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INVESTIGATION OF ANTICOCCIDIAL ACTIVITY OF METHANOLIC STEM BARK RXTRACT OF TERMINALIA MACROPTERA FULL & PEER AGAINST A IAN COCCIDIOSIS IN BROILER CHICKEN

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ABSTRACT

Avian coccidiosis remains a major challenge in poultry production, often leading to economic losses and reduced productivity. The emergence of drug-resistant strains of *Eimeria* spp. necessitates alternative therapeutic approaches. This study evaluates the phytochemical composition, GC-MS profile, and anti-coccidial efficacy of *Terminalia macroptera* stem bark extract in broiler chickens. Methanol extraction and chromatographic fractionation yielded three distinct fractions, which were subjected to phytochemical screening and GC-MS analysis. In vivo studies were carried out using broiler chickens infected with *Eimeria tenella*. The birds were divided into four pens. Pens I–III contained four treatments each as follows, 50mg/Kbw,100mg/Kbw,150mg/Kbw, and 200mg/Kbw, and treated with Fractions 1, 2, and 3, respectively. Pen IV served as the control with three treatments: (1) infected but untreated, (2) infected and treated with a standard drug, and (3) uninfected and untreated. Oocyst counts, prophylactic activity, and acute toxicity studies were conducted to evaluate efficacy and safety. The phytochemical analysis revealed the presence of diverse secondary metabolites such as alkaloid, phenolics, tannins, terpenoids and flavonoids, which are known to possess antimicrobial and antiparasitic activities. GC-MS profiling confirmed the presence of bioactive compounds with potential anticoccidial properties such as 2,4-Di-tert-butylphenol, palmitic, stearic, oleic, linoleic with their methyl/ethyl esters and unsaturated aldehydes/ketones which reported to have antimicrobial/antioxidant properties and could act synergistically with fatty acids or alter parasite oxidative stress response. Fraction 2 demonstrated significant reduction in oocyst count (0.6×10^3/g), comparable to the standard anticoccidial drug (0.7×10^3/g). The presence of bioactive compounds such as fatty acids, esters, and phenolics suggests a multifaceted mechanism of action. These findings support the potential of *T. macroptera* as a natural alternative in coccidiosis management.

KEYWORD: Pytochemical, *Avian-coccidiosis, Terminalia macroptera*, anticoccidia, Broiller chicken.

INTRODUCTION

Avian coccidiosis is a complicated ailment of the intestines, caused by the obligatory protozoan parasites that are the members of the genus Eimeria. This is a disease that causes huge losses even in the most advanced countries of the world (Garba *et al*., 2018). A recent approximation suggested that coccidiosis alone might cost the US chicken industry around 127 million dollars and 1.4 billion Indian Rupees every year, while other places would face similar losses in proportion (Shola, 2017). From this approximation, it is clear that if such an enormous loss could be incurred in countries like the United States of America (USA), then how much more fatal and uneconomical could it be in Asian and Sub-Saharan Africa, where the health management practices are still very rudimentary. Hence, coccidiosis is probably the most costly and widespread infectious disease in the poultry industry, which is a major obstacle to be overcome if sufficient progress is to be made in protein supply, particularly in the developing areas of Sub-Saharan Africa and Asia. To make matters worse, the emergence of drug resistance has made the situation

even more complicated regarding the success that has been achieved with the use of chemoprophylaxis and anticoccidial feed additives in controlling this disease, as these methods also often have poisonous side effects on the health of the animals (Nogueira *et al*., 2009).

The use of botanicals over the years has played a major part in the control of avian coccidiosis, as they are not only natural products but may comprise new therapeutic molecules to which resistance has not yet developed (Thangarasu *et al*., 2016). Natural products are the substances which originate from plants, animal, microbial and marine sources. The constituents which are identified and isolated from plants have been useful as a lead for a variety of drugs from many decades. About 40% of pharmaceutical products which are used in the present time are mainly derived from natural sources (Calixto *et al*., 1998). In the recent years, because of increasing interest in the use of pharmaceuticals, natural substances are the major source of complementary or alternative medicines, which are used in the treatment of many diseases (Calixto *et al*., 1998). In the 19th century, due to the advancements in the field of pharmaceutical chemistry especially the medicinal chemistry more than 25% of drugs used in well developed countries are of plant origin and about 120 plant derived substances are used in modern system of medicines worldwide (Sharman *et al*., 2021). The use of botanicals as anticoccidial remedies, therefore, holds promise as an alternative in the control of coccidiosis. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. Despite the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still like drugs from plants. In many of the developing countries, the use of plant drugs is increasing because modern life-saving drugs are beyond the reach of three-quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed presently and, in the future, (Thangarasu *et al*., 2016).

Terminalia macroptera whose authority name is Guill. & Perr., which belong to the family of (*Combretaceae*) and genus is *Terminalia* (Ibrahim, 2005) is a tree which grows in Western Africa from Senegal to Cameroon, and occasionally as far as Sudan. The tree is mostly found in Guinean and Sudanese-Guinean savannahs, preferably in moist areas and clayey ground, where it is stated to be common (Burkil, 1985). Terminalia spp. ranges from small and medium sized shrubs or trees to large deciduous forest trees, ranging in height from 1.5 to 75 m tall (Schmidt *et al*., 2002). Traditionally, the plant has been used in combination with Anogeissus leiocarpa for colouring cotton fabric yellow or ochre. This is then used for treatment of newly circumcised children due to its putative antimicrobial effect (Jansen *et al.*, 2005). The roots of the plant are regarded as an efficient antimicrobial remedy and are sold in markets in Guinea-Bissau (Silva *etal*,. 2002). In Burkina Faso, the plant is employed against malaria (Sanon *et al*., 2003) The bark is used against diarrhoea and dysentery in Nigeria (Burkill, 2004) A decoction of the leaves is used against hepatitis, ringworm, and skin diseases (Sanon *et al*., 2003). The leaves are also used in gastritis, colic, and hypertension, against fever, leprosy and tuberculosis (Arbonnier, 2004). The timber of *T. macroptera* is regarded as strong and resistant and is popular as building material and in furniture. The aim of the study is to determine phytochemicals constituents, *avian coccidiosis* effect and GC-MS analysis of methanolic back stem extract of *T. macroptera.* The study addresses the urgent need for alternatives to synthetic anticoccidial drugs, which are increasingly ineffective because of the emergence drug- *resistant Eimeria* strain and unsafe for human consumption, it also builds upon traditional knowledge which is not scientifically proof by subjecting *T. macroptera* extracts to rigorous phytochemical screening, chromatographic fractionation, and GC-MS analysis, thereby identifying specific bioactive compounds responsible for anticoccidial activity.

