### Phytochemical analysis, GC-MS profiling, and assessment of antioxidant, antibacterial, and antimycobacterial properties of extracts of *Brillantaisia owariensis* leaves

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Abstract: **Brillantaisia** *Owariensis (B.* owariensis) is a medicinal plant that grows in home gardens in Abia State, southeastern Nigeria. A research study was considered to determine the bioactivity of B.owariensis leaves. Air dried B.owariensis leaves were macerated in a methanol solvent for three days. The filtrate was concentrated to obtain the methanolic crude extract, which was then partitioned to yield hexane, chloroform, and ethyl acetate extracts. The results obtained from the phytochemical screening revealed the abundancy of cardiac glycoside, followed by phenols and saponins. The antibacterial analysis of B.owariensis extracts revealed activity against Streptococcus pneumoniae and Klebsiella pneumoniae, with methanol and hexane extracts potentially having the highest antibacterial potency. The extracts' free radical scavenging, reducing and chelating activities were investigated, and the results revealed significant antioxidant activity. The antimycobacterial assay of different fractions of the *B*. *owariensis* revealed that the methanol and hexane extracts are extremely sensitive to the various drug resistant species of Mycobacterium tuberculosis: drug susceptible and drug resistant varieties. The volatile constituents of the methanolic extracts were determined using GCMS. These compounds have established biological relevance in supporting B.owariensis, the plant's widespread ethnobotanical use.

**Keywords**: Brillantaisia owariensis, antibacterial, antioxidant. Antimycobacterium, GC-MS

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### 1.0 Introduction

A healthy and functioning society is the foundation of a viable and productive nation. Medicinal plants remain central to global healthcare due to their rich repository of metabolites-phytochemicalssecondary which are known for their diverse pharmacological properties. These naturally occurring compounds, such as alkaloids, flavonoids. tannins, phenols, glycosides, saponins. and anthraquinones, possess significant therapeutic potential and form the basis of many modern pharmaceuticals (Adegoke et al., 2010; Kamba and Hassan, 2010; Ujowundu et al., 2010). Herbal medicines, used since antiquity in forms such as decoctions, teas, poultices, and granules (Samuelsson, 2004), continue to play a vital role in primary healthcare across the world.

Research has shown that approximately 25% of prescription drugs are derived from plant sources (Adewusi and Afolayan, 2010), yet only an estimated 15% of the world's 300,000 higher plant species have been investigated for their pharmacological properties (De Luca et al., 2012). This highlights the urgent need to underutilized or lesser-known explore medicinal plants for potential therapeutic applications. Numerous studies have validated the antimicrobial, antidiabetic, antipyretic, analgesic. antioxidant. antiviral. and antiprotozoal activities of medicinal plant extracts (Olaleye et al., 2006; Antia et al., 2015; Dar et al., 2017; Igwe and Echeme, 2013). Ethnopharmacological and natural product research has increasingly turned its attention to phytochemicals identifying capable of combating infectious diseases, especially

tuberculosis, due to the increasing incidence of drug-resistant strains of *Mycobacterium tuberculosis* (Duraipandiyan et al., 2006; Cragg and Newman, 2009; Balunas and Kinghorn, 2005).

Brillantaisia owariensis (Acanthaceae), a perennial shrub commonly found in Nigeria, Togo, West Cameroon, and Uganda, is one such plant with promising ethnomedicinal uses. It is known locally in southeastern Nigeria as Oberi Iburu or Ekere and is frequently cultivated in home gardens. Morphologically, it is characterized by glandular, sticky stems up to 2 meters tall, petiolate leaves, dark purple calyces with dense glandular hairs, and a pale to deep purple corolla with a white neck measuring 25-53 mm. Its fruit is capsuleshaped. Ethnobotanical evidence supports the use of B. owariensis leaves in treating tuberculosis (Tabuti, 2010), anemia. rheumatism, menstrual disorders, and stomach pain in Congo and other African regions (Ngbolua et al., 2013; Makambila-Koubemba et al., 2011; Asai et al., 2012; Mbatchi et al., 2006). A more recent validation by Mac-Kalunta et al. (2021) confirms the plant's use in anemia treatment in parts of Nigeria, where local midwives and traditional birth attendants also administer its leaf extract to regulate postpartum bleeding and facilitate conception in newly married or infertile women.

Despite this widespread traditional usage, there is limited scientific data to support the bioactive properties of *B. owariensis*, particularly concerning its antibacterial, antioxidant, and antimycobacterial potential. Most studies have focused on its folkloric uses with scant phytochemical profiling and in vitro validation of its efficacy.

This study was therefore designed to address this knowledge gap by conducting a detailed phytochemical screening, GC-MS profiling, and evaluating the antioxidant, antibacterial, and antimycobacterial activities of different solvent extracts of *Brillantaisia owariensis* leaves.





The aim of the study is to isolate and characterize the bioactive constituents of B. owariensis. evaluate their free radical scavenging, reducing and metal chelating activities, assess their antibacterial effects against selected pathogens (Streptococcus pneumoniae, Klebsiella pneumoniae), and determine their antimycobacterial efficacy against both drug-susceptible and drugresistant strains **Mycobacterium** of tuberculosis.

