RESEARCH PAPER

Evaluation of Antioxidant, Anthelmintic, Antibacterial Activities and Pharmacognostic and Phytochemical Properties of Terminalia Ivorensis A. Chev. Leaves

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ABSTRACT

For thousands of years, plants have been used as natural medicines in traditional medicinal systems. Alkaloids, flavonoids, tannins, saponins, and steroids are examples of active chemicals that affect a plant's therapeutic effectiveness. This study looks into Terminalia ivorensis leaf extraction, phytochemical composition, anthelmintic, antibacterial, and antioxidant qualities. The maximum extraction yield was obtained from the hydro methanolic extract (30:70). Through phytochemical analysis, several beneficial compounds were discovered, including tannins, phenols, flavonoids, saponins, steroids, glycosides, carbohydrates, and proteins. The hydro methanolic extract (HME) exhibits the highest diversity of phytochemicals. The evaluation of anthelmintic activity of HME against Pheretima posthuma revealed dose-dependent action. HME shown strong antibacterial activity when tested against S. aureus and E. coli. With an IC 50 value of 74.501 µg/ml, the extract showed a significant capacity for radical scavenging when the antioxidant activity of HME was evaluated using the DPPH technique. An opportunity to preserve and research this effective medicinal herb for possible future usage in pharmaceuticals is presented by this study.

Keywords: Terminalia ivorensis, anthelmintic activity, antioxidant activity, antibacterial activity.

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INTRODUCTION

India possesses a wealth of knowledge about cultures, medicinal plants, and biodiversity (Shilpi et al., 2025). An estimated 40,000 different plant species exist on Earth (Ni et al., 2025). Plant-based therapy has evolved from traditional cures to contemporary pharmaceuticals, demonstrating the amazing healing ability of nature (Deepika et al., 2024). Secondary metabolites such as alkaloids, phenols, flavonoids, glycosides, tannins, resins, triterpenoids, carbohydrates, steroids, and essential oils are responsible for the medicinal qualities of plants (Ali et al., 2025). They're generally intended to help plants protect themselves from negative environmental factors as well as the effects of microorganisms, insects, and animals (Padmalatha et al., 2023). A member of the Combretaceae family, *Terminalia ivorensis* A. Chev. is commonly referred to as Idigbo. It is a huge, tall, straight deciduous forest tree found in South Asia, Africa, and Central America. Different portions of this plant have been used for a variety of traditional medical purposes by numerous civilisations (Norgrove et al., 2000). The antioxidant, antibacterial, anthelmintic, phytochemical composition, and pharmacognostic properties of *T. ivorensis* leaf extract are all investigated in this work.

MATERIALS AND METHODS

Plant Collection and Authenticity

Using Voucher No. 0771, Dr. K. Madhava Chetty, Assistant Professor in the Botany Department of S.V. University in Tirupati, Andhra Pradesh, confirmed *Terminalia ivorensis* A. Chev. I saved that voucher specimen for later use. The plant leaves were dried in

the shade for several days before being coarsely powdered and extracted.

Analysis of Organoleptic

The term "organoleptic" describes something that is detectable by the senses of taste, smell, touch, and appearance. Sense and thereby determine specific characteristics of the substance, which can be considered a first step in identifying its identity and degree of purity (Vikrant et al., 2012; Jarald et al., 2010). The dried leaves and extracts of the *T. ivorensis* leaves were gathered, analysed, and enumerated for several attributes, such as yield, size, colour, taste, touch, odour, and consistency.

Examination of Foreign Substances

A thin layer of the drug sample (100–500 g) or the maximum amount recommended in the monograph should be spread out. The unaided eye or a 10x lens should be used for inspection in order to identify the foreign material. Once it has been separated and weighed, determine what percentage is present (Khandelwal et al., 2016; WHO Press, 1998).