MATERIALS AND METHOD

Materials

The following are materials used during this course of this research project work: Organic solvent (methanol, ethanol, n-hexane, ethyl acetate), Heating mantle, Laboratory mortar and pestle, Glass column, Glass wares, Silica gel, Cotton wool, Aluminum foil, Whitman No 2 sterile filter paper, Iced water, Big bucket, Rotary vacuum evaporated (RE100 model), Water bath, Glass wool, Nose mark (PPP1), TLC plate, Capillary tube.

Experimental Animals

Day old broiler chicks were purchased at Ibadan. They were kept in the research pen and fed with broilers feed with unrestricted access to clean water ad-libitum for brooding period in two weeks. High standard of health/sanitary conditions was maintained throughout the course of the research work.

Sample Collection

It was consisted of bark *Terminalia macroptera* Guill. et Perr. (*combretaceae*). This bark was collected in Federal College of Wildlife Management New Bussa Niger State botanical garden, where all experimental trees were labelled by expert.

Sample Identification

The sample was identified and authenticated with a voucher number (LHO 993) at Pure and Applied Biology Ladoke Akintola University of Technology Ogbomoso by Dr. A.F Ogundola.

Drugs
Toltrazuril was purchased from State Veterinary Clinic, Bosso, Minna as oral solution (2.5%) under trade name TolacoxR.

Experimental Site
The study was carried out in the college farm of Federal College of Wildlife Management, New Bussa. Niger-State North Central Nigeria. New Bussa sits at 9^0 53`N $4^0$ 31`E.

Sample Preparation
The stem bark of the plant (*Terminali macroptera*) was washed with clean water and dried at room temperature before and after drying and then chopped into smaller fragments and pulverised using a grinder. Cold extraction was performed using 70% methanol.

Packing of Column with *silica gel*
A glass column was thoroughly rinsed with small quantity of n-hexane in other to make sure that the column was free from contaminants. The column was blocked with black wool so as to avoid the stationary phase from falling off. After that, 200g of silica gel was mixed with 500cm^3 of n-hexane and added into the column to serve as a stationary phase. 1g of stem bark extract of *Terminalia macroptera* was added into the column.

Column chromatography of stem bark extract of *Terminalia macroptera*
This elution was carried out by using organic solvent of various polarities and was done based on ratios, for the purpose of eluting different compounds present in the extract of *Terminalia macroptera.*

Series Elution with N-Hexane and Ethyl Acetate
100% N-hexane will be firstly used to elute the sample and a fraction was collected into 250ml conical flask, then Second elution was carried out using 90% of n-hexane and 10% ethyl acetate and fraction was collected as well.

80% to 20% of n-hexane and ethyl acetate respectively for the third elution and the eluate was collected into the 250ml conical flask. Then70% to 30% of n-hexane and ethyl acetate was used for the fourth elution and the fraction was collected. 60% to 40% of n-hexane and ethyl acetate respectively was used for the fifth elution and the eluate was collected. Then 50% to 50% of n-hexane and ethyl acetate was used for the sixth elution and eluate was collected from the glass column.40% to 60% of n-hexane and ethyl acetate was used in the seventh elution and fraction was obtained. 30% to 70% of n-hexane and ethyl acetate respectively was used for the eighth elution and the eluate was collected. Then 20% to 80% of n-hexane and ethyl acetate respectively was used in the ninth elution and the eluate was collected.10% to 90% of n-hexane and ethyl acetate respectively was used in the tenth elution and the eluate was collected. 100% of ethyl acetate was used in the eleventh elution and the eluate was collected.

Series Elution with Ethyl Acetate and Ethanol
About exactly 90% to 10% of ethyl acetate and ethanol respectively was used for the 11^{th} elution and the eluate was collected. 80% to 20% of ethyl acetate and ethanol respectively was used for the 12^{th}elution and the eluate was collected. 70% to 30% of ethyl acetate and ethanol respectively was used for the 13^{th} elution and the eluate was collected. 60% to 40% of ethyl acetate and ethanol respectively was used for the 14^{th} elution and the eluate was collected. 50% to 50% of ethyl acetate and ethanol respectively was used for the 15^{th} elution and the eluate was collected.40% to 60% of ethyl acetate and ethanol respectively was used for the 16^{th} elution and the eluate was collected. 30% to 70% of ethyl acetate and ethanol respectively was used for the 17^{th} elution and the eluate was collected. 20% to 80%of ethyl acetate and ethanol respectively was used for the 18^{th} elution and the eluate was collected. 10% to 90% of ethyl acetate and ethanol respectively was used for the 19^{th} elution and the eluate was collected. 100% of ethyl acetate and ethanol respectively was used for the 20^{th} elution and the eluate was collected.

Thin Layer Chromatography (TLC)
Thin layer chromatography (TLC) was used to group the eluates with same or marginally close "RF" values together.

Phytochemical Screening of the Fractions
Phytochemical screening was carried out according to (Traore Youssouf *et al*., 2015). On the crude extract using standard procedures to identify the phytochemicals present.