The significance of this study lies in its scientifically validate potential to the ethnobotanical relevance of B. owariensis. identify new compounds with pharmacological interest, and contribute to the development of plant-based therapeutics. especially for antibiotic resistance managing and tuberculosis. Furthermore, the identification of volatile compounds through GC-MS analysis will offer deeper insights into the chemical makeup of the plant and help establish a baseline for further drug development efforts. Fig. 1 shows the photograph of the plant



Fig. 1: photograph of *Brillantaisia* owariensis leaves

### 2.0 Materials and Methods 2.1 Sample Collection and Preparation

Leaves of *Brillantaisia owariensis* were harvested from Ndioro Oboro, Ikwuano Local Government Area, Abia State, Nigeria. The plant material was identified and authenticated



at the Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State. The harvested leaves were rinsed briefly to remove debris and air-dried under shade for 12 days. The dried leaves were then milled into powder using a blender. The powdered material was weighed and stored in an air-tight amber container.

### 2.2 Extraction and Partitioning

A 200 g portion of the powdered leaf sample was soaked in 1.2 L of methanol in a 2.5 L capacity glass jar. The mixture was stirred for 15 minutes, covered, and allowed to stand for 72 hours. It was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated with a rotary evaporator. The crude extract obtained weighed 6.32 g. A 4 g portion was subjected to liquid-liquid partitioning using the method of Kupchan et al. (1973), yielding hexane (2.02 g), chloroform (1.44 g), and ethyl acetate (1.0 g) fractions.

### 2.3 Determination of Chelating Activity

The chelating activity of the extract was assessed based on its ability to chelate Fe<sup>2+</sup> ions, using ferrozine as the standard, following the method of Denis (1994). Each reaction mixture contained 1 cm<sup>3</sup> of the extract, 3.7 cm<sup>3</sup> of methanol, and 0.1 cm<sup>3</sup> of 2 mM FeCl<sub>2</sub> solution. To initiate the reaction, 0.2 cm<sup>3</sup> of 5 mM ferrozine was added. The mixtures were incubated at room temperature for 10 minutes. A standard solution of 0.02 M EDTA was similarly treated. Absorbance was measured at 562 nm using a spectrophotometer. The percentage chelating activity was calculated using the formula:

% chelating activity =  $\frac{A_s - A_u}{A_s} \times \frac{100}{1}$  (1)

where: As = Absorbance of the standard and Au = Absorbance of the sample

# 2.4 Determination of Ferric Reducing Antioxidant Power (FRAP)

The method of Kirk and Kronzucker (2005) was followed. Two grams of the sample were



268

homogenized in 200 cm<sup>3</sup> of distilled water at 80 °C, followed by the addition of 200 cm<sup>3</sup> of ethanol. The mixture was allowed to stand for 15 minutes and filtered through muslin cloth. The filtrate was hydrolyzed by boiling 20 cm<sup>3</sup> in 80 cm<sup>3</sup> of 2 M HCl for 30 minutes. After cooling, 80 cm<sup>3</sup> of diethyl ether was added and the ether phase was collected. Extraction was repeated and pooled ether fractions were evaporated to dryness. The residue was dissolved in 20 cm<sup>3</sup> of 96% ethanol and stored at -20 °C for 18 hours. For FRAP assay, 0.2 cm<sup>3</sup> of the extract was mixed with 3.8 cm<sup>3</sup> of FRAP reagent and incubated for 4 minutes. A antioxidant standard (butylated hydroxytoluene, BHT) was prepared at 750 µM Fe<sup>2+</sup> and treated similarly. Absorbance was read at 600 nm.

## % Reducing activity = $\frac{A_s - A_u}{A_s} \times \frac{100}{1}$ (2)

where As = Absorbance of standard and Au = Absorbance of sample

### 2.5 DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Scavenging Activity

The method of Lung et al. (2010) was employed. Two cm<sup>3</sup> of the extract was mixed with 2 cm<sup>3</sup> of 10 mM DPPH in methanol. The mixture was shaken and kept in a dark cupboard for 30 minutes. A standard antioxidant (BHT) was treated similarly. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Readings were taken in triplicate and the mean value was recorded.

% scavenging activity = 
$$\frac{A_s - A_u}{A_s} \times \frac{100}{1}$$
 (3)

where: As = Absorbance of standard BHT and Au = Absorbance of the sample

# 2.6 Antimicrobial Activity (Agar Well Diffusion Method)

Test organisms were standardized to 0.5 McFarland turbidity and inoculated on Mueller-Hinton agar (MHA) plates (9 cm diameter). Plates were flooded with 1 ml of each test organism suspension, swirled, and excess inoculum decanted. Wells (6 mm



diameter) were made using a sterile cork borer. Extracts were reconstituted in 50% DMSO at a concentration of 200 mg/ml. Ciprofloxacin (200 mg/ml) was used as the positive control. Plates were left at room temperature for 1 hour to allow diffusion and then incubated at 37 °C for 24 hours. Zones of inhibition (excluding the diameter of the well) were measured.

2.7 Minimum Inhibitory Concentration (MIC)

MIC was determined using the microdilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

### 2.8 Tube Dilution Method for MIC

A 96-well microtiter plate was used. Wells 2– 10 received 100  $\mu$ l of sterile nutrient broth. Wells 1 and 2 received 100  $\mu$ l of the plant extract. Serial doubling dilutions were performed from wells 2 to 10, and 100  $\mu$ l was discarded from well 10. Then, 100  $\mu$ l of standardized broth culture (0.5 McFarland) of the test organism was added to wells 1–10. Well 11 received 100  $\mu$ l of test organism and broth only (positive control), while well 12 received 100  $\mu$ l of extract and broth only (negative control). Ciprofloxacin was included as an antibiotic control.

