

# **Antioxidant and Antimicrobial Studies of Some Hemi-parasitic West African Plants**

## **ABSTRACT**

The study investigated the antioxidant and antimicrobial activities of some West African hemi-parasitic plants, which comprised *Tapinanthus bangwensis* (TB), *Tapinanthus globiferus* (TG) and *Globimetula braunii* (GB), used in Nigerian ethnomedicine for the management of skin and other microbial infections. This was with a view to determining the most active plant extract and fraction. The leaf and stem of each plant was separately air-dried, powdered and macerated in ethanol-H<sub>2</sub>O (8:2). The extracts were subjected to *in vitro* antioxidant tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), Fe<sup>2+</sup> chelating ability (FIC) and total antioxidant capacity (TAC) assays, with L-ascorbic acid, quercetin and ethylenediaminetetraacetic acid (EDTA) as positive controls. The antimicrobial test was carried out using micro-broth dilution method against reference strains of methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, where ciprofloxacin and ketoconazole were the positive controls. An activity-guided fractionation approach was adopted in the study. The most active GB leaf extract was fractionated to obtain *n*-hexane, dichloromethane, ethylacetate, *n*-butanol and aqueous fractions, and were subsequently tested. The results showed that the extract of GB leaf demonstrated the highest bioactivities with inhibitory concentration (IC<sub>50</sub>) of DPPH at 31.21±1.11 µg/mL, FRAP value of 109.30±0.76 mg AAE/g, TAC value of 178.15±3.54 mg AAE/g and MIC of 5.0 – 10.0 mg/mL. The Ethylacetate fraction of GB leaf demonstrated the highest bioactivities, which were four-times, thrice and twice better than its GB leaf extract in the DPPH, TAC, and antimicrobial studies respectively. The EtOAc fraction (IC<sub>50</sub> = 8.58±1.39 µg/mL) was comparable (*P* > 0.05) in antioxidant activity with quercetin (IC<sub>50</sub> = 7.72±0.88 µg/mL). The EtOAc fraction also exhibited broad-spectrum antimicrobial activity with inhibitory concentration of 1.25 mg/mL against MRSA and *C. albicans*. In conclusion, the extract of *G. braunii* leaf demonstrated considerable bioactivities, and the moderately polar EtOAc fraction was the most active fraction; hence, validates the folkloric use *G. braunii* leaf as a remedy for microbial infections.

*Keywords:* Hemi-parasitic plant, *Globimetula braunii*, *Tapinanthus bangwensis*, *Tapinanthus globiferus*, antioxidant, antimicrobial

## **1. INTRODUCTION**

Antioxidants are substances which when present at low concentrations compared to oxidizable substrates, significantly delay or prevent the oxidation of those substrates [1]. Antioxidants are able to counteract the deleterious effect posed by free radical release in the biological system by breaking off the cascade of chains generated by reactive oxygen species (ROS, e.g. O<sub>2</sub><sup>•-</sup>, OH<sup>•</sup>, HO<sub>2</sub><sup>•</sup>, LOO<sup>•</sup>) and reactive nitrogen species (RNS, such as •NO, ONOO<sup>-</sup>, •NO<sub>2</sub>, •N<sub>2</sub>O<sub>3</sub>), thus, preventing free radical induced tissue damage and the manifestation of many chronic health problems such as microbial infections, tumours,

cardiovascular diseases and a host of others [2,3]. Microbial infections are among the top ten leading cause of death worldwide, accounting for about 3 million global deaths in the year 2016 [4]. Unfortunately, antimicrobial drug resistance (AMR) is currently a major challenge to reducing this burden. For instance, it has been reported that individuals suffering from infections caused by multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) are 64 % more likely to die than those with non-resistant form of infections [4,5]. This and other challenges of drug toxicity have triggered the search for more efficacious and less toxic drug leads from natural sources. Mistletoes are hemi-parasitic plants in the sense that though they are photosynthetic in nature, they still depend on other plants for mineral nutrients [6,7]. They are found growing on trees by getting themselves attached to their hosts by means of their modified roots, called haustoria [7].

