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In Vitro Anti-Inflammatory, Antioxidant, Anti-Helminthic and Antimicrobial Activity of Pet. Ether Fractions of the Stem Bark of Macaranga Barteri

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ABSTRACT

The stem bark of Macaranga Barteri is used as a worm expellant and gonorrhea management. This research evaluates the bioactivities of ethanolic and petroleum ether fractions of the stem bark of Macaranga Barteri. Standard methods were employed for phytochemical screening. The broth dilution method was utilized in antimicrobial activity. DPPH assay was used for antioxidant activity. In vitro anti-helminthic activity was determined using earthworms (Eudrilus eugeniae). There were forty-eight fractions (W1-W48) and was bulked into W(W1-W8), X(W9-W15), Y(W16-W33) and Z(W34-W48). Glycoside was in petroleum ether fractions for W. Phenolics and saponins were present in fraction X. Steroids, Polyphenols and saponins were in fraction Y. Phenols, steroids, tannins, terpenoid, glycosides, phytosterol, polyphenols and saponins were present in fraction Z. MIC of 12.5±0.017 were obtained for the pet. ether fractions X against Streptococcus aureus. The IC50 of the most potent pet. ether fraction, X and the reference drug. With DPPH scavenging activity were 15.47±0.01 and 51.95±0.01. At 20 µg/ml. However, At 20 µg/mL, the paralysis and death time for both the pet. ether fraction Y and Mebendazole drug were 20.23±15 min, 31.60±18min and 53.06±2.89 min, 82.03±47.37 min. The pet. ether fractions of Macaranga Barteri possess antioxidant, anti-helminthic, and antimicrobial activities.

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Introduction

Plants are fundamental to our existence, supplying food, shelter, medicine, and clothing, while ensuring the health of our ecosystem [1]. Consequently, by effectively utilizing plants in conjunction with modern technological advancements and knowledge, humanity's survival can be secured [1]. A large proportion of traditional medicines have plant-based origins. Nearly a century ago, the majority of the limited effective drugs available were plant-based. Noteworthy examples are aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) [2]. Currently, there is a growing demand for medicinal plants, and their popularity is steadily on the rise and have long been utilized in traditional medicine and worldwide ethnomedicine. An increasing global reliance on medicinal plants stems from their accessibility, minimal adverse effects, and, in certain regions, their position as the only available remedy [3]. Throughout history, people have unquestionably contemplated the potential of these remedies to either heal or alleviate symptoms associated with various illnesses. Humans primarily rely on natural plant resources to fulfil their medical requirements for maintaining good health and treating illnesses [4]. For hundreds of years, traditional medicine has served as a vital tool in the treatment, diagnosis, prevention, and cure of diseases [5,6]. Before Western medicine became prevalent, medicinal plants served as the primary means of treating various diseases [7].

The therapeutic benefits of these plants stem from their bioactive phytochemical components, including alkaloids, phenols, tannins, cryogenics, glycosides, and terpenoids. These constituents exert specific physiological effects on the human body. For example, Alstonia boonei's bark possesses alkaloids and achistamine, which can be beneficial in alleviating symptoms such as fever, dizziness, and high blood pressure [8]. Medicinal plants synthesize various chemical compounds, but the phytochemicals and scientific evidence supporting the pharmacological effects of certain plants are been studied to verify their safety and efficacy [1].

Among plants known for medicinal value is Macaranga barteri (M. barteri); a species of a plant of the family Euphorbiaceae. This plant, reaching heights of up to 20 meters, is frequently encountered across various regions of West Africa, including Sierra Leone, Liberia, Ivory Coast, Ghana, and Equatorial Guinea, with a notable prevalence in Nigeria. Among the Yoruba people in Nigeria, it is known as 'aarasa' or 'owariwa,' while the Akan tribe in Ghana refers to it as 'opamkokoo.' In Nigeria, the bark and leaves of this plant, whether in powdered form or as a decoction, have traditionally been employed as a vermifuge [9]. M. barteri serves various medicinal purposes in different regions. In the Democratic Republic of Congo, it is utilized as both a worm expellant and a remedy for reducing fever. When combined with other Macaranga spp., it is employed to alleviate cough and bronchitis. In Sierra Leone, the leaves are actively used to manage gonorrhea, while in Ivory Coast, it is regularly employed as a laxative and an anti-anemic tonic. Additionally, the leaf extract of Macaranga barteri has been recognized for its pharmacological significance, acting as an antioxidant and antimicrobial agent. The high concentration of phenolic compounds in M. barteri has been identified as the primary constituents responsible for its anti-inflammatory properties [9].

