixed with 5mL of 2N HCl, heated, and filtered. A few drops of Mayer's reagent were added to the mixture. The presence of alkaloids was indicated by a colored precipitate.

2.3.7 Carbohydrates

One mL of extract was combined with 0.2mL of Molisch's Reagent. After mixing, the tube was tilted. Later without stirring, 0.5mL of sulphuric acid was slowly added through the test tube's edge. At the interface between the aqueous (upper) and acid (bottom) layers, carbohydrates were identified as a reddish-violet ring.

2.4 Evaluation of *in vitro* antioxidant potential of the plant extract

The *in vitro* antioxidative potential of the hydromethanolic*Terminalia arjuna* bark extract (TAE) was evaluated by total antioxidative capacity and reducing power capacity.

2.4.1 Total antioxidant capacity

Total antioxidant capacity of the plant extracts was determined using the Phosphomolybdenum technique using ascorbic acid as standard and represented as mg ascorbic acid equivalent/g extract as per the protocol (Sharma and Singh, 2012) with slight modifications. For the calibration curve, ascorbic acid ranging from 2 to 20µg was employed. Stock solution of TAE was prepared at a concentration of 2mg/mL. 1mL of freshly made phosphomolybdate reagent solution was added to 100µL of the plant extract. TAE reacted with a freshly made phosphomolybdate reagent solution (0.6 M sulphuric acid, 28mM tri sodium phosphate, and 4 mM ammonium molybdate) and reduced molybdenum (VI) to form greenish phosphate molybdenum (V) complex. After sealing the tubes, the reaction mixture was incubated at 95°C for 90 minutes in a boiling water bath. A UV-visible spectrophotometer was used to detect the absorbance at 695 nm after the samples had cooled to room temperature. The blank was the reaction mixture that contained all of the chemicals except TAE. Three replicates of the entire experiment were conducted. The straight-line equation derived from the ascorbic acid standard curve was used to compute the total antioxidant capacity.

2.4.2 Reducing power capacity

The reducing power of the TAE was determined as per the method of (Chand *et al*., 2024) with slight modifications.1mg/mL stock solutions of TAE and ascorbic acid were prepared. One mL of various concentrations of TAE and ascorbic acid, ranging from 20 to 200µg/mL, were incubated for 30 minutes at 50°C with 2.5mL of phosphate buffer (0.1 mol/L pH 6.6) and 2.5mL of 1 percent (w/v) potassium ferricyanide. 2.5mL of 10% (w/v) trichloroacetic acid (TCA) was added to the mixture to halt the reaction, and the mixture was centrifuged for 10 minutes at 3000 g. The top layer (2.5mL) was diluted with equal parts water and stirred with 0.5mL of fresh 0.1% ferric chloride. The absorbance at 700 nm was measured against blank with the help of a UV-visible spectrophotometer. The entire experiment was run three times, with three replicates. A higher absorbance suggested greater reducing power.

2.5 Statistical Analysis

Data analysis was done using Prism Software, with results presented as mean values accompanied by their standard deviations (±SD). The assessment of statistical differences between experimental and control groups was performed through one-way analysis of variance (ANOVA). Results were deemed statistically significant when probability values fell below 0.05 ($p < 0.05$).

3. RESULTS

3.1 Percentage Yield of Hydromethanolic Extract of *Terminalia arjuna*

The percent yield of authenticated *Terminalia arjuna* bark extract was obtained as 13.16% (Table 1, Fig. 1).

Table 1: Percentage Yield of TAE

Plant name	Weight of dried bark powder (g)	Weight of the extract obtained (g)	Percent yield
Terminalia arjuna (TAE)	100	13.16	13.16

Fig. 1: A) *Terminalia arjuna* (B) Dried bark powder of *Terminalia arjuna* (C) Hydromethanolic bark extract of *Terminalia arjuna*

3.2 Qualitative phytochemical Analysisof TAE

Preliminary qualitative screening of TAE indicated the presence of various phytochemicals, including phenols, flavonoids, tannins, steroids, saponins, alkaloids, carbohydrate. The findings from the phytochemical analysis are tabulated in Table 2.

Table 2: Results of phytochemical Analysis

S. No.	Phytochemical Analysis	TAE
1.	Phenolics	+
2.	Flavonoids	+
3.	Tannins	+
4.	Phytosterol	+
5.	Saponins	+
6.	Alkaloids	+

7.	Carbohydrate	+
8.	Proteins	+

3.3 Total antioxidant capacity of TAE

A key feature of the total antioxidant capacity assay is the reduction of Mo (VI) to Mo (V) in acidic conditions, resulting in the formation of a dark bluish-green Mo (V) complex. Total antioxidant capacity (TAC) of the different extracts was evaluated by the Phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. The results showed that TAE exhibited great antioxidant capacity.TAE has a total antioxidant capacity with value of 133.04 mg ascorbic acid equivalents g-1 extract (Table 3).