*Tapinanthus bangwensis* (K. Krause) Danser, *T. globiferus* (A. Rich) Tiegh, and *Globimetula braunii* (Engler) van Tiegh belong to the family Loranthaceae [8]. They are commonly called “match-stick” plants due to the appearance of their inflorescence like that of a match stick. They are of the West African coast, and so are classified as the “African Mistletoes” or “West African Mistletoes” [8]. They are known as “àfómó onńsánó” in South Western part of Nigeria. They are implicated in Nigerian ethnomedicine for the management of many infections and diseases among which are rheumatic pain, hypertension, diabetes, ulcer and tumours [8,9,10]. The plant extracts have been reported for some biological activities such as antioxidant [11,12], antibacterial [13], anti-inflammatory [14], anti-lipemic and hypocholesteremic [15], anti-diabetic [16], cytotoxic [17], and anticancer [18]. Some lupane-type triterpene esters have been reported in the *n*-hexane and dichloromethane fractions of *G. braunii* leaf via phytochemical isolation approach [19]. However, bioactivity-guided phytochemical studies of the three plants against free radicals and pathogenic microbes are yet to be exploited, hence this report.

The biochemical and biological testing methods used for the determination of the antioxidant and antimicrobial activities of the studied plants are reliable, fast and easy to use. The *in vitro* antioxidant method such as the DPPH assay is based on the spectrophotometric determination of the free radical scavenging capacity of antioxidants in the plant extracts towards the DPPH free radical by decrease in absorbance. Therefore, the test provides information on the potentials of primary antioxidants in plants to exhibit hydrogen atoms transfer (HAT) resulting from free radical reaction of the purple colored DPPH with each extract to form a decolorized 2,2-diphenyl-1-picrylhydrazine. The ferric reducing antioxidant power (FRAP) method determines the free radical reducing abilities of plant extracts as potential natural antioxidants. FRAP measures the ability extracts to reduce ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ ). The formation of Pearl's Prussian blue at 593 nm indicates the formation of  $\text{Fe}^{2+}$ . Also, the total antioxidant capacity (TAC) method is one important tool used for the determination of the additive antioxidant properties of plant extracts. Plant extracts act by single electron transfer (SET) to exhibit strong antioxidant activities in FRAP, TAC and metal chelating methods [20]. The micro-broth dilution method of antimicrobial susceptibility testing allows the option of qualitative and quantitative analyses of samples. It also allows for the testing of and analysis of small volumes of plant extracts in micro-titer plates, thus reducing wastages which would normally be encountered in agar dilution and diffusion methods [21]. Therefore, the afore-mentioned assay methods were used to potentiate the antioxidant and antimicrobial activities of the plants.

## 2. EXPERIMENTAL DETAILS

### 2.1 General

The AnalaR grade of solvents (BDH, Poole, England) were used for plant extraction and fractionation. A 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, *L*-ascorbic acid (Vitamin C), Quercetin, acetate buffer (pH 3.6), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,

sodium phosphate, ammonium molybdate, ethylenediaminetetraacetic acid (EDTA), 70149 Nutrient Broth No 3 and S3306 Sabouraud Dextrose (Sigma-Aldrich Co., St. Louis, MO 63103 USA). The bacteria and fungi used for the antimicrobial screening were obtained from the culture collections of the Microbiology Laboratory in the Department of Pharmaceutics, Obafemi Awolowo University where the experiment was conducted.

## 2.2 Plant Materials

The three studied plants were collected from the Obafemi Awolowo University, Ile-Ife campus, Nigeria in the month of October, 2013. *Tapinanthus bangwensis*, *T. globiferus* and *Globimetula braunii* were found parasitizing the trees of *Citrus paradisi* (Rutaceae), *Ficus sur* (Moraceae), and *Leucena leucocephala* (Caesalpinoideae) respectively. They were authenticated at the Ife Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. Herbarium specimens were deposited and Voucher numbers IFE 17228, IFE 17230 and IFE 17229 respectively. The leaves and stems were separately dried inside the Screen House, Faculty of Pharmacy, OAU, Ile-Ife. They were separately milled into powder and kept air-tight prior to extraction.