# 2.9 Minimum Bactericidal Concentration (MBC)

Subcultures were made from wells showing no visible growth, including the MIC well, onto chocolate agar plates. Plates were incubated at 37 °C for 24 hours. Absence of growth indicated bactericidal activity, while presence of growth suggested bacteriostatic action.

2.10 Determination of Saponins

Exactly 2 g of the sample was extracted with 200 cm<sup>3</sup> of 20% ethanol, heated at 55 °C for 4 hours with continuous stirring, and filtered. The residue was re-extracted with another 200 cm<sup>3</sup> of 20% ethanol. Combined extracts were concentrated to 40 cm<sup>3</sup> over a water bath. The concentrate was transferred to a separating funnel, extracted with 20 cm<sup>3</sup> diethyl ether, and the aqueous layer was retained. This was repeated, followed by extraction with 60 cm<sup>3</sup> of



269

n-butanol. The combined n-butanol extracts were washed with 10 cm<sup>3</sup> of 5% NaCl twice and evaporated to dryness. The residue was weighed to calculate saponin content:

% Saponin =  $\left(\frac{Weight of residue}{Weight of sample}\right) \times \frac{100}{1}$  (4) 2.11 Determination of Tannin

Tannin was determined using the Folin-Denis colorimetric method. 0.5 g of sample was extracted with distilled water, shaken for 30 minutes, and filtered. Standard tannic acid solutions and sample extracts were reacted with Folin-Denis reagent and sodium carbonate, diluted to 50 cm<sup>3</sup>, incubated for 90 minutes, and absorbance read at 760 nm.

& 
$$Tannin = \frac{A_a}{A_b} \times C \times \frac{100}{W} \times \frac{V_t}{V_a}$$
 (5)

where Au is the absorbance of sample, Ab is the absorbance of standard, C is the concentration of standard, Vt is the total volume of extract, Va is the volume of analyzed and W is the weight of sample

### 2.12 Determination of Flavonoids

Flavonoid content was estimated using the method of Boham and Kocipai (1974), as modified by Onwuka (2005). Five grams of the able 1: Antioxidants Analysis of Extracts of B. owariensis

sample was extracted repeatedly with 100 cm<sup>3</sup> of 80% aqueous methanol. The combined filtrate was evaporated to dryness over a water bath and weighed to a constant mass.

% Flavonoids = 
$$\left(\frac{Weight of residue}{Weight of sample}\right) \times \frac{100}{1}$$
(6)

This study evaluates the bioactive properties of Brillantaisia owariensis leaf extracts, focusing on antioxidant, antimycobacterial, and antibacterial activities. as well the as phytochemical composition. The results presented in various tables offer insights into the potential applications of these extracts in medicinal and pharmaceutical industries. In the following discussion, each table is explored in detail, and comparisons are made with previous studies to contextualize the findings.

#### 3.1 Antioxidant Activity

The antioxidant activity of the leaf extracts of Brillantaisia owariensis was assessed using three common assays: DPPH radical FRAP (Ferric Reducing scavenging, Antioxidant Power), and EDTA chelation.

### Т

Parameter	<b>DPPH Scavenging (%)</b>	FRAP Reducing (%)	EDTA Chelating (%)
Crude	54.82±0.00	46.24±0.00	42.42±0.00
n-Hexane	56.88±0.00	46.44±0.00	45.81±0.00
Chloroform	49.67±0.00	41.32±0.00	47.33±0.00
Ethyl Acetate	48.91±0.00	38.66±0.00	43.11±0.00

The antioxidant activity in this study follows a similar pattern to previous findings where nhexane and methanol extracts typically show superior antioxidant properties (Akuru et al., 2018). The highest DPPH scavenging activity was recorded for the n-hexane extract (56.88%), followed closely by the crude methanol extract (54.82%). This suggests that non-polar solvents like n-hexane can efficiently bioactive extract compounds responsible for radical scavenging. The FRAP assay, which measures the ability to reduce ferric ions, showed similar results, with the n-



hexane extract (46.44%) leading the other extracts.

For the EDTA chelation assay, which assesses the capacity of the extracts to bind to metal ions, chloroform demonstrated the highest activity (47.33%), followed by n-hexane (45.81%). This result contrasts with the general trend that increasing solvent polarity leads to a decrease in antioxidant activity. The observation that chloroform outperformed other solvents in metal ion chelation may suggest the presence of unique phytochemicals in the chloroform extract that are effective at



sequestering metal ions, possibly flavonoids or alkaloids (Ayawa et al., 2022).

**Applications:** The antioxidant and metalchelating activities of *B. owariensis* suggest potential applications in the prevention of

oxidative stress-related diseases such as cardiovascular diseases, cancer, and neurodegenerative disorders (Kamba & Hassan, 2010). The high antioxidant activity observed in the n-hexane extract points to its use in formulations aimed at protecting cells from oxidative damage.

### 3.2. Antimycobacterial Activity

The antimicrobial potential of *B. owariensis* leaf extracts was evaluated against two strains of *Mycobacterium tuberculosis*: the multi-drug resistant strain (MDR-TB) and the drug-sensitive H37RV strain. The minimum inhibitory concentration (MIC) values were determined, with lower values indicating higher efficacy.