Table 3: Total antioxidant capacity of TAE in terms of ascorbic acid equivalents. Each valuere presents mean ± SD (n=3)

Plant Extract	**Total Antioxidant Capacity (AAE mg/g of dry matter)**			**Mean Total Antioxidant Capacity (AAE mg/g of dry matter) ± SD**
	I	**II**	**III**	
TAE	133.5	131.37	134.26	133.04±1.5

3.4 Reducing power capacity of TAE

The reducing power served as a significant indicator of the antioxidant activity. The reducing power analysis is based on the concept that antioxidant molecules present in plant extract develop a coloured complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which absorbs the most at 700nm wavelength. Enhanced absorbance is an indication of high reducing power, hence a greater absorbance values indicated that the plant extracts have significant reducing potential. Reducing power TAE was compared with ascorbic acid and is presented in Fig. 2. As we can see there is not much difference in the reducing power capacity of TAE and Ascorbic acid.

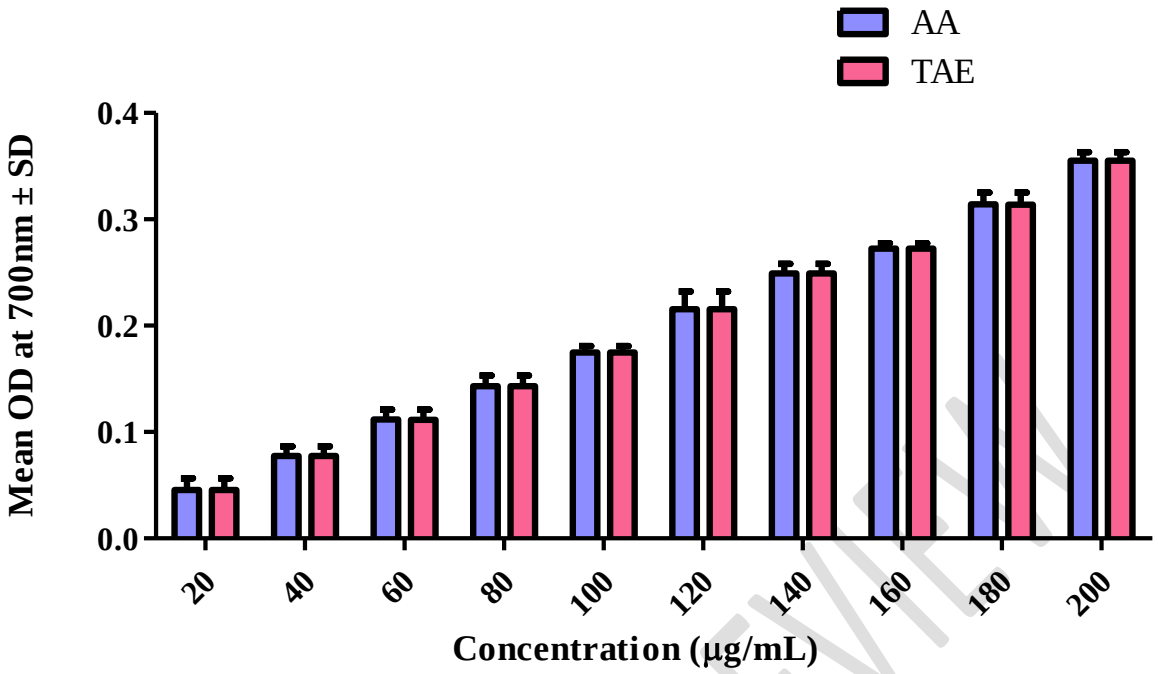

Fig. 2: Reducing power for TAE and Ascorbic acid. Each value represents mean ± SD (n=3)

