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Synergistic Antioxidant and Antimicrobial Effects of Antrocaryon Micraster and Panda Oleosa

Tawiah Eunice[1], Bright Y. Vigbedor[1], Aquel Rene Lopez[2*]
[1]*Department of Biomedical Sciences, University of Health and Allied Sciences, Ho*
[2]*Department of Medical Laboratory Science, School of Health and Allied Sciences, Baldwin University College, Accra*

Abstract

Antrocaryon micraster and Panda oleosa are medicinal plants used in ethnomedicine for treating various ailments, owing to their rich phytochemical profiles and reported bioactivities. This study investigated the antioxidant and antimicrobial properties of these plants, individually and in combination, to assess their therapeutic potential. The study aimed to extract bioactive compounds from A. micraster and P. oleosa using cold maceration, screen for phytochemicals, and evaluate their antioxidant and antimicrobial activities. Plant samples (stem bark) were collected from designated locations in Ghana. Cold maceration with methanol and chloroform was employed for extraction. Phytochemical screening and thin-layer chromatography (TLC) were conducted to identify bioactive constituents. Antioxidant activity was assessed using 2,2-Diphenyl-1-picrylhydrazy (DPPH) and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging assays. Antimicrobial activity was tested against six microorganisms: Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Aspergillus niger, and Escherichia coli. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, steroids, and glycosides. Both extracts exhibited notable antioxidant properties, with radical scavenging activity ranging from 52.79% to 100% (DPPH) and 90.91% to 100% (ABTS). Antimicrobial assays demonstrated inhibitory effects against the tested microorganisms. A. micraster extract had the lowest Minimum Inhibitory Concentration (MIC) (3.125 mg/mL) and highest Minimum Bactericidal Concentration (MBC) (12.5 mg/mL), while the combined extracts exhibited bactericidal and bacteriostatic effects, as well as fungicidal activity against A. Niger. This study confirms the antioxidant and antimicrobial potential of A. micraster and P. oleosa. Their combined use enhances bioactivity, suggesting potential as sources of natural therapeutic agents. Further research is recommended to explore their clinical applications.

Keywords: *Antimicrobial, Bacillus Subtilis, Enterococcus Faecalis, Escherichia Coli.*

Introduction

Natural products, particularly those derived from plants, have long served as vital sources of bioactive compounds with therapeutic potential. Their use in traditional medicine spans generations and continues to be integral in primary healthcare systems, particularly in developing regions. It is estimated that over 80% of populations in underdeveloped areas rely on herbal remedies for primary health needs [1]. Among the wealth of medicinal plants, *Antrocaryon micraster* and *Panda oleosa* have garnered attention for their promising biological activities, particularly their antioxidant and antimicrobial properties.

Antrocaryon micraster, commonly used in West African traditional medicine, has been

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****Corresponding Author: lonnet2001@yahoo.com***

reported to possess a diverse phytochemical profile, including flavonoids, tannins, saponins, and steroids [2]. It is traditionally used to manage conditions such as malaria, arthritis, and respiratory ailments, highlighting its potential for broader therapeutic applications. Similarly, *Panda oleosa* has been traditionally employed for treating diabetes, HIV/AIDS, and other conditions, with its bioactivities linked to the presence of alkaloids, tannins, saponins, and glycosides [3].

The increasing global burden of antimicrobial resistance and oxidative stress-related diseases has amplified interest in natural plant-derived compounds as potential sources of novel therapeutics. Oxidative stress, driven by an imbalance between free radicals and antioxidants in the body, is implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disease, cancer, and neurodegenerative disorders [4]. The antioxidant properties of natural products, therefore, play a critical role in neutralizing free radicals and mitigating their harmful effects. Likewise, the rise of antimicrobial-resistant strains of bacteria and fungi underscores the urgent need for alternative antimicrobial agents. Plant extracts with antimicrobial properties offer a promising avenue for addressing these challenges.

Despite the documented traditional use and preliminary phytochemical analyses of *A. micraster* and *P. oleosa*, there is a scarcity of comprehensive studies evaluating their combined antioxidant and antimicrobial effects. Investigating the interaction between these two plant species is critical, as synergistic combinations of bioactive compounds may enhance therapeutic efficacy, reduce resistance development, and contribute to the formulation of new, natural health products. Furthermore, understanding the phytochemical composition and bioactivities of these plants will provide scientific validation for their continued use in traditional medicine and contribute to the discovery of new drug leads.