The Evaluation of Physicochemical

As far as possible, medicinal plants should be free of mildew, insects, and other animal pollutants. The pharmacopoeia monograph states that the amount of foreign materials in a product should not exceed the standard that is controlled. To undertake the physical evaluation of crude medications, a variety of physico-chemical techniques are employed to ascertain certain physical constants (Khandelwal et al., 2016). The drying loss, extractive values, and ash values were calculated in accordance with the formal protocols described in WHO guidelines on quality control methods for medicinal plant materials (WHO Press, 1998).

Index of Froth and Swelling

Gums and medications that include high levels of mucilage, pectin, or hemicelluloses are particularly useful for therapeutic or medical purposes due to their capacity to swell. The foaming index measures how much an aqueous decoction of extracts of medicinal plants foams. An agitated aqueous infusion may produce a persistent froth due to saponin (Khandelwal et al., 2016). The WHO guidelines' methods were used to calculate the swelling and foaming indices (WHO Press, 1998).

Observation of Fluorescence

When various chemicals and solvents are applied to plant material, the chemical components present display a phenomenon called fluorescence. The current work investigated the fluorescence of powdered *T. ivorensis* plant leaves under UV and visible light/daylight settings following treatment with different agents and solvents. A little amount of dried and finely powdered material was treated with a variety of solvents, alkaline solutions, and freshly prepared acids. The powder sample was treated with acids such as concentrated HNO_3, 1N HCl and 1N H_2SO_4, and acetic acid in addition to alkaline solutions such as 1N alcoholic & aqueous NaOH and different solvents such as ammonia, distilled water, 5% $FeCl_3$, iodine, methanol, and ethanol. Their fluorescence in visible and ultraviolet light was studied (Kavitha et al., 2014; Sofowara et al., 1993).

Preparation of Plant Extract & Phytochemical Screening

Soxhlation is required when the contaminant is insoluble in a solvent and the desired substance has limited solubility in that solvent. If the prescribed component is highly soluble in a solvent, it can be extracted from the insoluble material using a simple filtration procedure. Instead of passing many quantities of heated solvent through the sample, this method has the advantage of reusing a single batch of solvent. This method cannot be used for thermolabile compounds since prolonged heating can degrade the substance. The leaves of T. ivorensis were successfully dried and extracted (100g) using a Soxhlet system and a sequential solvent extraction process, which used solvents with increasing polarity such as petroleum ether, chloroform, ethyl acetate, hydro methanol (30:70), and water. The residue was dried and extracted using the subsequent solvent once each extraction was finished. To remove any debris, the extracts were filtered through muslin fabric and concentrated. Afterward, the extracts were allowed to air dry. Colour, consistency, and yield percentage were calculated. The concentrated extracts were put through a qualitative test in accordance with standard protocols to identify different phytochemical ingredients (Khandelwal et al., 2016; Kokate et al., 2017; Prakash et al., 2019).

Microscopic Powder Analysis

With the use of an electric grinder, shade-dried leaves were ground into a fine powder. Using the standard methods, powder microscopy was performed on this

fine powder (Khandelwal et al., 2016; The Ayurvedic Pharmacopoeia of India, 2004).

Assessment of Anthelmintic Action

The efficacy of hydro methanolic extract of leaves as anthelmintics against *Pheretima posthuma* was investigated. The paralysis and death periods of the worms were assessed using a bioassay at extract concentrations of 10, 25, and 50 mg/ml. The control was saline water, and the standard reference was albendazole. Adult Indian earthworms were used for the test. Before being employed for anthelmintic research, earthworms were thoroughly cleaned with normal saline after being removed from damp soil. The earthworms were divided into four groups of six each. Small amounts of the extract were mixed with water, and the volume was then adjusted to 20 millilitres using saline water. In petri dishes, extract and standard drug solutions were added. All of the earthworms were placed in a 20 ml solution that contained hydro methanolic extract (ratios of water and methanol 30:70) and albendazole in different concentrations. The duration required for individual worms to become paralysed and die was recorded. It was time for paralysis when there was no movement keep when the worms were agitated violently. When immersed in heated water (50^0C), the worms lost their ability to move, and their body colours started to fade, indicating their death (Neha et al., 2011; Shagana et al., 2021).