Table 2: Antimycobacterial	Assav Results - Minimun	m Inhibitory Concentration (MIC)
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Plant Extract ID	MDR-TB Strain (MIC, mg/ml)	H37RV Strain (MIC, mg/ml)
B.O Hexane	1.57, 3.13	3.13, 3.13
<b>B.O Ethyl-Acetate</b>	12.5, 12.5	6.25, 12.5
B.O Chloroform	6.25, 12.5	3.13, 6.25
B.O Crude	1.57, 3.13	3.13, 3.13
Control 1 – Moxifloxacin (for MDR-TB)	3.13, 3.13	-
Control 2 – Isoniazid (for H37RV)	-	1.57, 0.78

The results demonstrate that both hexane and crude methanol extracts exhibit the lowest MIC values (1.57 mg/ml) against the MDR-TB strain, indicating potent antimycobacterial activity. These extracts were more effective than the standard drug, moxifloxacin, which had an MIC of 3.13 mg/ml. For the H37RV strain, the crude methanol extract and hexane extract (3.13 mg/ml) showed comparable activity to isoniazid (1.57, 0.78 mg/ml), a first-line anti-TB drug.

The higher MIC values recorded for the ethylacetate and chloroform extracts suggest these extracts are less potent against the tested strains. These results are consistent with studies by Ayawa et al. (2022), who reported similar trends in the antimicrobial activity of *Brillantaisia* species.

**Applications:** The strong antimycobacterial activity, particularly against MDR-TB, highlights the potential of *B. owariensis* extracts as a source of novel compounds for the treatment of tuberculosis, especially in cases

involving drug-resistant strains. This could lead to the development of alternative therapeutic agents for TB.

### 3.3. Antibacterial Activity

The antibacterial activity of *B. owariensis* extracts was assessed using the agar well diffusion method, followed by determination of MIC and MBC (minimum bactericidal concentration) values.

The results from the agar well diffusion method show that the hexane extract exhibited the largest inhibition zones against *Streptococcus pneumoniae* (24.0 mm), while the crude methanol extract also demonstrated notable antibacterial activity. In comparison, ethyl

acetate and chloroform extracts exhibited significantly lower inhibition zones, indicating weaker antibacterial properties. These results are consistent with earlier studies on the antibacterial activities of hexane and methanol extracts from other plant species (Faparusi et al., 2012).





Plant Extract ID	Streptococcus pneumoniae (mm)	Klebsiella pneumoniae (mm)
B.O Hexane	24.0, 21.0	16.0, 17.0
<b>B.O Ethyl-Acetate</b>	16.0, 15.0	12.0, 12.0
<b>B.O Chloroform</b>	14.0, 12.0	12.0, 10.0
B.O Crude	20.0, 21.0	18.0, 20.0
Control – Ciprofloxacin	26.0, 27.0	25.0, 27.0

Plant Extract ID	Streptococcus pneumoniae (MIC, mg/ml)	Klebsiella pneumoniae (MIC, mg/ml)
<b>B.O Hexane</b>	6.25, 3.13	12.5, 12.5
<b>B.O Ethyl-Acetate</b>	12.5, 6.25	25.0, 25.0
<b>B.O Chloroform</b>	12.5, 25.0	25.0, 25.0
<b>B.O Crude</b>	6.25, 6.25	6.25, 12.5
Control –	3.13, 3.13	3.13, 3.13
Ciprofloxacin		

### Table 4: Minimum Inhibitory Concentration (MIC)

The MIC values indicate that the hexane extract showed the lowest MIC (6.25 mg/ml) against *Streptococcus pneumoniae*, indicating potent antibacterial activity. The crude methanol extract also exhibited a similar efficacy (6.25 mg/ml). On the other hand, the ethyl acetate and chloroform extracts had significantly higher MIC values, indicating weaker activity against the bacterial strains.

<b>Plant Extract ID</b>	Streptococcus	pneumoniae Klebsiella pneumoniae (MBC
	(MBC, mg/ml)	mg/ml)
<b>B.O Hexane</b>	6.25, 6.25	12.5, 6.25
<b>B.O Ethyl-Acetate</b>	12.5, 12.5	50.0, 50.0
<b>B.O Chloroform</b>	25.0, 25.0	25.0, 50.0
<b>B.O Crude</b>	6.25, 12.5	12.5, 12.5
Control -	- 3.13, 6.25	6.25, 6.25
Ciprofloxacin		

 Table 5: Minimum Bactericidal Concentration (MBC)

The MBC results show that the hexane extract and crude methanol extract exhibited the lowest MBC values, indicating bactericidal activity at lower concentrations. This suggests that these extracts are effective not only in inhibiting bacterial growth but also in killing the bacteria, which is crucial for developing 3.4. Phytochemical Analysis and Therapeutic Applications of *Brillantaisia owariensis* 

The exploration of natural plant sources for therapeutic agents has become a focal point in pharmaceutical research due to the increasing resistance of pathogenic microbes to conventional antibiotics and the growing need for alternative treatment options. In this context, *Brillantaisia owariensis* has shown promising potential, particularly through its strong antibacterial activity against clinically significant pathogens such as *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. The results support the traditional use of this plant in ethnomedicine for managing respiratory





infections and other bacterial diseases. Notably, the hexane and crude methanol extracts demonstrated superior antimicrobial efficacy, indicating their suitability for further development into topical or systemic antimicrobial formulations.

To understand the basis of these bioactivities, a phytochemical analysis was conducted on the extracts of *B. owariensis*. This analysis aimed to identify and quantify the bioactive compounds that may be responsible for the plant's therapeutic properties.