This study aims to bridge the knowledge gap by investigating the phytochemical profiles, antioxidant, and antimicrobial activities of *Antrocaryon micraster* and *Panda oleosa*, both individually and in combination. By assessing the synergistic potential of these plants, this research seeks to contribute to the development of novel natural therapeutics with potential applications in addressing oxidative stress and microbial infections.

Methodology

Study Design

This laboratory-based cross-sectional study involved the in vitro evaluation of the phytochemical constituents, thin layer chromatography (TLC) profiles, and the antimicrobial and antioxidant activities of stem bark extracts from *Panda oleosa* and *Antrocaryon micraster*.

Study Site

The study was conducted at the Microbiology Laboratory and the Analytical and Organic Chemistry Laboratory of the School of Basic and Biomedical Sciences, University of Health and Allied Sciences (UHAS), Ho, Ghana.

Sample Collection

Stem bark samples of *Antrocaryon micraster* were collected from Kwahu Tafo (6.65666°N, –0.65975°W), and those of *Panda oleosa* from Afram Plains (6.9209°N, 0.1495°W), both in the Eastern Region of Ghana. Botanical identification and authentication were carried out by a certified botanist. The collected samples were transported to the School of Basic and Biomedical Sciences, University of Health and Allied Sciences, Ho, where they were air-dried at ambient temperature (25–28 °C) for one to two weeks.

Sample Preparation and Extraction

The air-dried stem bark samples of *Antrocaryon micraster* and *Panda oleosa* were

pulverized into fine powder using a mechanical grinder. Extraction was performed using a cold maceration method adapted from [5] with slight modifications.

For each plant, 50 g of powdered material was soaked in a total of 250 mL of solvent mixture. *A. micraster* was extracted using methanol:chloroform in a 70:30 (v/v) ratio, while *P. oleosa* used a 50:50 (v/v) ratio. The mixtures were left to macerate at room temperature for 72 hours with occasional agitation. The supernatants were filtered using Whatman No. 1 filter paper and concentrated by oven drying. The resulting crude extracts were weighed and stored at 4°C for subsequent analysis.

Ethical Consideration

The Research Ethics Committee of the University of Health and Allied Sciences, Ho granted the study ethical approval with a protocol identification number **UHAS-REC A.10 [176] 23 24.**

Thin Layer Chromatography (TLC) Analysis

TLC was conducted to assess the chemical profile of each extract. A silica gel-coated TLC plate (3 cm × 5 cm) was used. For *P. oleosa*, petroleum ether:ethyl acetate (70:30) served as the mobile phase, while for *A. micraster*, a 50:50 ratio with three drops of methanol was employed. Samples were spotted 1 cm from the base, developed until the solvent front was 1–2 cm from the top, then visualized under UV light. Retardation factor (Rf) values were calculated as:

$$Rf = \frac{Distance\ traved\ by\ solute}{Distance\ traveled\ by\ solvent}$$

Phytochemical Screening

The presence of key phytochemicals was qualitatively assessed using standard protocols [6] with slight modifications. Crude extracts were dissolved in methanol and filtered prior to analysis.

1. Flavonoids: Detected by reaction with NH_4 and H_2SO_4, producing a pale-yellow color.
2. Alkaloids: Detected using Mayer's reagent; an orange precipitate indicated presence.
3. Tannins: Ferric chloride ($FeCl_3$) was used; dark green coloration signified presence.
4. Saponins: Persistent froth formation after shaking with distilled water confirmed presence.
5. Steroids: Identified via red ring formation when mixed with chloroform and H_2SO_4.
6. Glycosides: Positive indication given by a brick red ring after treatment with glacial acetic acid, $FeCl_3$, and H_2SO_4.

Evaluation of Antioxidant Activity

Antioxidant properties were assessed in vitro using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging assays.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay.

Following the method of Vigbedor et al. (2022), 160 µL of 0.1 M DPPH was added to 40 µL of each extract or ascorbic acid (positive control) at concentrations of 0.0625–1.0 mg/mL. Plates were incubated for 1 hour at room temperature and absorbance measured at 517 nm using a VERSAmax microplate reader.

$$\%\ DPPH\ Scavenging\ Activity\ = \left(\frac{(Ao\ -\ A)}{Ao}\right) \times\ 100$$

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay.