Assessment of Microbial Inhibition

All Petri plates and graduated measurement pipettes were sterilised by heating them to 120 degrees Celsius for one hour in an autoclave. An autoclave was used to steam sterilise the material for 20 minutes at 121 degrees Celsius (15 psi). The nutritional agar used to make each plate was the same thickness. The well plate method was used to conduct the antimicrobial test. Through dissolution in water, three test concentrations of the hydro methanolic extract of leaves were created: 200, 400, and 600 µg/ml. The standard gentamycin concentration was 10 µg/ml. The control used was water. A sterile borer was used to scrape off media from a petri dish that had been injected with the organisms, creating bores with a diameter of 5 mm. Petri dishes were then incubated at 37 ° C after the solutions of each test substance and reference standards were added individually. The zones of inhibition were assessed following an overnight incubation period (Sandhya et al., 2010).

Assessment of Antioxidant Activity by DPPH Method

1. Making a Hydro Methanolic Solvent (30:70 V/V)

Add thirty millilitres of distilled water to seventy millilitres of methanol. The extract and DPPH solution will be dissolved using this.

2. Making a Solution of Plant Extract

Weigh a reasonable amount of the powdered plant extract (10 mg). Dissolve it in ten millilitres of hydro methanolic solvent to create a stock solution containing 1 mg/mL. For testing, create serial dilutions at 10, 20, 40, 60, 80, and 100 µg/mL.

3. Making a Solution of DPPH

To make a 0.1 mM solution, weigh 3.94 mg of DPPH and dissolve it in 100 mL of hydro methanol (30:70 v/v). Keep the solution away from light.

4. The Process of Making Ascorbic Acid

Weigh out 1.76 mg of ascorbic acid and dissolve it in 100 mL of hydro methanol (30:70 v/v) to create a 0.1 mM solution. Ascorbic acid is air and light sensitive. The solution should be kept in an amber-colored bottle.

5. The Method of Assay

With a few modifications, the plant extract's ability to scavenge free radicals was assessed using the technique described by Swapnil et al. (2024) using DPPH. To 1 mL of plant extract solution in different strengths, add 2 mL of DPPH solution. For half an hour, incubate at room temperature in the dark. The absorbance at 517 nm should be measured with a UV-Vis Spectrophotometer.

The Blank Solution

For base line correction, use 3 mL of hydro methanol solvent (absorbance: 0.000).

Solution of Control

Combine 2 mL of DPPH solution with 1 mL of hydro methanol solvent. Measure absorbance after incubating as described above.

Sample: 1 mL of plant extract plus 2 mL of DPPH solution.

Standard: Ascorbic acid (As with the plant extract, use the same series of dilutions).

The percentage scavenging activity of the plant extract was calculated using the formula below.

Scavenging activity is expressed as (Ab control − Ab sample /Ab control) × 100.

where the absorbance of the sample is indicated by Ab sample and the absorbance of the control by Ab control.

Calculation of IC_{50}

Plot the concentration (X-axis) against the percentage inhibition (Y-axis).

OUTCOMES AND DISCUSSION

Sensory Evaluation

It is a crucial metric for the qualitative characterisation of the morphological and sensory features of plant leaves and extracts. The characteristics, colour, taste, smell, shape, consistency, and texture listed in Tables 1 and 2 were found during the investigation.