Test for alkaloids
About 0.5 g of extract was diluted into 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggen dorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggen dorff's reagent) was regarded as positive for the presence of alkaloids.

Test for polyphenols and tannins
About 0.5 g of the extract was boiled into 10 ml of water in a test tube and then filtered. A few drops of 0.1% of ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for terpenoids (Salkowski test)
To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2SO4 (3 ml) was carefully

added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for glycosides
Extracts were treated with 2 ml of glacial acetic acid, 1 drop of $FeCl_3$ and 1 ml of concentrated H_2SO_4 was added to give appearance of brown coloration which indicates the glycosides.

Test for flavonoids
Three methods was used to test for flavonoids. First, dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1ml) was then added. A yellow coloration that disappears on standing indicates the presence of flavonoids. Secondly, a few drops of 1% aluminium solution was added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. Next, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture will be filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins
To 0.5 g of extract was added 5 ml of distilled water in test tube. The solution was shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken after which it was observed for the formation of an emulsion.

Test for steroids and terpenoids
Nine millilitre (9 ml) of ethanol was added to 1 g each of the extracts and refluxed for a few minutes and filtered. Each of the filtrates was concentrated to 2.5 ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 h and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5 ml of chloroform using a separating funnel. To each 0.5 ml of the chloroform extracts in a test tube will be carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface showed the presence of steroids. To another 0.5 ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 min on a water bath. A grey color indicates the presence of terpenoids.

Pharmacological Studies
Anticoccidial effect
Total number of 45 chickens was divided into fifteen (15) groups, each group consisting of 3chickens as thus.

PEN A
Treatment with Fraction A in different concentrations
Group I= 50 mg/kgbw
Group II= 100 mg/kgbw
Group III= 150 mg/kgbw
Group IV= 200 mg/kgbw

PEN B
Treatment with Fraction B in different concentrations
Group I= 50 mg/kgbw
Group II= 100 mg/kgbw
Group III= 150 mg/kgbw
Group IV= 200 mg/kgbw

PEN C
Treatment with Fraction C in different concentrations
Group I= 50 mg/kgbw
Group II= 100 mg/kgbw
Group III= 150 mg/kgbw
Group IV= 200 mg/kgbw

PEN D
Group I= infected and not treated (negative control)
Group II= not infected and not treated (Normal control)
Group III= treated with the standard drug

Infection of chicks with coccidia Oocytes
About 100mls of fresh faecal sample containing over 48,000 viable oocytes per ml of Eimeria *tenella* were purchased and collected from National Veterinary Research Institute, parasitology laboratory Vom, Jos Plateau state. The fresh faecal sample was transported overnight in a sealed container under 4^0c. The infective dose of the culture (i.e 48,000 Oocyst/g) was administered orally using cannulation tube. Then the treatment was carried out in each group for 21 days (3 weeks).

Preparation of *Terminalia macroptera* into solution from solid concentrated plant fractions
From the solid fraction, 1gram was fetched and dissolved in 10mls of dimethyl sulfoxide
(DMSO). So, 1 gram in 10mls is equivalent to1000mg in 10mls.
It means every 1ml contain 100mg.
Therefore, for the administration of concentration of 50mg/KgBw to a chick of 500gram of weight.
The average weight of the chicks was exactly 500g at the time of administration of the fractions.
Then if 100mg is equivalent to 1ml.
Therefore $50mg = 1000g$

$$mg = 500g$$

$$50\ x\ 500 = 25000/1000$$

$$x = 25mg$$

So then, $100mg = 1ml$

$$25mg = xml$$

$$25mg = 100ml$$

Therefore, 0.2 5mls was administered to a chick of 500gram.

Parasitological Examination (Oocyst Count)
A fresh faecal sample was collected from freshly evacuated faeces of induced chicks. This was lightly macerated and subsequently washed and filtered through

gauze and the final clear liquid sample containing the Oocyst was poured into a beaker containing normal saline and kept for oocyst count using compound microscope. The count was carried out at the beginning and at the end of the treatments to check the efficacy of the fractions of *Terminalia macroptera* administered to the chicks using haematocytometer and compound microscope.

RESULTS AND DISCUSSION

Column Fractionation and Thin Layer Chromatography

The column fractionation of *Terminalia macroptera* extract demonstrated a clear solvent-dependent separation pattern, reflecting the chemical complexity and polarity distribution of constituents present in the plant material. Using a gradient elution system consisting of n-hexane, ethyl acetate, and absolute ethanol, compounds were effectively resolved according to their increasing polarity. At the initial stages of elution, solvent systems dominated by n-hexane (100:0 to 70:30 n-hexane/ethyl acetate) produced little or no separation, indicating a limited presence of highly non-polar constituents in the extract. This observation suggests that lipophilic hydrocarbons or waxy substances are either minimal or strongly retained within the stationary phase under these solvent conditions. The lack of elution at this stage confirms that the majority of phytochemicals in *T. macroptera* stem bark possess moderate to high polarity.

Noticeable separation commenced when the solvent polarity increased, particularly from the 60:40 ethyl acetate/absolute ethanol ratio through to 100% ethanol. This progressive elution pattern indicates the presence of semi-polar to polar compounds such as flavonoids, tannins, phenolic acids, and glycosides, which are known to exhibit stronger interactions with polar eluents. Ethyl acetate serves as an intermediate solvent, efficiently mobilizing moderately polar constituents, while absolute ethanol enables the elution of more strongly adsorbed polar metabolites.

Thin layer chromatography (TLC) further validated the effectiveness of the column fractionation process. Seven eluates were analyzed with these retention factors as follows 0.241, 0.242,0.241, 0.120, 0.134, 0.101, 0.122, 0.021, and 0.011respectively. these were subsequently grouped into three distinct fractions based on closely related retention factor (Rf) values. The clustering of eluates into three major fractions suggests that compounds with similar polarity and chemical characteristics were successfully pooled together. This grouping reflects a reduction in chemical complexity from the crude extract to fewer, more chemically coherent fractions, which is essential for downstream bioactivity evaluation and compound isolation.