ABTS solution (160 µL, 0.1 M) was mixed with 40 µL of the test extract or ascorbic acid. Following incubation at room temperature for 1 hour, absorbance was measured at 734 nm.

$$\%\ ABTS\ Scavenging\ Activity\ = \left(\frac{Ao - Ao}{AO}\right) \times 100$$

Antimicrobial Analysis

Microbial Strain Isolation and Growth Conditions

Microbial strains used include *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (NCTC 13440), *Staphylococcus aureus* (NCTC 29212), *Bacillus subtilis*, *Enterococcus faecalis*, and *Aspergillus niger*. Isolates were sub-cultured on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 48 hours for purity confirmation.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of *Panda oleosa* and *Antrocaryon micraster* extracts were determined using the microbroth dilution method in 96-well microtiter plates, following protocols described by [7-10] with slight modifications.

Stock solutions of the extracts were prepared in DMSO and serially diluted to obtain concentrations ranging from 0.0976 to 50 mg/mL. In each well of a 96-well microtiter plate, 100 µL of double-strength Mueller Hinton Broth (Oxoid Ltd., UK) was mixed with 100 µL of each extract concentration.

Wells 11 and 12 served as controls:

1. Positive control**:** broth with test organism only
2. Negative control**:** broth without organism

Tetracycline (for bacteria) and nystatin (for fungi) at 1 mg/mL were included as reference antibiotics.

A standardized microbial suspension (0.5 McFarland standard) was prepared for each test organism, and 100 µL was added to each well. Plates were incubated at 37°C for 48 hours for both bacterial and fungal strains.

After incubation, 10 µL of 0.1% (w/v) tetrazolium chloride (TTC) solution was added to each well and allowed to react for 10 minutes. Wells that remained colorless or light yellow (indicating no microbial growth) were considered inhibitory. The MIC was recorded as the lowest concentration of extract that showed no visible color change (i.e., no growth).

All tests were performed in duplicates, and the average MIC values were reported.

MIC was determined by broth microdilution using 96-well plates. Extracts were serially diluted (0.0976–50 mg/mL) in DMSO and added to Mueller Hinton broth. Positive (broth + organism) and negative (broth only) controls were included. After inoculation and incubation at 37°C for 48 hours, 0.1% TTC dye was added. MIC was recorded as the lowest concentration that inhibited color change.

Determination of Minimum Fungicidal Concentration (MFC)

The Minimum Fungicidal Concentration (MFC) of each plant extract was determined using a standard broth microdilution method as described by Clinical and Laboratory Standards Institute (CLSI, 2008), with slight modifications. Following MIC determination, 10 µL aliquots from wells showing no visible fungal growth were subcultured onto sterile Sabouraud Dextrose Agar (SDA) plates. The plates were then incubated at 37°C for 48 hours.

Post-incubation, the plates were examined for fungal colony formation. The MFC was defined as the lowest concentration of the extract at which no visible fungal growth was observed on the agar surface, indicating fungicidal activity.

To distinguish between fungistatic and fungicidal effects, the MFC/MIC ratio was calculated:

1. An MFC/MIC ratio ≤ 4 was interpreted as fungicidal
2. An MFC/MIC ratio > 4 was interpreted as fungistatic

All tests were performed in duplicate to ensure accuracy and reproducibility.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of each plant extract was determined using a standard broth microdilution method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012). Following Minimum Inhibitory Concentration (MIC) assessment, 10 µL aliquots were taken from each well that exhibited no visible bacterial growth and subcultured onto fresh Mueller-Hinton Agar (MHA) plates.

The plates were incubated at 37°C for 24 hours, after which they were examined for colony formation. The MBC was defined as the lowest concentration of the extract that yielded no visible bacterial growth on the agar surface, indicating bactericidal activity.

To interpret the nature of the antimicrobial effect, the MBC/MIC ratio was calculated:

1. An MBC/MIC ratio ≤ 4 was considered bactericidal
2. An MBC/MIC ratio > 4 was considered bacteriostatic

All assays were performed in duplicate to ensure reproducibility and accuracy

Results

Percentage yield of A. micraster and P. oleosa extracts

Table 1. below present the percentage yields of stem bark extracts from *Antrocaryon micraster* and *Panda oleosa* obtained using a methanol-chloroform solvent system. The extraction yield for *P. oleosa* was higher (8.36%) compared to *A. micraster* (4.24%). This disparity may be due to species-specific differences in phytochemical content and matrix structure. Specifically, *P. oleosa* may contain a greater proportion of polar and semi-polar secondary metabolites such as flavonoids, terpenoids, and saponins that are more readily solubilized in methanol-chloroform mixtures [11]. Additionally, plant anatomical features such as bark density and resin content may influence the extractability of bioactive compounds [12].