Table 1: Organoleptic Properties of *T. ivorensis* Leaves

Attributes	Description
Colour	Green
Smell	Mild
Taste	Astringent
Shape	Broadly obovate to elliptic
Texture	Smooth

Table 2: Organoleptic results of various extracts of leaves

Properties	Pet. Ether	Chloroform	Ethyl acetate	Hydro methanolic	Water
Colour	Blackish-brown	Jet black	Brownish black	Dark brownish black	Reddish brown
Odour	Agreeable	Agreeable	Agreeable	Agreeable	Agreeable
Consistency	Sticky	Sticky	Sticky	Semisolid	Sticky paste
% Yield	1.5	1.99	0.95	10.98	4.53

Figure 1: Pet. ether extract

Figure 2: Chloroform extract

Figure 3: Ethyl acetate extract

Figure 4: Hydro methanolic extract

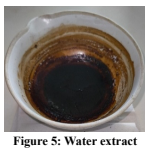

Figure 5: Water extract

Examining foreign substances

The presence of undesired and extraneous material that is not a part of the original plant material is indicated by this quality control test. The quality and purity of plant materials are evaluated using this test. Table 3 tabulates the result obtained.

Table 3: Foreign matter analysis

Analysis of foreign matter	Percentage (%) W/W
Total foreign matter	Below 0.001

Physical-chemical analysis

In order to accomplish the various goals of evaluating crude medical products, including determining their identity, purity, and quality, physical evaluation procedures are effectively employed. Data on drying loss, ash values, extractive values in different solvents, swelling index, and foaming index are shown in Table 4.

Table 4: Physicochemical Specifications

Attributes	Values % (w/w)
LOD	9
Total amount of ash	6
Water soluble ash	3.4
Acid insoluble ash	1.7
Index of Swelling	0
Index of Foaming	0
Values of Extraction % (w/w)	
Petroleum ether	0.092
Chloroform	0.122
Ethyl acetate	0.058
Hydro methanolic	0.675
Water	0.278

Observation of fluorescence

The powder sample of leaf was treated with a variety of compounds to examine its fluorescence behaviour under visible, short, and long light; table 5 summarises the results of the study.

Table 5: Examination of fluorescence observations

Treatments	Visible	UV short (254nm)	UV long (365nm)
Powder	Olive-brown	Greyish green-brown	Greenish brown
Powder + Distilled water	Greenish brown	Light green	Dull green
Powder+ 5% FeCl3	Olive green	Brownish green	Dark green
Powder + Iodine	Greenish brown	Reddish brown	Blackish green
Powder +1N NaOH (aqueous)	Yellowish-green	Dark green	Citrus green
Powder +1N NaOH (alcoholic)	Yellowish-green	Olive green	Greenish yellow
Powder + CH_3COOH	Pale brown	Dull yellow	Yellowish greenish
Powder + Conc. HNO_3	Cinnamon-brown	Dull red	Reddish-brown
Powder + 1N HCl	Brown	Dull green	Faded green
Powder + 1N H_2SO_4	Greenish brown	Brownish green	Deep green
Powder + Ammonia	Brownish green	Pale green	Emerald green
Powder + Ethanol	Brownish green	Pale yellow green	Yellow green
Powder + Methanol	Greenish-brown	Pale green	Green

The colour that plant materials release when exposed to UV light as a result of reagent reactions or natural compounds can be used to identify them using fluorescence analysis. Flavonoids, phenolics, and tannins are frequently indicated by green, yellow, or red fluorescence. The presence of starch is confirmed when iodine turns black or blue. Polyphenols and other polar chemicals are frequently extracted by methanol and ethanol. Tannin concentration is indicated by a ferric chloride reaction that turns green or black. Strong fluorescence is frequently produced by phenolic groups in alcoholic and aqueous NaOH (Table 5). Every kind of material has a distinct, vivid colour. Diverse plant materials yield diverse colours when exposed to various chemicals and solvents (Sumithra et al., 2016; Suriyavathana et al., 2018).

Testing for phytochemicals

The bioactive components varied depending on the extract type and extraction method. Therefore, using the normal procedure outlined in the methodology section, the extracts underwent initial phytochemical screening. Verification of the chemical makeup of the active ingredients in the leaf extract is aided by chemical testing. The hydro methanolic extract had the highest concentration of secondary metabolites, according to the results, as indicated in table 6. It was therefore used further in each of the examinations that followed.