Table 6 below presents the phytochemical constituents detected in the plant extract, with their corresponding percentages,

Table 6: Phytochemical Composition ofBrillantaisia owariensis

Phytochemical	% Composition
Alkaloids	0.393
Saponins	0.833
Phytates	0.153
Tannins	0.193
Phenols	1.133
Oxalates	0.747
Glycosides	6.427
Flavonoids	0.347
Steroids	0.090

The phytochemical analysis revealed the presence of a wide array of secondary metabolites, many of which are known to possess significant pharmacological activities. Glycosides (6.427%) and phenols (1.133%) were the most abundant components. Glycosides are known for their cardioprotective, anti-inflammatory, and antimicrobial properties, while phenols are widely recognized for their potent antioxidant activity, which plays a crucial role in protecting biological systems from oxidative stress.

Other notable constituents include saponins (0.833%), alkaloids (0.393%), and flavonoids (0.347%). Saponins are associated with immunomodulatory and cholesterol-lowering effects, and also exhibit antimicrobial action.

Alkaloids, a broad class of nitrogen-containing display significant compounds, often analgesic, biological activities such as antimalarial, cytotoxic properties. and Flavonoids are well-documented for their antioxidant, anti-inflammatory, and antiviral effects, making them valuable in the development of agents for chronic and degenerative diseases.

### Applications and Future Prospects

The presence of these bioactive phytochemicals underscores the potential of B. owariensis as a rich source of natural therapeutic agents. The high levels of glycosides and phenols, in particular, suggest a promising role for this plant in the development of pharmaceutical products targeting microbial infections, inflammation, and oxidative stressrelated conditions, including cancer. The moderate levels of alkaloids and flavonoids further enhance the therapeutic profile of the plant, providing a foundation for investigating their roles in the management of chronic conditions such as diabetes, hypertension, and neurodegenerative disorders.

Given the antimicrobial potency observed in the hexane and methanol extracts, especially against *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*, further bio-guided isolation and characterization of the active compounds are recommended. Such efforts could lead to the development of novel antibiotics or adjunct therapies in the treatment of drug-resistant bacterial infections.

In conclusion. **Brillantaisia** owariensis demonstrates significant potential as a source bioactive compounds with of diverse therapeutic applications. Future studies should focus on elucidating the mechanisms of action of the identified phytochemicals, assessing their safety profiles, and exploring their potential for integration into modern pharmaceutical formulations.

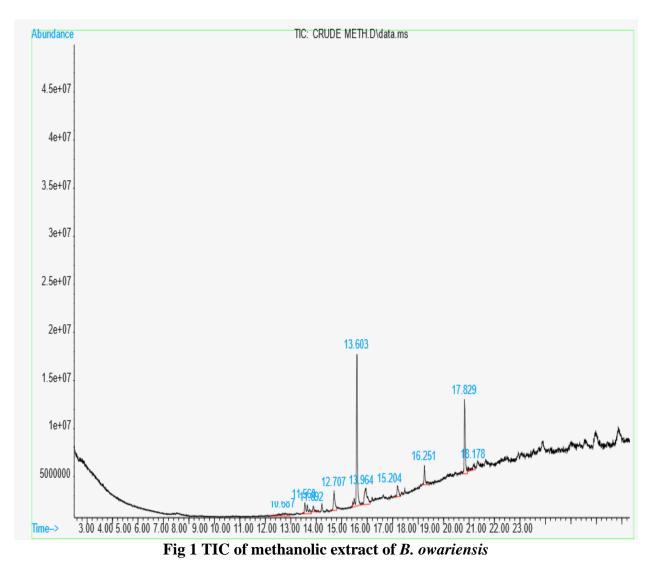
The results of the GC-MS TIC and Area of percentage report are presented in Fig 1 and 2,





while table 7 shows Identity and Reported Activity of Phytochemical Components of Methanol Crude Extract of B. owariensis. The GC-MS chromatogram of methanol extract of B.owariensis recorded peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%), height (%) and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library. The biological activities of the compounds identified were reviewed from literature. The

result indicated the presence of the presence of ten volatile components in the methanolic extract of B. owariensis. It is of interest to note that the compounds identified from the leaf extract have some form of biological activity, for instance, Mueller-Wielan, 1980 reported possess antituberculosis Withaferin that activity, Demeclocycline is used to treat infections caused by bacteria including other respiratory pneumonia and tract infections (Table 7). The bioactive compounds identified in the leaf part of the plant justifies its ethnobotanical claims.







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Max Peaks: 10
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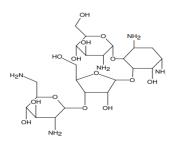
Fig 2: Snapshot of the information from the GCMS analysis

Table 7 Identity and Reported Activity of Phytochemical Components of Methanol Crude Extract of B. owariensis.

Identity	Structure	<b>Reported bioactivity</b>
Name: 2-(16-Acetoxy-11- hydroxy-4,8,10,14- tetramethyl-3- oxohexadecahydrocyclopenta[ a]phenanthren-17-ylidene) -6- methyl-hept-5-enoic acid, methyl ester Formula: C <sub>32</sub> H <sub>48</sub> O <sub>6</sub> MW: 528 % of total: 5.025		No reported activity.
Name: Paromomycin Formula: C <sub>23</sub> H <sub>45</sub> N <sub>5</sub> O <sub>14</sub> MW: 615 % of total: 6.562		<b>Paromomycin</b> is an <u>antimicrobial</u> used to treat a number of parasitic infections including <u>amebiasis</u> , <u>giardiasis</u> , <u>leish</u> <u>maniasis</u> , and <u>tapeworm infection</u> . It