The presence of three distinct Rf groupings also implies that *T. macroptera* stem bark extract contains at least three dominant classes of phytochemicals differing in polarity. Lower Rf values are typically associated with more polar compounds that interact strongly with the stationary phase, while higher Rf values correspond to less polar constituents with greater mobility on the TLC plate.

Table 1: Eluotropic series of n-hexane, Ethyl acetate and Absolute Ethanol with their retention factors.

n-Hexane	Ethyl acetate	Absolute Ethanol	Retention factor
100			
80			
70			
60			
50			
40			
30			
20			
10			
0	100	0	
	90	10	
	80	20	
	70	30	
	60	40	0.241
	50	50	0.242
	40	60	0.241
	30	70	0.120, 0.134
	20	80	0.101, 0.122
	10	90	0.021
	0	100	0.011

Retention Factor = distance moved by the solute/ distance moved by the solvent.

The retention factor 0.011, 0.021, were grouped as fraction 1

The retention factor 0.101, 0.120, 0.134, 0.122, were grouped as fraction 2
The retention factor 0.241, 0.242, 0.241, were grouped as fraction 3 respectively.

Phytochemicals Screening In Three Fractions (1, 2, And 3) Of The *Terminalia Macroptera* Extract

- **Broad-spectrum phytochemical presence**: Most bioactive groups are consistently found across all three fractions, suggesting a rich and diverse chemical profile.
- **Absence of glycosides**: This is notable, as glycosides often play roles in cardioprotective and anti-inflammatory activities. Their absence may influence the therapeutic scope.
- **Therapeutic potential**: The presence of flavonoids, alkaloids, terpenoids, and tannins points to strong antioxidant, antimicrobial, and anti-inflammatory properties.

What This Means for *Terminalia macroptera*

Terminalia macroptera stem bark extract shows a robust phytochemical profile across all tested fractions, with consistent presence of key bioactive compounds like flavonoids, alkaloids, and tannins. This supports its traditional use in herbal medicine and suggests potential for pharmacological applications, especially in antioxidant, antimicrobial, and anti-inflammatory therapies.

Table 2: Phytochemical Screening Across Fractions 1–3.

Phytochemical	Fraction 1	Fraction 2	Fraction 3	Interpretation
Tannins	+	+	+	Present in all fractions; known for antioxidant and antimicrobial properties.
Saponins	+	+	+	Present throughout; may contribute to immune modulation and cholesterol-lowering effects.
Flavonoids	+	+	+	Consistently present; potent antioxidants with anti-inflammatory and anticancer potential.
Phenols	+	+	+	Found in all; key contributors to antioxidant activity.
Phlorotannins	+	+	+	Present; typically found in brown algae, but here may indicate complex polyphenols.
Chalcones	+	+	+	Present; precursors to flavonoids, with anti-inflammatory and antimicrobial roles.
Glycosides	–	–	–	Absent; suggests limited sugar-bound bioactive compounds in these fractions.
Alkaloids	+	+	+	Present; often linked to analgesic, antimalarial, and anticancer effects.
Anthraquinones	+	+	+	Present; known for laxative and antimicrobial properties.
Terpenoids	+	+	+	Present; diverse bioactivities including anti-inflammatory, antiviral, and anticancer.

Table 3: Phytochemicals, Retention Time, And Abundance in Fraction 1.

Peak	Retention Time (RT)	Phytochemical(s) Identified	Abundance (Area%)
1	3.288	Ethane, 1,1-dichloro-; 2-Chloroethyl methyl sulfoxide	0.72%
2	6.200	Acetamide derivatives; L-Tyrosine derivative	0.65%
3	7.379	Undecanoic acid, 2-methyl; Decanoic acid, 2-methyl	0.65%
4	8.123	3-Tetradecene (Z); 5-Octadecene (E); 1-Tetradecanol	1.38%
5	9.559	Dodecanoic acid, methyl ester	5.42%
6	10.217	2-Tetradecene (E); 9-Octadecene (E); 1-Nonadecene	1.89%
7	11.098	Octahydro-naphthalenone; Guaia-1(10),11-diene	0.64%
8	11.344	Carbonic acid ester; Octadecane, 2-methyl	0.72%
9	11.504	Methyl tetradecanoate; Tridecanoic acid, methyl ester	1.46%
10	12.088	1-Octadecene; 5-Octadecene (E); 9-Eicosene (E)	0.81%
11	12.145	Heptadecane, 8-methyl; Nonadecane; Hexacosane	1.50%

12	13.009	Nonadecane; Heneicosane; Hexadecane	3.86%
13	13.255	Hexadecanoic acid, methyl ester; Pentadecanoic acid ester	3.69%
14	13.610	n-Hexadecanoic acid; Pentadecanoic acid	4.57%
15	13.828	Eicosane; Octadecane; Octacosane	7.10%
16	14.434	Pentatriacontene; Nonyl ethers	1.09%
17	14.617	Heneicosane; Heptadecane; Eicosane	8.47%
18	14.663	Octadecenoic acid, methyl esters (cis/trans)	2.63%
19	14.852	Methyl stearate	0.80%
20	15.172	Octadecenoic acids (cis/trans); Vaccenic acid	3.93%
21	15.367	Hexacosane; Heptadecane; Nonadecane	7.18%
22	15.624	Methanoazulenol; Octadecadienol; Fluorooctanoic acid ester	0.69%
23	15.830	Hexadecanoic acid esters; Palmitoyl chloride	0.83%
24	16.179	Eicosane; Heptadecane; Methoxyacetic acid ester	8.58%
25	16.517	Cyclotetradecane; Cyclohexylmethyl cyclohexane	0.92%
26	16.774	Heneicosane; Octadecyl ethers	0.68%
27	17.164	Tetracosane; Heptadecane	7.06%
28	17.890	Octadecyl propyl esters; Nonyl tetradecyl ether	1.27%
29	18.176	Tetrahydro-1-Naphthalenol acetate; Indole, 3-methyl	1.25%
30	18.388	Eicosane; Nonadecane; Hexacosane	7.49%
31	18.497	Fluorooctanoic acid ester; Dodecanol derivative	1.20%
32	18.749	Heptadecenal; Oxirane; 1-Octadecene	1.04%
33	19.246	Bis(2-ethylhexyl) phthalate; Phthalic acid esters	1.92%
34	19.962	Hexacosane; Methylnonadecane; Eicosane, 9-octyl-	7.93%