Table 6: Preliminary assessment of various leaf extracts' phytochemical composition

Name of compound	Petroleum ether	Chloroform	Ethyl acetate	Hydro methanolic	Water
Tannins & Phenolics	−	−	+	++++	+++
Flavonoids	−	+	−	++	--
Steroids	−	−	+	++	--
Saponins	−	−	−	++	+++
Glycosides	−	−	−	++	−
Alkaloids	+	+	−	−	−
Amino acids	−	−	−	−	−
Proteins	−	+	−	++++	−
Carbohydrates	−	−	−	−	++

(Where – Absent, + Slightly significant, ++ Moderately significant, +++++ Highly significant)

Powder microscopic examination

Reticulate epidermal cell: The leaf's epidermis, which might be uneven or polygonal, has a net-like look. It is employed for leaf identification (Fig.6).

Unicellular covering trichome: It is a non-glandular, covering, unicellular trichome. It has a curved base and a pointed end (Fig. 7).

Reticulate xylem vessel: It is classified as a xylem vessel and has thick-walled, irregular or ladder-like vessels. Among their roles are water conduction and plant mechanical support (Fig.8).

Anomocytic stomata: In this kind, stomata are encircled by a variable number of epidermal cells that are identical to one another (i.e., no separate subsidiary cells) (Fig.9).

Brownish matter (tannin-rich content): One distinguishing characteristic of Terminalia species' leaves is their amorphous brown substance. Which possess astringent, antibacterial, or antioxidant qualities. The identity and quality of crude drug evaluation are supported by all of these characteristics (Fig. 10).

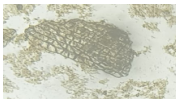
Figure 6: Reticulate epidermal cell


Figure 7: Unicellular covering trichome


Figure 8: Reticulate xylem vessel


Figure 9: Anomocytic stomata

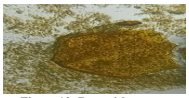
Figure 10: Brownish matter

Anthelmintic efficacy

Higher extract concentrations decreased the time to death for all worms and produced paralysis significantly earlier, according to the current study. The hydro methanolic extract of leaf demonstrated the quickest time of paralysis (P) and death (D) at a dosage of 50 mg/ml, suggesting dose-dependent anthelmintic activity. Albendazole was utilised as a reference standard to evaluate the anthelmintic activity (Table 7). These results imply that leaf extract is high in these substances, such as tannins, phenolic compounds, saponins, flavonoids, steroids, and glycosides, may be a useful substitute for synthetic anthelmintic medications, providing a safer and more natural means of managing parasitic diseases. Tannins have been found to be responsible for preventing the development and movement of several helminth stages. Direct damage to the worm's cuticle and hypodermis may cause it to become immobile and eventually die. Phenolic substances' potent antioxidant properties can enhance the host's overall health and indirectly aid in the anthelmintic activity. Additionally, they have the ability to disrupt the parasites' metabolic functions. Known for their anti-inflammatory and antioxidant properties, flavonoids can disrupt the energy metabolism of helminths, decrease their viability and make removal easier (Hoste et al., 2006; Barrau et al., 2005; Brunet et al., 2006; Waller et al., 2004). Moreover, saponins can paralyse the worm by preventing it from absorbing nutrients or by increasing membrane permeability, which can allow vital internal components to seep out and ultimately lead to the parasite's demise. Steroids may cause paralysis and impaired muscle coordination by interfering with parasite neuroreceptors. Glycosides are also paralysing and neurotoxic (Nwosu et al., 2011; Saha et al., 2013; Satheesh et al., 2012).