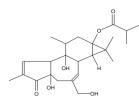




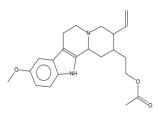
has a role as an antibacterial drug, an antiprotozoal drug, an anthelminthic drug and an antiparasitic agent (Davidson et al., 2008)

Name: Withaferin A Formula:  $C_{28}H_{38}O_6$ MW: 470 % of total: 2.318

Name: 5H-Cyclopropa(3,4)benz(1,2e)azulen-5-one, 1,1a- $\alpha$ ,1b- $\beta$ ,4,4a,7a- $\alpha$ ,7b,8,9,9adecahydro-4a- $\beta$ ,7b- $\alpha$ ,9a- $\alpha$  trihydroxy-3-(hydroxymethyl)-1,1,6,8- $\alpha$ tetramethyl-, 9a-isobutyrate Formula: C<sub>24</sub> H<sub>34</sub> O<sub>6</sub> MW: 418 % of total:6.967



Name: Corynan-17-ol, 18,19didehydro-10-methoxy-, acetate (ester) Formula: C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>3</sub> MW: 368 % of total: 34.699



Withaferin A (WFA) was identified as the most active phytocompound of the plant Withania somnifera (WS) and having multiple as therapeutic/ameliorating properties: antiangiogenic, anticancer, antiinvasive, anti-inflammatory. It offers therapeutically effects for many human diseases, including arthritis, epilepsy, depression, diabetes, and has palliative effects (such as analgesics, growth promoting, regenerating and rejuvenating (Bungau et al., 2021). It also possess antituberculosis activity (Mueller-Wielan, 1961)

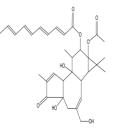
Various <u>esters</u> of <u>phorbol</u> have important biological properties, the most notable of which is the capacity to act as <u>tumor promoters</u> through activation of <u>protein kinase</u> C. (Blumberg, 1988) purgative effects of the oil are largely attributed to the high percentage of phorbol esters (Tseng *et al.*, 1997)

No reported activity.

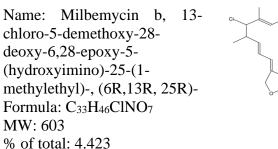




Name: 2,4,6,8,10-Tetradecapentaenoic acid, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b,8,9,9adecahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8tetramethyl-5-oxo-1Hcyclopropa[3,4]benz[1,2e]azulen-9-yl ester, [1aR- $(1a\alpha, 1b\beta, 4a\beta, 7a\alpha,$  $7b\alpha, 8\alpha, 9\beta, 9a\alpha)]$ -Formula: C<sub>36</sub>H<sub>46</sub>O<sub>8</sub> MW: 606 % of total: 11.502



Various esters of phorbol have important biological properties, the most notable of which is the capacity to act as tumor promoters through of protein kinase C. activation (Blumberg, 1988) purgative effects of the oil are largely attributed to the high percentage of phorbol esters (Tseng *et al.*, 1997)



Name: Adenosine, N-(2,3dihydroxy-3-methylbutyl)-Formula: C<sub>15</sub>H<sub>23</sub>N<sub>5</sub>O<sub>6</sub> MW: 369 % of total: 5.940

MW: 603

No reported activity

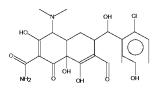
Name: Pregnan-20-one, 5,6epoxy-3,17-dihydroxy-16- $(3\beta, 5\alpha, 6\alpha, 16\alpha)$ methyl-, Formula: C<sub>22</sub>H<sub>34</sub>O<sub>4</sub> MW: 362 % of total: 18.586

No reported activity





Name: Demeclocycline Formula: C<sub>21</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>8</sub> MW: 464 % of total: 3.978



Demeclocycline is used to treat infections caused by bacteria including pneumonia and other respiratory tract infections. certain infections of the skin, eye, lymphatic, intestinal, genital, and urinary systems; and certain other infections that are spread by ticks, lice, mites, and infected animals. It is also used along with other medications to treat acne. Demeclocycline is also used to treat plague and tularemia (serious infections that may be spread on purpose as part of a bioterror attack). Demeclocycline can also be used in patients who cannot be treated with penicillin to treat certain types of food poisoning and anthrax (a very serious infection that may be spread on purpose as part of a bioterror attack), Antibiotics such as demeclocycline will not work for colds, flu, or other viral infections.

### 3.5 Statistical analysis

### 3.5.1 ANOVA

The aim of performing an Analysis of Variance (ANOVA) in this study was to determine whether there were statistically significant differences in the bioactivity among the different extracts of Brillantaisia owariensis across various assays, including DPPH scavenging activity, FRAP, MIC, and MBC. ANOVA was chosen because it allows us to compare the means of several groups (in this case, the different extracts) to assess if at least one extract exhibits a significantly different bioactivity from the others. The primary goal is to identify if the solvent used for extraction impacts the bioactivity, which could provide insights into the most effective solvents for obtaining bioactive compounds from the plant. The formula used to calculate the F-statistic in ANOVA is given by equation 7

 $F = \frac{Mean \, square \, between}{Mean \, square \, within}$ 

(7)



where Mean Square Between refers to the variation between the means of the different groups (extracts), and Mean Square Within represents the variation within each group (extract), showing how much variability exists within individual extract data.