Table 3 maps each compound's retention time to its relative abundance, and also pinpoint which phytochemicals dominate the sample. Notably.

- Eicosane, Hexacosane, and Octadecane appear frequently and abundantly.
- Fatty acid esters and alkanes dominate the profile, suggesting lipid-rich phytochemical content.
- Retention times between 13–19 minutes show the highest abundance peaks.

Major classes and prominent components shown across the peak of fraction 1 according to the library matches are;

a. Medium- and long-chain fatty acids and their methyl esters (e.g. dodecanoic acid methyl ester / lauric acid methyl ester, hexadecanoic acid methyl ester, methyl tetradecanoate, methyl stearate).
b. Free fatty acids (n-hexadecanoic acid = palmitic acid, cis/trans octadecenoic acid
c. Alkanes and long chain hydrocarbons (eicosane, nonadecane, hexacosane, etc.).
d. Terpenoid / sesquiterpene-like hits (e.g. guaia-1(10),11-diene, naphthalenone derivatives).
e. Aromatic esters / phthalate esters and other esters (bis(2-ethylhexyl) phthalate and related hits — these may sometimes be contaminants or plasticizer artifacts but show in the library matches).
f. Some indole / naphthalenol derivatives and other minor compounds appear in later retention times.

Major constituents included:

- Fatty acid esters and hydrocarbons (e.g., methyl laurate, methyl palmitate, octadecene, eicosane)
- Terpenoid derivatives (guaia-1(10),11-diene)
- Indole compounds

These compounds are linked to membrane-disrupting and antimicrobial actions, consistent with moderate antiparasitic activity. This is consistent with (Obukhova. and Murzina. 2024).

Table 4: Phytochemicals, Retention Time, and Abundance in fraction 2.

Peak	Phytochemical Name	Retention Time (RT)	Abundance (Area %)
1	2-Pyrrolidinone, 1-methyl-	4.048	1.20%
2	4-Tetradecene (E), 6-Tetradecene (Z), Cyclotetradecane	8.122	0.75%
3	2,4-Di-tert-butylphenol	9.553	4.09%
4	9-Octadecene (E), Cetene, 7-Hexadecene (Z)	10.217	1.16%
5	1-Pentadecene, Cyclododecanol, 7-Heptadecene	11.206	0.55%
6	Methyl tetradecanoate	11.498	0.84%

7	Tetradecanoic acid	11.910	0.61%
8	1-Nonadecene, Eicosyl methyl ether, 1-Octadecene	12.088	2.14%
9	1-Hexadecanol, n-Pentadecanol, n-Nonadecanol-1	12.889	1.66%
10	Hexadecanoic acid, methyl ester	13.255	10.05%
11	n-Hexadecanoic acid	13.638	17.22%
12	Hexadecanoic acid, ethyl ester	13.804	4.02%
13	Carbamic acid derivative	13.970	0.52%
14	5-Eicosene (E), 5-Octadecene (E), Cyclotetradecane	14.537	1.52%
15	9,12-Octadecadienoic acid, methyl ester	14.623	10.36%
16	9-Octadecenoic acid (Z), methyl ester	14.663	8.42%
17	Methyl stearate	14.851	1.21%
18	cis-13-Octadecenoic acid, cis-Vaccenic acid	15.017	10.85%
19	Octadecanoic acid	15.178	9.88%
20	Henicos-1-ene, 1-Heneicosanol	15.332	2.26%
21	cis-10-Heptadecenoic acid, methyl ester	15.853	0.77%
22	cis-13-Octadecenoic acid, Palmitoleic acid	16.213	0.86%
23	Methyl trans-9-(2-butylcyclopentyl) nonanoate	16.471	0.68%
24	1-Octadecene, Carbonic acid ester	17.106	1.06%
25	13-Octadecenal (Z), Dimethyl-tetradecen-1-ol acetate	17.569	1.38%
26	Palmitoleic acid, Hexadecenoic acid	18.496	0.57%
27	Cyclopropaneoctanal, Palmitoleic acid	18.731	1.11%
28	2-Heptadecenal, Oleic Acid	18.885	0.72%
29	Bis(2-ethylhexyl) phthalate	19.234	2.92%
30	Trifluoroacetic acid ester, Dichloroacetic acid ester	19.887	0.62%

Major Classes and Prominent Components Shown Across The Peak of Fraction 2

i. **Long-chain fatty acids & esters (palmitic, stearic, oleic, linoleic + their methyl/ethyl esters)** — these dominate F2 by area (~60%+ combined). Fatty acids and some FAMEs are known to have broad antimicrobial and antiparasitic effects (membrane disruption, detergent-like action, inhibition of metabolic enzymes) and can damage protozoan membranes or interfere with oocyst viability. Given their high abundance, they are likely major contributors to vivo activity

ii. **2,4-Di-tert-butylphenol** — a substituted phenol, presents at smaller but noticeable abundance (~4%). This compound has reported **antimicrobial/antioxidant** properties and could act synergistically with fatty acids or alter parasite oxidative stress response.

iii. **Fatty alcohols and minor unsaturated aldehydes/ketones** can contribute to membrane activity or act as penetration enhancers increasing the effectiveness of other actives.