These results highlight the importance of selecting appropriate solvent systems and understanding plant matrix characteristics in maximizing extraction efficiency. The higher yield from *P. oleosa* suggests a potentially richer phytochemical reservoir, making it a suitable candidate for further pharmacological investigations.

Table1. Percentage yield of A. micraster and P. oleosa extracts

EXTRACTS	% YIELD	APPEARANCE
AMB	4.24	Brownish-red
POB	8.36	Brownish-red and Sticky

**(AMB) = Antrocaryon micraster bark (POB) = Panda oleosa bark.*

Thin Layer Chromatography (TLC) Profile of the Extracts

Table 2 presents the Thin Layer Chromatography (TLC) profiles of *Antrocaryon micraster* and *Panda oleosa* stem bark extracts, revealing the presence of multiple phytochemical constituents as indicated by distinct spots and their corresponding Rf values. For *A. micraster*, four distinct spots were observed with Rf values of 0.20, 0.30, 0.50, and 1.00**,** while *P. oleosa* displayed four spots with Rf values of 0.10, 0.50, 0.70, and 0.90**.**

The variation in Rf values reflects differences in the polarity and solubility of the phytochemicals present in the two plant extracts. Compounds with lower Rf values tend to be more polar and migrate less on the TLC plate, while higher Rf values indicate the presence of non-polar or less polar compounds

[13]. The appearance of multiple spots confirms the crude nature of the extracts and suggests a rich phytochemical composition, which may account for the observed biological activities such as antioxidant and antimicrobial effects.

TLC remains a widely used, cost-effective preliminary screening method for evaluating plant extract composition, especially when investigating the presence of classes of bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolics [14]. The distinct TLC profiles of both extracts also offer a foundation for future compound isolation and purification using advanced chromatographic techniques.

Table 2. Thin Layer Chromatography (TLC) profile of the extracts

Extract	Solvent System	Method of Extraction	Number of Spots	R F Values in (cm)
A.micraster	Pet ether/ ethyl acetate (50:50) + 3 drops of methanol	UV	4	0.2,0.3,0.5
P. oleosa	Pet ether/ ethyl acetate (70: 30)	UV	4	0.1, 0.5, 0.7, 0.9

Phytochemical Screening of Crude Extracts

Table 3 summarizes the results of the qualitative phytochemical screening conducted on the crude stem bark extracts of *Antrocaryon micraster* and *Panda oleosa*. The analysis revealed the presence of several classes of bioactive secondary metabolites, including flavonoids, tannins, saponins, and steroids in both extracts. Notably, alkaloids and glycosides were detected only in *P. oleosa*, suggesting species-specific phytochemical variation.

The presence of flavonoids and tannins, which are well-documented for their antioxidant, anti-inflammatory, and antimicrobial properties, supports the observed biological activities of the extracts [15, 16]. Saponins, known for their membrane-disruptive activity, and steroids, which exhibit diverse pharmacological effects, may also contribute to the extracts' antimicrobial potential.

The detection of alkaloids and glycosides exclusively in *P. oleosa* could partly explain its relatively higher antimicrobial efficacy in some assays. Alkaloids are known to interfere with microbial DNA replication and protein synthesis, while glycosides have been implicated in cytotoxic and antimicrobial mechanisms [17].

These results provide a biochemical basis for the traditional use of these plants in ethnomedicine and offer preliminary justification for further isolation and characterization of specific active compounds.

Table 3. Phytochemical screening of crude extracts

Phytochemicals Analyzed	A. micraster	P. oleosa
Flavonoids	+	+
Alkaloids	ND	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Glycosides	ND	+

**(+) = Presence of the Phytochemical ND = Not detected.*

Determination of Antioxidant Activity of the Extracts

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of *Antrocaryon micraster*, *Panda oleosa*, and their combined extract (AM+PO) was evaluated at varying concentrations and compared to a standard antioxidant (Vitamin C). All three samples exhibited concentration-dependent scavenging activity, with percentage inhibition increasing as the concentration of the extracts increased in table 4 below.