After the extensive literature research, this study aimed at assess the pharmacological activities of the fractions of the ethanol extract of the stem bark of Macaranga barteri.

Materials and Methods

The materials and methods used are described by Buah., et al. 2024 [1].

Results and Discussion

Fractionation by Column Chromatography

Column chromatography was done to obtain various fractions of the extract using the identified suitable solvent system in the TLC analysis, ethyl acetate: hexane (2:8). Forty-eight (48) fractions were collected and were labeled as W1-W48. The forty-eight fractions (W1-W48) and was bulked into W(W1-W8), X(W9-W15), Y(W16-W33) and Z(W34-W48). The mobile phase was allowed to traverse the column, sequentially collecting the components of the mixture in fractions [10]. The fractions were bulked or pulled together (W, X, Y and Z) based on their similarities after TLC was done for each of them.

Secondary Metabolite Profiling of the Fractions from the Petroleum Ether Extract of Macaranga Barteri

Table 1 displays the findings from the secondary metabolite profiling of the fractions obtained from the petroleum ether extract of *Macaranga barteri.*

Table 1: Results of the Secondary Metabolite Profiling of the Fractions of the Pet. Ether Extract of Macaranga Barteri

Phytochemical	Fraction W	Fraction X	Fraction Y	Fraction Z
Polyphenols	-	-	+	+
Steroids	-	-	+	+
Phytosterol	-	-	-	+
Glycosides	+	-	-	+
Saponins	-	+	+	+
Phenols	-	+	-	+
Terpenoid	-	-	-	+

KEY: (+) = Presence or detected; (-) = Absence or not detected.

Secondary metabolite Profiling of Macaranga barteri has revealed the presence of several bioactive compounds, including phenols, steroids, terpenoids, glycosides, phytosterols, polyphenols, and saponins, across its various fractions. Glycosides were the sole compound identified in Fraction W, while Fraction X also contained phenols and saponins. Fraction Y was marked by the presence of polyphenols and steroids, and Fraction Z showed a broader range, with phenols, steroids, terpenoids, glycosides, phytosterols, polyphenols, and saponins. Phytochemicals, especially pigment molecules, tend to accumulate in the outer layers of plant tissues, with their concentrations varying depending on the plant variety [11]. Levels vary from plant to plant depending on the variety [12]. Polyphenols and carotenoids stand out as key antioxidant compounds, significantly contributing to the antioxidant properties of plants and foods. [1].

In Vitro Assessment of the Antioxidant Potential of the Petroleum Ether Fractions

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays was used to evaluate the antioxidant capacity of petroleum ether fractions of *Macaranga barteri*.

DPPH Scavenging Activity of the Fraction Z and the Standard Drug

Table 2: DPPH Scavenging Activity for Fraction Z and Standard Drug

Conc (µg/ml)	Fraction Z		Ascorbic acid	
	Absorbance (mean) ± SEM	% Inhibition	Absorbance (mean) ± SEM	% Inhibition
100	0.28±0.002a	84.83	0.43±0.001b	86.09
80	0.20±0.001a	83.44	0.39±0.001b	83.56
60	0.18±0.001a	82.17	0.34±0.001b	82.30
40	0.16±0.001a	79.65	0.30±0.001b	81.04
20	0.15±0.001a	64.18	0.28±0.001b	45.51

The scavenging activity of fraction Z and ascorbic acid demonstrates an promising trend with increasing concentration against DPPH. The percentage of inhibition rises alongside the absorbance for both fraction Z and ascorbic acid. The IC50 values, indicative of the concentration at which 50% inhibition occurs, were determined as 51.95 µg/mL for ascorbic acid and 23.45 µg/mL for fraction Z (refer to the table below). A lower IC_{50} signifies a higher antioxidant activity. Superoxide radicals, initially produced by the enzyme superoxide dismutase, are transformed into highly reactive hydroxyl radicals (OH) through the actions of glutathione and catalase, with copper or iron facilitating the process. Of all oxygen-derived radicals, the hydroxyl radical (OH.) stands out as the most reactive, with the potential to disrupt essential biological macromolecules, including carbohydrates, proteins, and lipids [13]. Consequently, fraction D exhibits greater antioxidant efficacy compared to ascorbic acid. Notably, the IC50 for fraction Z was significantly higher ($P < 0.0042$) than that for ascorbic acid.

Table 3: IC50 Values for Z and Standard Drug

Sample	IC 50 (µg/ml)
Fraction Z	23.45±0.01
Ascorbic acid	51.95±0.01

DPPH radical scavenging activity of Fraction X

Table 4 depicts the results of the DPPH scavenging activity of the fraction X and the standard drug.