Dominated by.

- Palmitic acid, oleic acid, linoleic acid, stearic acid, and their esters
- 2,4-di-tert-butylphenol (antioxidant and antimicrobial)

These long-chain fatty acids and esters are well-documented for anticoccidial and antimicrobial properties. They act by:

- Disrupting parasite membranes
- Inhibiting energy metabolism
- Enhancing host immunity

Their high abundance (~60%) likely explains the superior efficacy and safety of Fraction 2.

Long-chain fatty acids (e.g., palmitic, oleic, linoleic, stearic) and their esters have documented antimicrobial activity according to (Obukhova ,and Murzina ,2024).

Table 5: Phytochemicals, Retention Time, and Abundance in fraction 3.

Peak	Phytochemical(s)	Retention Time (RT)	Abundance (Area %)
1	Dimethyl Sulfoxide	3.282	1.11%
2	1,2-Dipentylcyclopropene, Oxazole, 2H-Pyrrol-2-one	5.571	0.90%
3	1-Tridecene, Cyclododecane, 1-Dodecanethiol	5.742	1.63%
4	Cyclandelate, N-p-Tolyl-succinamic acid	6.200	1.00%
5	Thiophene, 3-Amino-5-t-butylisoxazole	7.402	1.52%
6	Cyclododecane, Cyclotetradecane	8.123	3.91%
7	4-Piperidinol, Diisopropyl methanephosphonate	8.718	1.34%

8	2,5-Cyclohexadiene-1,4-dione (various isomers)	9.050	2.70%
9	4-Methoxy-3-methylphenylacetonitrile	9.153	0.91%
10	3-(But-3-enyl)-cyclohexanone	9.313	1.26%
11	Butylated Hydroxytoluene	9.507	3.53%
12	2,4-Di-tert-butylphenol, Dodecanoic acid methyl ester	9.559	10.03%
13	5-Octadecene, 2-Tetradecene, 9-Octadecene	10.217	2.22%
14	γ-Muurolene, Germacrene D	11.098	1.34%
15	Cyclododecane, Dichloroacetic acid ester	11.213	0.91%
16	2-Thiopheneacetic acid ester	11.344	1.25%
17	Methyl tetradecanoate, Tridecanoic acid ester	11.504	1.84%
18	Acetic acid ester, Pentafluoropropionic acid ester	12.895	1.43%
19	Hexadecanoic acid, methyl ester	13.255	3.86%
20	n-Hexadecanoic acid	13.616	7.31%
21	Oleic Acid, Isobutyl tridecyl carbonate	13.805	1.52%
22	1-Nonadecene, Behenic alcohol	14.543	1.50%
23	9,12-Octadecadienoic acid methyl ester	14.623	3.04%
24	9-Octadecenoic acid methyl ester	14.669	3.25%
25	Heptadecanoic acid methyl ester	14.852	0.95%
26	2-Chloropropionic acid esters	15.012	1.52%
27	Octadecanoic acid	15.178	3.83%
28	Octacosane, Eicosane	15.584	11.05%
29	9-Octadecenal, 2-Piperidinone	15.836	0.97%
30	4-Quinolinecarboxylic acid, 2-Ethylacridine	16.214	1.54%
31	Indole-2-one, 2-Pyridinamine	16.677	0.92%
32	Hexahydropyridine, N-Methyl-1-adamantaneacetamide	16.780	2.64%
33	1H-imidazole-2-methanol, Benzo[h]quinoline	17.890	3.49%
34	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluoropropionate	18.748	1.01%
35	Bis(tridecyl) phthalate, Eicosane	19.235	12.78%

Phytochemicals Present and Their Relative Abundances In Fraction 3

i. Antioxidants / Phenolics

Butylated Hydroxytoluene (BHT) – 3.53%

2,4-Di-tert-butylphenol –10.03%

These are strong free-radical scavengers and membrane stabilizers. They can reduce oxidative stress caused by *Eimeria* infection.

ii. Fatty Acids & Esters

Hexadecanoic acid (Palmitic acid) – 7.31%

9,12-Octadecadienoic acid methyl ester (Linoleic acid ester) – 3.04%

9-Octadecenoic acid methyl ester (Oleic acid ester) – 3.25%

Octadecanoic acid (Stearic acid) – 3.83%

Methyl stearate, methyl palmitate, methyl tetradecanoate (minor amounts) Fatty acids and esters have been reported to possess anticoccidial, antimicrobial, and membrane-disrupting activity.

iii. Quinones

2,5-Cyclohexadiene-1,4-dione,(benzoquinone-derivative) –2.70%

Quinones are known to interfere with parasite respiration and metabolism.

iv. Sterols & Hydrocarbons

Cyclododecane, Octacosane, Eicosane –total>14% Hydrocarbons often have structural/membrane effects, though less bioactive compared to phenols and fatty acids.

v. Phthalates

Bis (tridecyl), phthalate –12.78% Reported with antimicrobial and cytotoxic effects, possibly contributing to parasite inhibition.

Contained

- Antioxidants: BHT (3.53%), 2,4-di-tert-butylphenol (10.03%)
- Fatty Quinones: interfere with parasite respiration
- Hydrocarbons and phthalates

This mixture suggests potential synergistic effects between antioxidants and fatty acid. This is in line with documented Evaluation of synergistic/antagonistic antibacterial activities of fatty oil by (Xiaofei Shang *et al*., 2025).

Synergistic Action: Multiple compounds act together to enhance efficacy and minimize resistance. This is consistent with Philippe R. *et al*., 2011 in the review whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions.