Table 7: HME *in vitro* anthelmintic properties

Group	Concentration (mg/ml)	Paralysis (min)	Death (min)
Normal	----------	----------	----------
Hydro methanolic extract	10 25 50	26 15 11	35 24 21
Albendazole	25	18	25

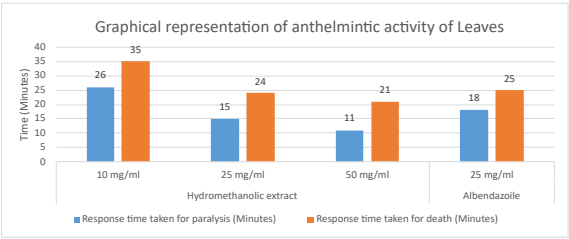

Figure 11: Bar graphical representation of anthelmintic activity of HME of leaf vs Albendazole

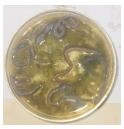
Figure 12: 10 mg/ml extract


Figure 13: 25 mg/ml extract

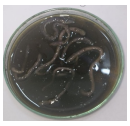
Figure 14: 50 mg/ml extract


Figure 15: 25 mg/ml (Albendazole)

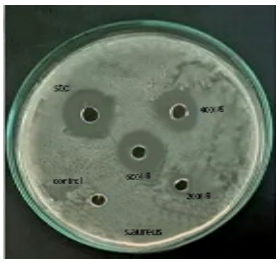

Figure 16: *S. aureus*

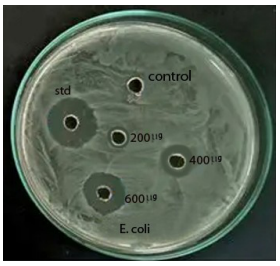

Figure 17: *E. coli*

Antimicrobial activity

Table 8: Inhibition zone

Organism	Plant extract (µg/ml)			Standard (µg/ml)
Concentration	200	400	600	10
S. aureus inhibition zone (mm)	5	10	12	14
E.coli inhibition zone (mm)	5	8	12	14

The antibacterial activity assessed on the bacterial strains *S.* aureus and *E. coli* using the agar well diffusion method. Table 8 displays the inhibition zone of the sample and standard against both S. aureus and E. coli. The minimum inhibitory concentration, or MIC, was determined to be 200 µg/ml, which is the value at which observable inhibition occurs (i.e., any noticeable inhibition zone >0 mm). At 600 µg/ml, the extract demonstrated significant efficacy against both S. aureus, and E. coli. According to the findings, this extract may be utilised to treat bacterial illnesses brought on by *S. aureus* and *E. coli*. Because the extract contains flavonoids, tannins, phenolic substances, steroids, saponins, and glycosides, it has antibacterial properties. Phenolic chemicals are what cause microbial DNA, protein, and lipids to be damaged; tannins work by disrupting cell walls and precipitating proteins. Flavonoids that disrupt cell membranes and prevent the production of nucleic acids (Scalbert et al., 1991; Daglia et al., 2012; Cushnie et al., 2005). Steroids can stop microorganisms from growing and replicating by altering metabolism, which causes cell lysis. By reacting with sterols in microbial membranes, saponins induce cell lysis. Glycosides prevent the production of nucleic acids and microbial metabolism (Ali et al., 2001; Akinpelu et al., 2008; Mandal et al., 2005; Igbinosa et al., 2009). These elements work together to boost the antibacterial potency.