4. DISCUSSION

Medicinal plants have long been recognized for their significance in promoting individual and community health. Phytochemical analysis of the medicinal plant extracts showed the presence of compounds known for their medicinal and physiological effects (Khan *et al*., 2022). Various studies have highlighted the antioxidant properties of different parts of medicinal plants, particularly those rich in phenolic compounds (Škrovánková *et al*., 2012; Chanda and Dave 2009; Lee *et al*., 2003). *Terminalia arjuna* is a commonly used medicinal plant in traditional medicine, known for its role in treating various degenerative diseases (Jain *et al*., 2009; Nema *et al*., 2012). This study aimed to assess the antioxidant properties of hydromethanolic bark extract of *Terminalia arjuna*. Antioxidant activity was evaluated using two methods: Total antioxidant capacity and Reducing power capacity. In this present study, preliminary phytochemical analysis revealed the presence of phytochemicals like phenols, flavonoids, tannins, steroids, saponins, alkaloids, carbohydrate in TAE. Natural antioxidants are primarily derived from plants in the form of phenolic compounds, including flavonoids, phenolic acids, tocopherols and others. The antioxidant effects of flavonoids result from various mechanisms, such as scavenging free radicals, binding to metal ions like iron and copper and inhibiting enzymes that generate free radicals (Ali *et al*., 2008). This hydromethanolic extract demonstrates strong antioxidant activity and also showed the presence of flavonoid. Flavonoids are hydroxylated phenolic compounds produced by plants in response to microbial infections, and they have been shown to possess antimicrobial

properties against a wide range of microorganisms (Evans and Cowan 2016). Recent studies have provided increasing evidence highlighting the role of reactive oxygen species (ROS) in the development of various diseases (Liu *et al*., 2018). Key cellular and extracellular targets for ROS include lipids, proteins, enzymes, DNA and RNA, with damage to these components being linked to degenerative conditions often referred to as "oxidative" diseases (Yang and Lian 2020). Most organisms are equipped with effective enzymatic and non-enzymatic defense mechanisms to regulate ROS levels (Hoffmann and Griffiths, 2018). However, external factors such as smoking, poor diet, alcohol consumption, certain medications and aging can impair these defense systems, disrupting the redox balance typically maintained in healthy states (Caliri *et al*., 2021). Consequently, antioxidants that neutralize ROS could play a crucial role in preventing or slowing the progression of oxidative diseases. Our research expands on current knowledge by specifically examining the antioxidant capacity of the bark extract of *Terminalia arjuna*. The findings reveal a notable total antioxidant capacity in TAE 133.04 mg ascorbic acid equivalents g^{-1} extract, consistent with observations from other studies on *Terminalia arjuna* extracts. *Terminalia arjuna* methanol extract was shown to have the highest overall antioxidant capacity, followed by ethanol. According to (Bodke *et al*.,2013), the maximum antioxidant capacity was identified in the ethanolic bark extract of *Terminalia arjuna*, with a value of 85.73 mg ascorbic acid/g extract, while the lowest capacity was found in the chloroform extract, with a value of 61.13 mg ascorbic acid/g extract. (Kumar *et al*., 2016) investigated the antioxidant activity of *Terminalia arjuna* bark and leaves and determined that bark extract had more antioxidative potential than leaves extract. The antioxidative activity of *Terminalia arjuna* leaves and bark was shown to be positively correlated with total phenolics and flavonoid content. In the present study reducing power of TAE was determined and measured as a function of their concentration. It was observed that TAE possesses potent reducing capacity. The reducing power of the extract was comparable to the ascorbic acid. Presence of various phytochemicals, predominantly polyphenolic content are responsible for the antioxidative activity of the plant. Shahriar *et al*. (2012) also observed the same trend in the reducing power of bark extract of *Terminalia arjuna*. (John 2024) conclude that stem bark powder of *Terminalia arjuna* can be supplemented levels upto 400g/kg of diet without affecting adversely the performance or health status of guinea fowl. (Sharma *et al*., 2024) suggested that 1% Terminalia arjuna bark powder have positively influenced egg quality and composition of Uttara chicken indicating its potential as a dietary supplement in poultry. The results suggested that TAE may strengthen the intracellular antioxidant defence by its antioxidant phytoconstituents to cope up with the free radical mediated oxidative stress. Thus, might be a good candidate for further investigation in developing new antioxidants.

5. CONCLUSION

This study revealed that the hydromethanolic bark extract of *Terminalia arjuna* possessed potential sources of natural antioxidants which was very evident through its great total antioxidant capacity and reducing power. The antioxidative effect of TAE is mainly attributed to phenolic components, such as flavonoids and phenolic acids, which have the ability to scavenge free radicals, donate hydrogen atoms or electron or chelate metal cations. Therefore, TAE can be used as a source of excellent natural antioxidants for health benefits and pharmacological properties. TAE can also be used as natural antioxidants in place of synthetic antioxidants in foods. However, they should be tested in the food systems under various processing and storage conditions. Further research also needed to determine their safety for human consumption. A further characterisation of biologically active compounds of plant extract showing high antioxidant capacity and total phenols would also be useful. It can also be used as a feed additive in the poultry industry.

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