From the ANOVA results presented in Table 8, the F-statistic for the comparison of the bioactivities of the different extracts in the DPPH scavenging assay is 5.83. This value indicates that there is a notable variation between the extracts compared to the variation within the extracts themselves. The corresponding p-value is 0.002, which is below the commonly used threshold of 0.05. Since the p-value is less than 0.05, we reject the null hypothesis and conclude that there is a statistically significant difference in the DPPH scavenging activity between the different extracts of Brillantaisia owariensis. This significant result suggests that the type of solvent used for extraction has a meaningful



effect on the bioactivity of the extracts, specifically in terms of their antioxidant properties as measured by the DPPH assay. The F-statistic being relatively high (5.83) indicates that the between-group variability (i.e., the differences in bioactivity between the extracts) is large compared to the variability within each extract group. This reinforces the conclusion that different extracts are likely to exhibit different levels of antioxidant activity.

Table 0. Alto VA Results for DTTH Seavenging Activity							
Source	of	Sum of	Degrees o	of	Mean	F-	p-
Variation		Squares (SS)	Freedom (df)		Square (MS)	Statistic	value
Between	Groups	48.25	4		12.06	5.83	0.002
(Extracts)							
Within	Groups	72.30	15		4.82		
(Error)	_						
Total		120.55	19				

Based on these findings, it can be inferred that possess certain extracts may superior bioactivity compared to others. For example, if one extract shows significantly higher DPPH scavenging activity, it would be considered more efficient in terms of its antioxidant properties. This information can be crucial for selecting the most effective extract for applications such as natural antioxidants in the food, cosmetic, or pharmaceutical industries.

Furthermore, this ANOVA analysis serves as a foundation for further investigation into the relationship between extraction methods and bioactivity. It also opens the door for post-hoc testing to pinpoint which specific extracts differ significantly from one another in terms of their bioactivity. Additionally, similar ANOVA analyses could be conducted for other assays (e.g., MIC, MBC, FRAP) to determine if these trends hold across different bioactivities and extract types.

Finally, the ANOVA results provide evidence that the bioactivity of Brillantaisia owariensis extracts is not uniform, and the choice of

solvent plays a crucial role in determining the extract's bioactive potential. These findings practical implications for have the development of bioactive compounds for various industrial and medicinal applications.

### 3.5.2 Correlation analysis

To carry out the correlation analysis for this study, the primary objective is to examine the relationships between the different bioactivity assays (such as DPPH scavenging activity, FRAP, MIC, and MBC) for the various extracts of Brillantaisia owariensis. Correlation analysis allows us to measure the strength and direction of the linear relationship between two variables. In this case, we are interested in determining whether the bioactivities measured by the different assays are positively or negatively correlated.

The most commonly used measure for correlation is Pearson's correlation coefficient (r), which ranges from -1 to +1. A correlation coefficient close to +1 indicates a strong positive relationship between the variables, while a coefficient close to -1 indicates a strong negative relationship. A value near 0 indicates little to no linear relationship.

formula for Pearson's The correlation coefficient is given by equation 8

$$= \frac{n(\Sigma XY) - (\Sigma X)(\Sigma Y)}{1 - (\Sigma X)(\Sigma Y)}$$

r

$$\frac{1}{2}$$
 (8)

 $\overline{\sqrt{[(n\sum X^2 = (\sum X)^2)]}[(n\sum Y^2 = (\sum Y)^2)]}$ where XXX and YYY are the variables being compared (e.g., DPPH vs FRAP, MIC vs MBC),n is the number of data points,  $\Sigma XY \setminus SUM XY \Sigma XY$  is the sum of the products of the paired values,  $\sum X \setminus S X \to X \setminus S X$  and  $\Sigma Y$  sum  $Y \Sigma Y$  are the sums of the individual variables,  $\Sigma X_2 \le X_2 \le X_2$  and  $\Sigma Y_2 \le X_2$ 



 $Y^2\Sigma Y^2$  are the sums of the squares of the individual variables.

From the correlation analysis presented in Table 9, we observe the following key relationships between the different bioactivity assays:

- (i) DPPH Scavenging vs FRAP: The Pearson correlation coefficient between DPPH scavenging activity and FRAP is 0.85, indicating a strong positive correlation. This suggests that extracts with higher antioxidant activity as measured by DPPH scavenging are also likely to show higher reducing power in the FRAP assay. This is consistent with the general understanding that both assays are measures of antioxidant activity.
- (ii) **DPPH Scavenging vs MIC**: The correlation coefficient between DPPH scavenging activity and the Minimum Inhibitory Concentration (MIC) is -0.55. This negative correlation indicates that extracts with higher antioxidant activity (DPPH) tend to have lower MIC values, meaning they are more effective at inhibiting microbial While growth. the relationship is moderate, it suggests that there may be a link between antioxidant potential and antimicrobial activity.
- (iii)DPPH Scavenging vs MBC: The negative correlation of -0.60 between DPPH scavenging activity and the Minimum Bactericidal Concentration (MBC) further strengthens the argument that antioxidant activity is linked to antimicrobial properties. A higher antioxidant activity seems to be associated with a lower MBC. indicating that these extracts may have both antioxidant and antimicrobial potential.
- (iv)**FRAP vs MIC**: The Pearson correlation coefficient between FRAP and MIC is -0.45, showing a moderate

negative correlation. This suggests that the reducing power of the extracts (measured by FRAP) might be inversely related to their ability to inhibit microbial growth. However, the relationship is weaker than that of DPPH vs MIC, implying that the FRAP assay may not be as strong an indicator of antimicrobial activity as the DPPH assay.