At lower concentrations (0.0625 mg/mL), the combination (AM+PO) showed the highest scavenging activity, achieving 98.47% inhibition, while *P. oleosa* alone showed the lowest activity. As the concentration increased, all extracts demonstrated near-complete radical scavenging, reaching 100% inhibition at concentrations ≥ 0.5 mg/mL. The observed activity of the combination extract suggests a possible synergistic effect, where the antioxidant components of both plants enhance each other's efficacy.

The strong DPPH radical scavenging ability observed supports the presence of phenolic compounds, flavonoids, and tannins identified in the phytochemical screening, all of which are known for their hydrogen-donating and free radical-neutralizing properties [18, 19]. The superior performance of the combined extract further indicates its potential as a natural antioxidant formulation.

Table 4. Mean ± Standard deviation percentage inhibition of all the extracts and Vitamin C from DPPH scavenging assay

DPPH Assay	**Concentrations (mg/mL)**					
Extracts	0.0625	0.125	0.25	0.5	1	Vitamin C
Percentage Inhibition (%) Mean ± SD						
AM + PO	98.47 ± 1.69	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
AM	60.99 ± 2.16	84.90 ± 0.59	94.85 ± 2.37	100 ± 0	100 ± 0	
PO	52.79 ± 1.63	62.64 ± 2.30	89.17 ± 0.41	100 ± 0	100 ± 0	100 ± 0

ABTS Radical Scavenging Activity

The ABTS radical scavenging assay was employed to evaluate the antioxidant capacity of *Antrocaryon micraster, Panda oleosa*, and their combined extract (AM+PO), with Vitamin C serving as the standard reference. Results demonstrated that all three extracts exhibited strong ABTS radical scavenging activity**,** consistent with their DPPH results.

At the lowest concentration tested (0.0625 mg/mL), *A. micraster* and *P. oleosa* individually exhibited complete scavenging activity **(**100% inhibition**),** while the combination extract (AM+PO) showed slightly lower activity **(**90.91 ± 0.88%**).** However, at concentrations ≥ 0.125 mg/mL, the AM+PO formulation reached 100% inhibition**,** indicating that synergism may be concentration-dependent.

The high antioxidant performance observed in the ABTS assay confirms the presence of potent hydrogen- or electron-donating phytochemicals, such as flavonoids, phenolics, and tannins, which were identified in the preliminary phytochemical screening. The ABTS assay is particularly suitable for both hydrophilic and lipophilic antioxidant compounds, making it effective for evaluating complex plant extracts [20]. The activity observed in both assays reinforces the potential of these plant extracts individually and in combination as promising natural antioxidants for therapeutic or nutraceutical applications. As shown in table 5.

Table 5. Mean ± Standard deviation percentage inhibition of all the extracts and Vitamin C from ABTS scavenging assay

ABTS Assay	**Concentrations (mg/mL)**					
Extracts	0.0625	0.125	0.25	0.5	1	Vitamin C
Percentage Inhibition (%) Mean ± SD						
AM + PO	90.91 ± 0.88	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
AM	97.85 ± 3.72	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
PO	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0