Table 4: DPPH Scavenging Activity of Fraction X and Standard Drug

Concentration (µg/ml)	Fraction X		Ascorbic acid	
	Absorbance (mean)	% Inhibition	Absorbance (mean)	% Inhibition
100	0.64±0.00018[a]	26.57	0.45±0.0019[b]	44.51
80	0.58±0.00019[a]	32.72	0.17±0.0020[b]	80.04
60	0.49±0.0001[a]	36.13	0.16±0.0018[b]	81.30
40	0.44±0.0002[a]	43.34	0.15±0.0020[b]	82.56
20	0.37±0.0015[a]	52.19	0.13±0.0017[b]	85.09

The DPPH scavenging assay results revealed that both fraction X and ascorbic acid exhibited enhanced radical scavenging activity as the concentration increased. At a concentration of 80 µg/ml, the petroleum ether extracts effectively neutralized a significant number of free radicals, reaching a mean absorbance of 0.64. The IC_{50} values for DPPH scavenging activity were determined to be 51.95 µg/mL for the reference drug and 18.23 µg/mL for fraction X, indicating that fraction X demonstrated a stronger antioxidant capacity than ascorbic acid. Furthermore, the IC_{50} of fraction X was found to be significantly lower ($P < 0.0022$) than that of ascorbic acid, suggesting its superior radical-scavenging potential.

Table 5: IC_{50} Values for Fraction X and Standard Drug

Sample	IC 50 (µg/ml)
Fraction X	18.23±0.01
Ascorbic acid	51.95±0.01

DPPH Radical Scavenging Activity of Fraction Y

Table 6. depicts the results of the DPPH scavenging activity of the fraction Y and the standard drug

Table 6: DPPH Scavenging Activity of Fraction Y and Standard Drug

Concentration (µg/ml)	Fraction Y		Ascorbic acid	
	Absorbance (mean) ± SEM	% Inhibition	Absorbance (mean) ± SEM	% Inhibition
100	0.53±0.0011[a]	75	0.43±0.0012[b]	45.51
80	0.18±0.0012[a]	60	0.15±0.0012[b]	81.04
60	0.15±0.0011[a]	45	0.14±0.0011[b]	82.30
40	0.14±0.0013[a]	30	0.13±0.0013[b]	83.56
20	0.02±0.0012[a]	15	0.11±0.0014[b]	86.09

As concentrations increased, both the standard drug and Fraction Y exhibited a greater capacity to scavenge free radicals. At a concentration of 100 µg/ml, Fraction Y showed an even higher ability to neutralize free radicals, with a mean absorbance of 0.53. The IC_{50} values for the reference drug and Fraction Y were 51.95 µg/mL and 28.78 µg/mL, respectively, indicating that Fraction Y has a more powerful antioxidant effect than ascorbic acid. This could be due to the synergistic interactions among the phytochemicals in Fraction Y. Furthermore, the IC_{50} for Fraction Y was significantly lower ($P < 0.0001$) than that of ascorbic acid, highlighting its superior ability to scavenge radicals.

Table 7: IC_{50} for DPPH Scavenging Capacity for Fraction Y and Standard Drug

Sample	IC 50 (µg/ml)
Fraction Y	28.78±0.01
Ascorbic acid	51.95±0.01

In terms of IC_{50} values, the extract and fractions were ranked as follows: petroleum ether extract (47.66 µg/ml) > Fraction Y (28.78 µg/ml) > Fraction X (18.23 µg/ml) > Fraction Z (23.45 µg/ml). Given that a lower IC50 indicates higher antioxidant activity, Fraction Z demonstrates the strongest antioxidant effect, while the petroleum ether extract shows the weakest. All fractions outperform the petroleum ether extract in terms of antioxidant activity. The IC_{50} of ascorbic acid (51.95 µg/ml) is higher than that of the fractions, indicating weaker antioxidant activity. The superior antioxidant activity of the fractions may be attributed to the synergistic effects of their phytochemicals [14].

In Vitro Antimicrobial Assessment of the Various Pet. Ether Fractions

The micro-well broth dilution method was employed for determining the minimum inhibitory concentration (MIC).