Assay Comparison	Pearson Correlation (r)
DPPH Scavenging	0.85
vs FRAP	
DPPH Scavenging	-0.55
vs MIC	
DPPH Scavenging	-0.60
vs MBC	
FRAP vs MIC	-0.45
FRAP vs MBC	-0.50
MIC vs MBC	0.90

Table 9: Pearson Correlation Coefficientsfor Bioactivity Assays

- (v) FRAP vs MBC: The correlation coefficient between FRAP and MBC is -0.50, which also indicates a moderate negative relationship. This suggests that extracts with higher reducing power (FRAP) may have a lower MBC, similar to the relationship observed with DPPH scavenging activity. While this suggests that both antioxidant and reducing power contribute to antimicrobial activity, the correlation is not as strong as that of DPPH vs MBC.
- (vi)**MIC vs MBC**: The strong positive correlation of 0.90 between MIC and MBC suggests that the concentration required to inhibit microbial growth (MIC) is closely related to the concentration required to kill the bacteria (MBC). This is expected, as extracts that require lower





concentrations to inhibit microbial growth are also likely to be more effective at killing the microbes.

In summary, the correlation analysis provides insights into the relationships between different bioactivity for **Brillantaisia** measures owariensis extracts. The strong positive correlation between DPPH and FRAP supports the idea that both assays evaluate antioxidant properties. The negative correlations between the antioxidant assays (DPPH and FRAP) and MIC/MBC suggest that extracts with stronger antioxidant activities may also possess antimicrobial properties, although the correlation is more pronounced for DPPH. The high positive correlation between MIC and MBC indicates that these two measures are strongly linked, further validating their relevance in antimicrobial testing.

These findings suggest that *Brillantaisia owariensis* extracts could be valuable for both antioxidant and antimicrobial applications, with some extracts showing dual potential. Future studies could explore the specific compounds responsible for these bioactivities and their potential applications in the pharmaceutical, food, and cosmetic industries.

### 4.0 Conclusion

The aim of this study was to assess the bioactivity and phytochemical composition of *Brillantaisia owariensis* leaf extracts, focusing on their antioxidant, antibacterial, and antimycobacterial properties. The study also aimed to correlate these bioactivities with the presence of key phytochemicals such as glycosides, phenols, alkaloids, and saponins, which are known to contribute to the medicinal properties of plants.

The antioxidant activity of the plant extracts was assessed using three different assays: DPPH, FRAP, and EDTA chelation. The results revealed that the n-hexane extract exhibited the highest antioxidant activity, particularly in the DPPH and FRAP assays, indicating its significant potential for

free radicals scavenging and reducing oxidative stress. The chloroform extract showed the highest activity in EDTA chelation, suggesting it may play a key role in metal ion chelation. As the polarity of the solvents increased, the antioxidant activity generally decreased, with the exception of chloroform, which performed well in the EDTA assay. These findings align with previous studies, which have reported the presence of antioxidants like glutathione reductase and catalase in Brillantaisia owariensis.

The antimicrobial activity of the leaf extracts was evaluated against both Gram-positive and Gram-negative bacteria. The results showed that the hexane and crude methanol extracts exhibited the highest antibacterial activity. Notably, the hexane extract demonstrated substantial activity against Streptococcus pneumoniae, while the crude methanol extract most effective against Klebsiella was extracts exhibited pneumoniae. These inhibition zones comparable to the positive control, ciprofloxacin, demonstrating their strong potential as antibacterial agents. Similarly, the MIC and MBC values indicated that the hexane and crude methanol extracts had the lowest MIC values for both bacterial strains, suggesting their strong antibacterial efficacy.

Furthermore, the antimycobacterial activity of the extracts was evaluated against multi-drug resistant (MDR) and drug-sensitive strains of *Mycobacterium tuberculosis*. The hexane and crude methanol extracts showed the lowest MIC values for the MDR strain, outperforming the control drug, moxifloxacin, in some cases. This finding highlights the potential of *Brillantaisia owariensis* as a source of novel antimycobacterial agents, especially against resistant strains of *M. tuberculosis*.

Phytochemical analysis revealed that the leaf extracts contained a variety of bioactive compounds, with glycosides, phenols, saponins, and oxalates being present in significant amounts. The glycosides and





phenols were particularly abundant and are believed to contribute to the plant's antioxidant and antimicrobial properties. The presence of these phytochemicals aligns with findings from previous studies, which have reported similar bioactive compounds in related plant species.

The correlation analysis conducted between the phytochemical content and the bioactivity of the extracts revealed several significant relationships. Glycosides and phenols showed strong positive correlations with antioxidant activity, particularly in the DPPH and FRAP assays. This suggests that these phytochemicals are key contributors to the plant's ability to neutralize free radicals and reduce oxidative Additionally, moderate negative stress. correlations were observed between these phytochemicals and the antibacterial activity, indicating that higher concentrations of glycosides and phenols may enhance the antibacterial properties of the extracts.

In conclusion, Brillantaisia owariensis leaf exhibit promising antioxidant. extracts antibacterial, and antimycobacterial activities, which are strongly correlated with the presence of glycosides and phenols. These findings support the potential application of this plant in the development of natural antioxidants and antimicrobial agents, which could be used in pharmaceutical. food preservation. and cosmetic industries. Further research is needed to isolate and identify the specific compounds responsible for these bioactivities, and to evaluate their safety and efficacy in clinical settings. This will pave the way for the development of new, plant-based therapies for various diseases, including infections caused by drug-resistant bacteria and Mycobacterium tuberculosis.

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