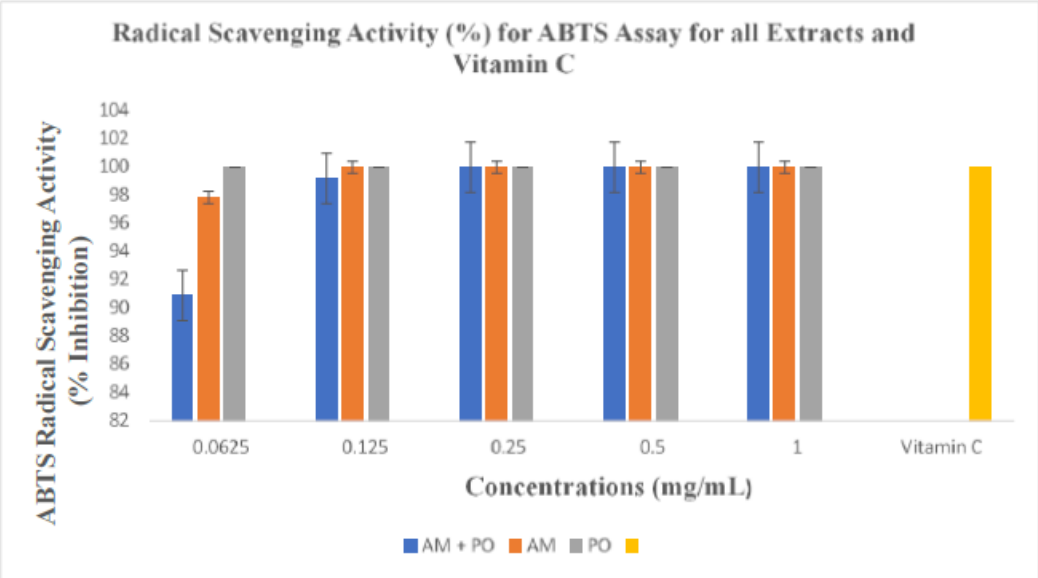

Figure 1. Radical Scavenging activity (%) of all the extracts and Vitamin C obtained from ABTS scavenging assay

Determination of Antimicrobial Activity

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Extracts Against Test Organisms

Table 6 summarizes the antimicrobial efficacy of *Antrocaryon micraster* (AM), *Panda oleosa* (PO), and their combination (AM+PO) against five bacterial strains: *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus subtilis*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth microdilution method, with the MBC/MIC ratio used to categorize the extracts as either bactericidal (R ≤ 4) or bacteriostatic (R > 4).

The MICs of the extracts ranged from 3.125 to 25 mg/mL**,** indicating varied antimicrobial potency across different organisms. *P. oleosa* demonstrated the lowest MICs (3.125 mg/mL) against most test organisms, suggesting potent inhibitory effects. However, despite its low MICs, *P. oleosa* generally exhibited bacteriostatic activity, as reflected by high MBC/MIC ratios (R > 4).

Conversely, *A. micraster* displayed bactericidal effects against *E. coli*, *E. faecalis*, *K. pneumoniae*, and *S. aureus*, with R-values ranging from 1 to 4. Its lowest MIC was against *S. aureus* (3.125 mg/mL), with an MBC of 12.5 mg/mL. The combination extract (AM+PO) also showed bactericidal properties against most strains, including *E. coli*, *E. faecalis*, *K. pneumoniae*, and *B. subtilis*, with R-values ≤ 4. This suggests that synergistic interactions

between the two plant extracts may enhance bactericidal efficacy.

The observed differences in antimicrobial activity may be attributed to variations in the phytochemical profiles of the extracts. The presence of flavonoids, tannins, and saponins which are known for their membrane-disruptive and protein-denaturing properties could underlie the bactericidal action of *A. micraster* [15, 21].

Table 6. MIC, MBC and MBC/MIC ratios for the crude extracts.

Test Organisms	**AM**			**PO**			**AM + PO**		
	MIC	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**
E. coli	25	25	1^{bc}	6.25	50	8^{bs}	6.25	25	4^{bc}
E. faecalis	6.25	12.5	2^{bc}	3.125	50	16^{bs}	12.5	25	2^{bc}
K. pneumoniae	6.25	12.5	2^{bc}	3.125	25	8^{bs}	6.25	25	4^{bc}
S. aureus	3.125	12.5	4^{bc}	3.125	25	8^{bs}	3.125	25	8^{bs}
B. subtillis	3.125	25	8^{bs}	3.125	25	8^{bs}	6.25	25	4^{bc}

R values were further calculated to classify if they are either bactericidal (kill the organisms) or bacteriostatic (inhibit bacterial growth). This is given by the formula below;

$$R = \frac{Minimum\ Bactericidal\ Concentration\ (MBC}{Minimum\ Inhibitory\ Concentration\ (MIC)}$$

With reference being $R \leq 4$ = bactericidal (bc) and $R > 4$ = bacteriostatic (bs)

Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the Extracts against the Test Organism

Table 7 summarizes the antifungal activity of *Antrocaryon micraster* (AM), *Panda oleosa* (PO), and their combination (AM+PO) against *Aspergillus niger* using Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and the MFC/MIC ratio (R). The results show that all three extracts had the same MFC value of 25 mg/mL, while the MIC values varied among them.

Antrocaryon micraster exhibited an MIC of 12.5 mg/mL and an MFC/MIC ratio of 2, indicating fungicidal activity **(**$R \leq 4$). In contrast, both *Panda oleosa* and the AM+PO combination had lower MIC values of 3.125 mg/mL but MFC/MIC ratios of 8, which classifies their activity as fungistatic ($R > 4$). This implies that while *P. oleosa* and the combined extract are potent in inhibiting fungal growth, they do not effectively kill the fungal cells at the concentrations tested.