Table 8: MIC of Fraction Z and Standard Drug

Test organisms	Fraction Z (mg/ml) ±SEM	Gentamicin (µg/ml) ±SEM
C. albicans	12.5±0.012	0.05±0.010
E. coli	25±0.014	0.05±0.010
S. aureus	25±0.013	0.05±0.014
S. pyogenes	25±0.011	0.05±0.011
S. typhi	25±0.013	0.05±0.013
P. aeruginosa	50±0.016	0.05±0.012

Fraction Z demonstrated notable antimicrobial properties against a range of microorganisms at different concentrations. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of microorganisms after overnight incubation, while the minimum bactericidal concentration (MBC) is the smallest concentration that completely halts microbial growth. Fraction Z exhibited antimicrobial effects against tested organisms, MIC = 12.5 mg/ml for Candida albicans, MIC = 25 mg/ml for other organism except for P. aeruginosa, which showed increased resistance (MIC = 50 mg/ml). These results highlight Fraction Z as a promising candidate for the development of new antimicrobial agents. Although gentamicin showed higher potency, Fraction Z may also offer potential activity against a broader spectrum of pathogenic microbes

Table 9: MIC of Fractions Y and Standard Drug

Test organisms	Fractions Y (mg/ml) ±SEM	Gentamicin (µg/ml) ±SEM
C. albicans	12.5±0.019	0.05±0.018
E. coli	25±0.015	0.05±0.015
S. aureus	25±0.020	0.05±0.011
S. pyogenes	12.5±0.016	0.05±0.013
S. typhi	12.5±0.018	0.05±0.013
P. aeruginosa	25±0.013	0.05±0.011

Fraction Y exhibited antimicrobial activity against all tested microorganisms at varying concentrations, with MIC values ranging from 6.25 to 25 mg/ml. *S. pyogenes* was the most susceptible, while *P. aeruginosa* demonstrated the greatest resistance. Fraction Y showed lower antimicrobial efficacy than gentamicin across all concentrations, with gentamicin exhibiting superior activity against *P. aeruginosa*.

Table 10: MIC of Fractions X and Standard Drug

Test organisms	MIC (mg/ml) ±SEM	Gentamycin (µg/ml) ±SEM
C. albicans	25±0.015	0.05±0.011
E. coli	25±0.014	0.05±0.013
S. aureus	12.5±0.017	0.05±0.012
S. pyogenes	6.25±0.016	0.05±0.014
S. typhi	25±0.019	0.05±0.012
P. aeruginosa	No inhibition	0.05±0.011

Fraction X exhibited antimicrobial activity against all the microorganisms tested, with MIC values ranging from 6.25 to 25 mg/ml. S. pyogenes was the most vulnerable, while P. aeruginosa showed the least susceptibility, with Fraction X demonstrating no inhibitory effect against it. Across all concentrations, Fraction B displayed less antimicrobial efficacy than gentamicin, which effectively inhibited P. aeruginosa.

The extract and fractions varied in their potency against different organisms. Against *E. coli*, both the extract and the fractions exhibited equivalent activity. For *S. aureus*, Fraction X and the extract showed similar potency, which were better than that of Fractions Y and Z, both of which exhibited equal activity. Regarding *S. pyogenes*, Fraction X was the most potent, proceeding with the extract, Fraction W, and Fraction Y, with Fraction Z showing the least activity. Fraction Y demonstrated superior activity against *S. typhi* compared to the extract, which was more potent than Fractions W and Z. In the case of *P. aeruginosa*, Fraction Y was the most effective, followed by the extract, Fraction W, and Fraction Z, while Fraction Y exhibited no inhibition. For *C. albicans*, Fractions X and Y displayed the strongest activity, whereas the extract and Fraction W showed less potency. Phytochemicals like tannins, steroids, and flavonoids may be responsible for the observed antimicrobial activity of these fractions [12].

In Vitro Anti-Helminthic of Pet. Ether Fractions

The anti-helminthic activity of fractions Z and Y was determined using earthworm (*Eudrilus eugeniae*)

Table 11: Mean Paralysis and Death Time of the Earthworm (Eudrilus Eugeniae) for Fraction Z and Standard Drug

Treatment	Fraction D		Mebendazole	
Concentration (mg/ml)	Mean Paralysis Time(min) ±SEM	Mean Death Time(min) ±SEM	Mean Paralysis Time(min) ±SEM	Mean Death Time(min) ±SEM
20	35.13±15[a]	62.16±2.89 [a]	50.60±18[b]	82.03±47.37 [b]
10	38.65±18 [a]	90.05±7.22 [a]	58.40±21[b]	135.01±72.55 [b]
5	40.75±24 [a]	150.01±5.09 [a]	63.12±15[b]	180.60±98.15 [b]
2.5	45.13±30 [a]	185.17±0.24 [a]	69.35±36 [b]	190.53±106.42 [b]
1.25	50.43±38 [a]	215.00±8.51 [a]	80.45±42 [b]	230.10±128.74 [b]
Normal saline	-	-	-	-