Evaluation of the resulted fractions using different administration doses on *avian coccidiosis*

Pharmacological studies

There were total number of four groups in each pen and each group was standing for one treatment. In the present study, after challenging the chicks with Eimeria *tenella* species especially in the control pen of (Infected non-treated group), birds showed severe forms of coccidiosis, represented by high mortality rate, lesion appearances and feacal abnormalities with obvious amount of blood in litter, while all the(non-infected group) chicks were all in good health throughout the investigation.

Treatment of Chicks by Using "Fraction 1" of *Terminalia macroptera* in Pen (A)

There were total number of four treatments administered to pen A, "fraction 1" *Terminalia macroptera* was administered in different concentrations of 50mg/kgbw, 100mg/kgbw, 150mg/kgbw and 200mg/kgbw making four treatments. These were equivalents of 0.3mls, 0.5mls, 0.7mls, and 1mls respectively. Calculations into volume was According to the OECD's (organization of economic corporation and development's) guidelines.

Table 6: Treatment Using "Fraction 1" of *Terminalia macroptera* in Pen A.

Treatments	Concentration of the fraction mg/kgbw	Equivalent vol(ml) of the fraction	No of chicks	No of mortalities	No of survivor
T1	50	0.3	3	3	0
T2	100	o.5	3	3	0
T3	150	0.7	3	1	2
T4	200	1	3	1	2

At **low doses (50–100 mg/kg)**, *100% mortality*.

At **higher doses (150–200 mg/kg)**, mortality dropped to ~33%.

Treatment of Chicks by Using Fraction 2 of *Terminalia macroptera* in Pen (B)

There were total number of four treatments administered to penB as well, "fraction 2" of *Terminalia macroptera* was administered in different concentrations of 50mg/kgbw, 100mg/kgbw, 150mg/kgbw and 200mg/kgbw making four treatments. These were equivalents of 0.3mls, 0.5mls, 0.7mls, and 1mls respectively as well.

Table 7: Treatment Using "Fraction 2" of Terminalia *macroptera* in Pen B.

Treatments	Concentration of the fraction mg/kgbw	Equivalent vol(ml) of the fraction	No of chicks	No of mortalities	No of survivor
T1	50	0.3	3	2	1
T2	100	o.5	3	1	2
T3	150	0.7	3	0	3
T4	200	1	3	0	3

- Mortality rates decrease as dose increases.
- At **150–200mg/kg**, *0%mortality*.

➡ **Fraction 2 appears safer** and possibly protective at higher doses.

Treatment of Chicks Using "Fraction 3" of *Terminalia macroptera* in PenC

There were total number of four treatments administered to pen C as well, "fraction 3" of Terminalia *macroptera* was administered in different concentrations of 50mg/kgbw, 100mg/kgbw, 150mg/kgbw and 200mg/kgbw making four treatments.. These were equivalents of 0.3mls, 0.5mls, 0.7mls, and 1mls respectively as well.

Table 8: Treatment Using "Fraction 3" of *Terminalia macroptera* in Pen C.

Treatments	Concentration of the fraction mg/kgbw	Equivalent vol(ml) of the fraction	No of chicks	No of mortalities	No of survivor
T1	50	0.3ml	3	2	1
T2	100	o.5ml	3	2	1
T3	150	0.7ml	3	0	3
T4	200	1ml	3	1	2

- High mortality at **50–100 mg/kg**.
- No mortality at **150 mg/kg** but rises again (1/3) at **200 mg/kg**. Shows a **non-linear effect**, suggesting possible narrow therapeutic window.
- The mortality at 200 mg/kg may also be traced to difference in immunity system of the chicks.

Control experiment Pen D, treated with standard drug, induced but not treated and not induced not treated.

Treatments	Concentration	No of chicks	No of mortalities	No of survivor
Treated with standard drug	(1ml) of 2.5% (Toltrazuril) manufacturer's prescription	3	1	2
Induced and not treated	-	3	3	0
Not induced not treated	-	3	0	3

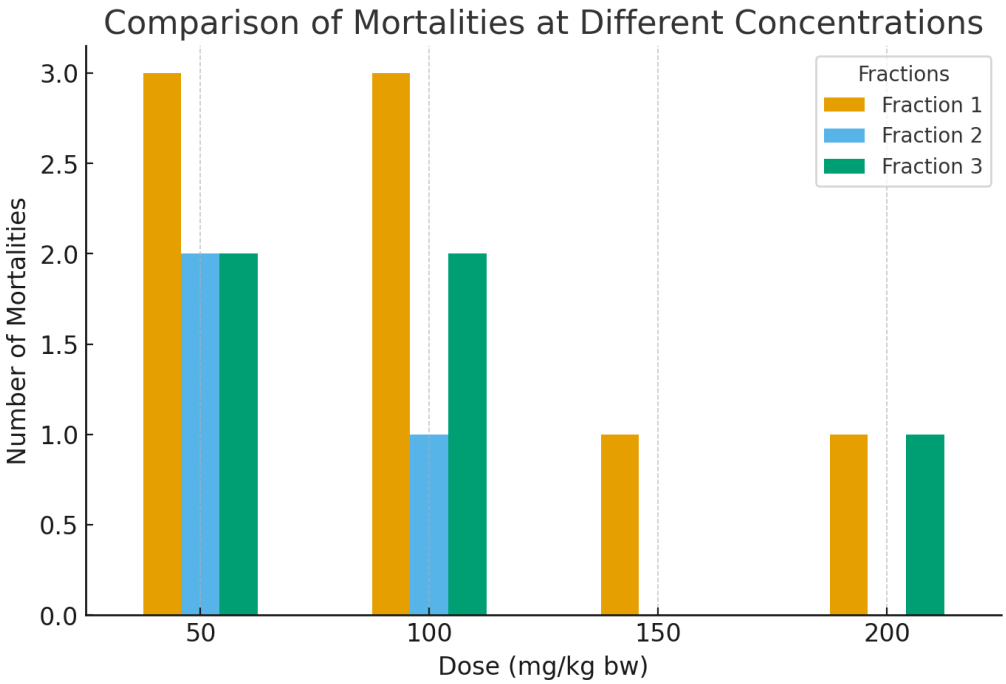

Figure 4: comparison showing motalities at different concentrations for fraction 1,2,3.