The results suggest that *A. micraster* contains fungicidal bioactive compounds that may act through mechanisms such as disruption of fungal cell membranes or inhibition of ergosterol synthesis, as observed with certain plant-derived compounds like saponins and flavonoids [22]. Meanwhile, the fungistatic effect of *P. oleosa* and AM+PO may still hold therapeutic relevance, especially in managing chronic fungal infections where growth suppression is beneficial.

The consistent MFC values but differing R-values highlight the importance of not relying solely on MFC or MIC in isolation when evaluating antifungal efficacy. The MFC/MIC ratio provides a clearer distinction between fungistatic and fungicidal effects**,** which is crucial for selecting suitable candidates for antifungal drug development.

Table 7. MIC, MFC and MFC/MIC ratios for the crude extracts

Test Organism	AM			PO			AM + PO		
	MIC	MFC	R	MIC	MFC	R	MIC	MFC	R
A. niger	12.5	25	2^{fc}	3.125	25	8^{fs}	3.125	25	8^{fs}

R values were further calculated to classify if they are either fungicidal (kill the organism) or fungistatic (inhibit fungal growth). This is given by the formula below;

$$R = \frac{Minimum\ Fungicidal\ Concentration\ (MFC)}{Minimum\ Inhibitory\ Concentration\ (MIC)}$$

With reference being R ≤ 4 = fungicidal (fc) and R > 4 = fungistatic (fs)

Discussion

The current study evaluated the antioxidant and antimicrobial activities of stem bark extracts of *Antrocaryon micraster* and *Panda oleosa*, both individually and in combination. The findings provide scientific validation for their ethnopharmacological use and demonstrate their potential as sources of natural therapeutic agents.

Extraction Yield and Phytochemical Composition

The percentage yield was higher for *P. oleosa* (8.36%) compared to *A. micraster* (4.24%), suggesting that *P. oleosa* may contain more extractable compounds or greater solubility in the methanol-chloroform system used. This observation aligns with [11], who reported that solvent polarity significantly affects extraction efficiency. The presence of flavonoids, tannins, saponins, and steroids in both extracts and alkaloids and glycosides in *P. oleosa* indicates a diverse phytochemical composition, supporting their observed biological activities. These findings are consistent with previous reports on the phytochemical richness of both species [2, 3].

Antioxidant Activity

The antioxidant assays (DPPH and ABTS) confirmed that both extracts possess significant free radical scavenging abilities, with near-complete inhibition at higher concentrations. The formulation of AM+PO exhibited enhanced DPPH inhibition (98.47% at 0.0625 mg/mL) and reached 100% inhibition at ≥0.5 mg/mL, indicating a possible synergistic interaction. This synergism may be attributed to the combined effects of flavonoids, phenolics, and tannins phytochemicals known for their strong antioxidant properties [18, 19]. The ABTS results further supported these findings, particularly highlighting that *P. oleosa* and *A. micraster* both exhibited 100% inhibition even at lower concentrations, confirming their potent antioxidant capacity.

Antibacterial Activity

The extracts demonstrated broad-spectrum antibacterial activity against *E. coli*, *E. faecalis*, *K. pneumoniae*, *S. aureus*, and *B. subtilis*. The MIC values ranged from 3.125–25 mg/mL, with *P. oleosa* showing the lowest MICs, yet often associated with bacteriostatic effects (MBC/MIC > 4). In contrast, *A. micraster* displayed bactericidal activity (MBC/MIC ≤ 4) against four of the five bacteria tested, supporting the claim that it may be more effective in microbial elimination rather than inhibition alone. These observations align with previous reports that highlight the membrane-disrupting effects of flavonoids and saponins [15, 21].

Notably, the combination extract (AM+PO) exhibited bactericidal activity against *E. coli*, *E.*

faecalis, *K. pneumoniae*, and *B. subtilis*, suggesting that synergistic interactions between the two extracts may enhance antibacterial efficacy. This is important, as synergistic formulations can lower the required dosage, reduce side effects, and help mitigate antimicrobial resistance.