Table 12: Mean Paralysis and Death Time of the Earthworm (Eudrilus Eugeniae) for Fraction C and Mebendazole

Treatment	Fraction Y		Mebendazole	
Concentration (mg/ml)	Mean Paralysis Time(min) ±SEM	Mean Death Time(min) ±SEM	Mean Paralysis Time(min) ±SEM	Mean Death Time(min) ±SEM
20	28.73±15[a]	55.06±2.89 [a]	31.60±18[b]	82.03±47.37 [b]
10	33.15±18 [a]	89.02±7.22 [a]	37.70±21 [b]	126.07±72.55 [b]
5	39.55±24 [a]	150.10±5.09 [a]	26.02±15 [b]	170.00±98.15 [b]
2.5	46.33±30 [a]	182.05±0.24 [a]	63.37±36 [b]	184.33±106.42 [b]
1.25	56.16±38 [a]	219.05±8.51 [a]	74.40±42 [b]	223.00±128.74 [b]
Normal saline	-	-	-	-

Tables 11 and 12 present the mean paralysis and death times of worms at various concentrations of Fraction C, Fraction D, and mebendazole. As the concentrations of both Fraction C, Fraction D, and mebendazole increased, a corresponding reduction in both the death time and mean paralysis time of the worms was observed, indicating enhanced anthelmintic activity. At all concentrations, except 5 mg/ml, both Fraction C and Fraction D exhibited superior paralysis potential compared to mebendazole. Fraction C demonstrated a greater capacity to kill the worms at all concentrations than Fraction D, while Fraction D displayed higher potency than mebendazole, as it resulted in a shorter death time.

These findings suggest that Fraction C is a more potent anthelmintic agent than both Fraction D and the standard drug, mebendazole. Furthermore, when comparing the extract to Fraction C, the latter showed superior efficacy in paralyzing the worms, as it induced paralysis in a shorter time. Fraction C also caused faster worm mortality compared to the extract, underscoring its enhanced anthelmintic potency. The increased effectiveness of Fraction C may be due to the reduction or elimination of antagonistic components during the fractionation process, leading to an improved synergistic effect of its active compounds. Notably, the mean paralysis and death times for Fraction C were significantly shorter ($P < 0.0004$ and 0.0002, respectively) compared to mebendazole.

In Vitro Anti-inflammatory Capacity Determination of Pet. Ether Fractions

Table 13: Percentage Inhibition of Fraction, D and Aspirin

Concentration (µg/mL)	% inhibition, D	% inhibition, Aspirin
1000	26.10	82.08
2000	50.63	88.44
3000	54.57	88.89
4000	60.42	89.25
5000	61.67	90.95

Table 14. Percentage Inhibition of Fraction, C and Aspirin

Concentration (µg/mL)	% inhibition, C	% inhibition, Aspirin
1000	20.11	82.08
2000	44.73	88.44
3000	47.65	88.89
4000	58.68	89.25
5000	60.37	90.95

The plant extract's anti-inflammatory properties refer to its capacity to hinder the thermal denaturation of protein (specifically egg albumen) [15]. The pet. ether fractions D, C, and aspirin exhibited a concentration-dependent anti-inflammatory response at test concentrations ranging from 1000 to 5000 µg/ml. In this context, aspirin exhibited the highest inhibition at the test concentrations, followed by the D and C respectively. The anti-inflammatory potential of fractions D is attributed to phytochemicals like flavonoids, glycosides, terpenoids, and steroids present in M. barteri, which have been reported to possess anti-inflammatory properties according to [16, 17].

Conclusion

The study was conducted to investigate the phytochemical profile, as well as the antioxidant, antimicrobial, anthelmintic, and anti-inflammatory activities of Macaranga barteri. Petroleum ether was employed as the solvent for extracting the stem bark, yielding 4.20%. The phytochemicals present in the powdered sample included phenols, steroids, tannins, glycosides, saponins, terpenoids, polyphenols, and phytosterols. The extract was then fractionated into four primary fractions—W, X, Y, and Z—using column chromatography. The results of the study validate the traditional use of Macaranga barteri in the treatment of various ailments. Further purification and isolation of specific compounds could provide valuable leads for the development of novel antioxidant, antimicrobial, and anthelmintic therapeutic agents

Data Availability

All the data has been included in the manuscript.

Conflict of Interest

The authors have no conflict of interest (financial, professional, or personal) to declare.

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