Antioxidant activity by DPPH method

Chemicals that have a single unpaired electron are called free radicals. Health problems are associated with these free radicals. Antioxidants, which are vital for protecting the body from oxidative stress, which is connected to several chronic diseases, are among the many health benefits of medicinal plants. The radical is neutralised when an antioxidant reacts with an unpaired electron. The free radical DPPH has a rich violet hue in solution and is stable at room temperature (Mensor et al., 2001). By contributing an electron or hydrogen, an antioxidant, like a plant extract, can reduce DPPH and cause a solution to become pale yellow. The degree of fading indicates the strength of the antioxidant. DPPH provides a rapid and easy way to evaluate antioxidants. These chemicals, which include flavonoids, phenolic compounds, terpenoids, and alkaloids, function to neutralise free radicals and protect cells from oxidative stress by scavenging reactive oxygen species (ROS), preventing lipid peroxidation, and metal ion chelation. For example, flavonoids neutralise free radicals by giving them hydrogen atoms. Polyphenols from stable antioxidant complexes, which stop more oxidative damage. The antioxidant defence systems of cells are strengthened by terpenoids, and alkaloids may shield mitochondria from oxidative damage. The combined effects of these bioactive substances in herbal extracts increase their potential as a treatment for illnesses linked to oxidative stress (Pankaj et al., 2025). The results of the plant extract and ascorbic acid's ability to scavenge free radicals against the DPPH radical are shown in Table 9, and the data is graphically represented in Figure 18.

Table 9: % inhibition of hydro methanolic extract of *T. ivorensis* leaves and standard ascorbic acid against DPPH at 517nm

Concentration µg/mL	Absorbance of extract	% inhibition of extract	Absorbance of Ascorbic acid	% inhibition of Ascor

				bic acid
10	0.807	12.756%	0.705	23.783%
20	0.715	22.702%	0.600	35.135%
40	0.615	33.513%	0.475	48.648%
60	0.535	42.162%	0.355	61.621%
80	0.435	52.972%	0.255	72.432%
100	0.345	62.702%	0.165	82.162%

According to table 9, ascorbic acid demonstrated a percentage inhibition of 82.162% at a concentration of 100 µg/mL, whereas hydro methanolic extract demonstrated a percentage inhibition of 62.702 percent. The concentration at which 50% inhibition occurs is known as the IC50. According to the above results, 50% inhibition of plant extract occurs between 60 and 80 µg/mL (74.501 µg/mL), while ascorbic acid occurs between 40 and 60 µg/mL (42.084 µg/mL). The extract had a good ability to scavenge radicals. Figure 18 illustrates the correlation between the concentration (µg/mL) and the percentage of inhibition for ascorbic acid and plant extract. For both samples, the percentage of inhibition rises with increasing concentration. At all doses, ascorbic acid has greater antioxidant activity than the extract, as evidenced by its steeper slope and higher inhibition values.

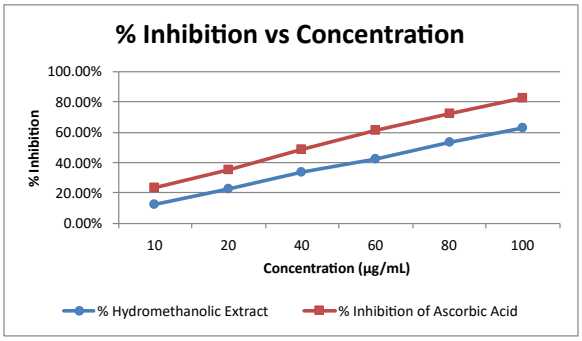

Figure 18: Relationship between concentration, and % inhibition of extract and ascorbic acid

CONCLUSION

This investigation revealed promising phytochemical and pharmacological potential. Bioactive components that are known to contribute to a variety of therapeutic benefits, including tannins, phenolics, flavonoids, saponins, steroids, and glycosides, were confirmed to be present by phytochemical screening. In order to standardise and control the quality of the plant material, pharmacognostic evaluation yielded crucial identifying factors. Based on its antioxidant qualities, the plant might be a natural source of antioxidants. Anthelmintic activity, which showed dose-dependent worm paralysis and death, validated the plant's traditional use to treat helminth infections. The antibacterial assay demonstrated significant inhibitory effects against certain bacterial strains, suggesting that it can be used to treat bacterial illnesses. Overall, the results demonstrate the potential of *T. ivorensis* leaves as a source of natural chemicals for pharmacological and therapeutic uses and support their use in ethnomedicine. To confirm and build on these findings, more thorough research is advised, including in vivo tests and the isolation of active molecules.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The writers of this article state that they have no relevant conflicts of interest.

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