Antifungal Activity

Against *Aspergillus niger*, *A. micraster* demonstrated fungicidal activity (MFC/MIC = 2), while *P. oleosa* and the AM+PO formulation were fungistatic (MFC/MIC = 8). This implies that while *P. oleosa* is effective in inhibiting fungal growth, *A. micraster* may be more suitable for complete fungal eradication. These results are consistent with previous findings that certain plant-derived compounds like flavonoids and saponins exert fungicidal effects by disrupting fungal membranes or inhibiting ergosterol synthesis [22].

Conclusion

This study explored the phytochemical composition, antioxidant activity, and antimicrobial potential of the stem bark extracts of *Antrocaryon micraster* and *Panda oleosa*, both individually and in combination. The findings revealed that both plants are rich in phytochemicals such as flavonoids, tannins, saponins, and steroids, with *P. oleosa* also containing alkaloids and glycosides. These bioactive compounds are known to contribute significantly to therapeutic effects, particularly in combating oxidative stress and microbial infections [6, 23].

The antioxidant evaluation using DPPH and ABTS assays demonstrated potent radical scavenging activities by all extracts, with the combination extract (*AM + PO*) showing enhanced activity at lower concentrations, suggesting possible synergistic interactions between the phytochemicals present in both plants [24, 25]. The observed antioxidant potential highlights the extracts' ability to neutralize free radicals, which are implicated in the development of chronic diseases such as cancer, cardiovascular, and neurodegenerative disorders [19, 24].

Antimicrobial analysis indicated that the extracts exhibited both bactericidal and bacteriostatic effects against clinically significant pathogens including *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, as well as fungicidal and fungistatic effects against *Aspergillus niger*. Notably, *A. micraster* showed superior antimicrobial efficacy in terms of minimum inhibitory and bactericidal concentrations, while *P. oleosa* demonstrated broader phytochemical diversity [2, 3, 5].

Implications and Future Directions

The demonstrated antioxidant and antimicrobial properties of these extracts underscore their potential as natural alternatives to synthetic drugs, particularly in the management of infections and oxidative stress-related diseases. The observed synergistic effects of the AM+PO combination are particularly noteworthy and could be explored in the development of phytopharmaceutical formulations.

Limitations of the Study

Despite the promising findings, several limitations should be acknowledged:

1. The study was limited to the use of crude plant extracts, which contain a complex mixture of compounds. Without purification, the specific phytochemicals responsible for the observed activities remain unidentified.
2. All antimicrobial and antioxidant evaluations were performed in vitro. While these provide preliminary insights, they may not fully reflect how the extracts behave in vivo due to bioavailability and metabolic factors.
3. The antimicrobial screening was conducted against a select panel of microbial strains. Broader screening, including multidrug-

resistant clinical isolates, would enhance the generalizability of the results.

4. The study did not assess the cytotoxicity or potential side effects of the extracts on normal cells, which is essential for determining safety for therapeutic use.
5. Plant samples were obtained from a single geographical location, which may limit the chemical profile diversity influenced by environmental factors like soil type and climate.

Recommendations

Based on the outcomes and limitations of the study, the following recommendations are proposed:

1. Future work should focus on isolating, identifying, and characterizing the specific bioactive constituents using chromatographic and spectroscopic techniques.
2. The promising in vitro activities warrant in vivo testing to assess pharmacokinetics, bioavailability, toxicity, and therapeutic efficacy in animal models.
3. Cytotoxicity assays using normal mammalian cell lines should be conducted to determine the safety profile of both individual and combined extracts.
4. Molecular investigations should be conducted to elucidate the mechanisms behind the antimicrobial and antioxidant effects.
5. The antimicrobial potential should be evaluated against a broader range of pathogens, including drug-resistant clinical isolates.
6. Standardization of extract doses and exploration of suitable formulation techniques (e.g., capsules, ointments) should be pursued for future pharmaceutical or nutraceutical applications.
7. The findings support the traditional uses of *Antrocaryon micraster* and *Panda oleosa*. Therefore, efforts should be made to integrate these plants into validated traditional medicine protocols.

Conflict of Interest

There is no conflict of interest

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Author Contributions

Tawiah Eunice, Bright Y. Vigbedor conceived and designed the study and data collection. Aquel Rene Lopez contributed to and analysis and interpretation. All authors contributed to data interpretation. Aquel Rene Lopez drafted the article. Tawiah Eunice, Bright Y. Vigbedor provided critiques and revisions to the drafted article. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

Availability of Data

Data is available upon request to the corresponding author.

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