Overall Analysis

Fraction 1: High mortality at low doses, safer at higher doses (paradoxical dose-response).

Fraction 2: Most promising — mortality decreases steadily, and none died at high doses.

Fraction 3: Mixed results — some toxicity persists even at higher doses.

Among the three, Fraction 2 of *Terminalia macroptera* appears to be the most effective and safest fraction, with better survival at higher doses. Fractions 1 and 3 show irregular dose-response relationships, raising concerns about toxicity or inconsistent pharmacological effects.

Table 9: Parasitological Examination results of feacal sample collected from induced chicks after 21 days.

Treatments	Pen A (Fraction 1)	Pen B (Fraction2)	Pen C (Fraction3)
50mg/kgbw	$4.2x10^3$/g	$2.1x10^3$/g	$2.0x10^3$/g
100mg/kgbw	$3.8x10^3$/G	$1.8x10^3$/g	$2.1x10^3$/g
150mg/kgbw	$2.8x10^3$/g	$1.6x10^3$/g	$1.9x10^3$/g
200mg/kgbw	$1.6x10^3$/g	$0.6x10^3$/g	$1.4x10^3$/g

Table 10: Parasitological Examination results of feacal sample collected from the experimental control chicks.

Treatments	Pen D
Treated with standard drug	$0.7x10^3$/g
Induced and not treated	$32.1x10^3$/g
Not induced not treated	$0.1x10^3$/g

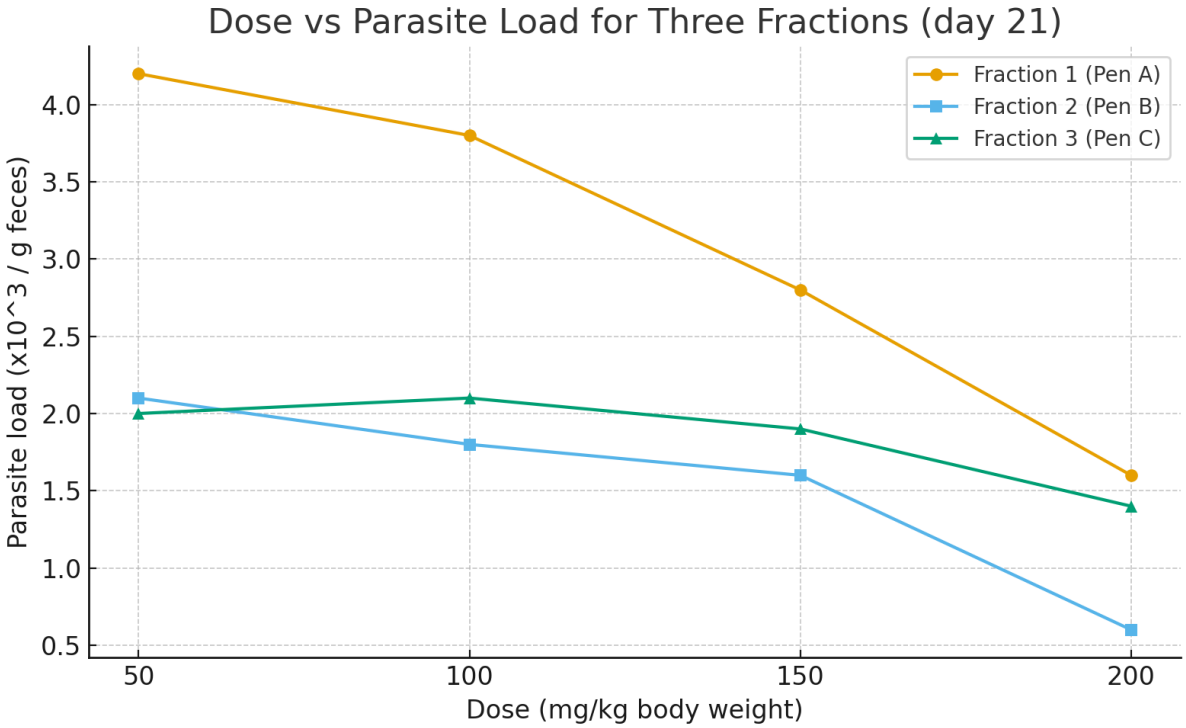

Figure 5: Graph of dose against parasite load in treated Chicken.

CONCLUSION

This study demonstrates that the methanolic stem bark extract of *Terminalia macroptera* possesses significant anticoccidial activity against *Eimeria tenella* infection in broiler chickens. Among the three chromatographic fractions tested, Fraction 2 consistently showed superior efficacy, reducing oocyst counts to levels comparable with the standard anticoccidial drug (Toltrazuril). The phytochemical screening reveal the presence of secondary metabolites such as phenolics, flavonoids, tannins, and terpenoids, and GC-MS analysis revealed the presence of diverse bioactive compounds, including fatty acids, 2,4-Di-tert-butylphenol, palmitic, stearic, oleic, linoleic with their methyl/ethyl esters which are known to exert antimicrobial, antioxidant, and immunomodulatory effects. These findings suggest that the anticoccidial activity of *T. macroptera* is likely due to a synergistic interplay of multiple phytoconstituents rather than a single compound.

The results also highlight a dose-dependent relationship, with higher concentrations of Fraction 2 (150–200 mg/kg) not only reducing parasite load but also improving survival rates, thereby underscoring its therapeutic potential. Importantly, the comparable efficacy of Fraction 2 to the standard drug positions *T. macroptera* as a promising natural alternative for coccidiosis management, particularly in resource-limited settings where synthetic drugs are costly, less accessible, and associated with resistance and residue concerns.

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