## 2.3 Plant Extraction

The stem and leaf (500 g each) of the three plants were separately extracted with 2.5 L each of ethanol/H<sub>2</sub>O (8:2), at room temperature for 72 hr. They were filtered and concentrated *in vacuo* at 50 °C, using rotary evaporator. The six hydro-ethanol extracts obtained (8.5 – 12.5 % yield) were kept dry inside a desiccator.

## 2.4 Antioxidant Tests

### 2.4.1 DPPH Spectrophotometric Assay:

The assay was carried out according to the method of Sanchez-Moreno *et al.* [22]. A 1 mL DPPH solution in methanol (0.05 mg/mL) was added to 1 mL samples [(positive controls: L-ascorbic acid and Quercetin) and (plant extracts)] at varying concentrations: 50.00, 25.00, 12.50, 6.25 and 3.13 µg/mL, in triplicate. The samples were incubated in the dark room for 30 minutes after which their absorbances were determined spectrophotometrically at 517 nm. Methanol was the negative control. The percentage inhibition of DPPH by each test sample was calculated as:

$$\% \text{ Inhibition of Sample} = \left[ \frac{Abs_{\text{sample}} - Abs_{\text{control}}}{Abs_{\text{control}}} \right] \times 100$$

where,  $Abs_{\text{sample}}$  = Absorbance of Plant Extract or Fraction, and  
 $Abs_{\text{control}}$  = Absorbance of negative control

For each sample, the concentration which inhibited the DPPH radical solution by 50% was taken as the IC<sub>50</sub> value.

### 2.4.2 Ferric Reducing Ability of Plasma (FRAP) Assay

This was carried out according to the method of Benzie and Strain [23], as a measure of antioxidant power of plant extracts. Here, a 30 mmol/L acetate buffer of pH 3.6 (3.1 g of Sodium acetate 3H<sub>2</sub>O with 16 mL of glacial acetic acid made up of 1 L with distilled water. 10 mmol/L 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) 98 % (3.1 mg/ml in 40 mmol/L HCl) and 20 mmol/L FeCl<sub>3</sub>.6H<sub>2</sub>O (5.4 mg/mL in distilled water) were mixed together in the ratio of 10:1:1, respectively, to give the working FRAP reagent. A 50 mL aliquot of test sample was added to 1 mL of FRAP reagent in duplicate. The absorbance of each sample was determined spectrophotometrically at 593 nm, exactly 10 minutes after mixing. To standardize 50 mL of the standard, FeSO<sub>4</sub>.7H<sub>2</sub>O, 1 mmol/L was added to 1 mL of FRAP reagent. All measurements were taken at room temperature, with samples protected from direct sunlight. The ferric reducing power of plant extracts were determined as ascorbic acid equivalent (AAE) from the calibration curve of the positive control (L-ascorbic acid) at concentrations 1000.00, 500.00, 250.00, 125.00, 62.50 and 31.25 µg/mL in methanol.

#### 2.4.3 Ferrous ion Chelating (FIC) Assay

The FIC was carried out according to the method of Singh and Rajini [24]. Solutions of 2 mM  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and 5 mM ferrozine were diluted 20 times. An aliquot (1 mL) of different concentrations of extract was mixed with 1 mL  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  in triplicate. After 5 min incubation, the reaction was initiated by the addition of ferrozine (1 mL). The mixture was shaken vigorously and after a further 10 min incubation period, the absorbance of the solution was determined on a UV spectrophotometer set at 562 nm. The percentage inhibition of ferrozine- $\text{Fe}^{2+}$  complex formation was calculated by using the formula:

$$\% \text{ Chelating ability of Sample} = \left[ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

where  $\text{Abs}_{\text{control}}$  = absorbance of control (the control contains  $\text{FeCl}_2$  and ferrozine, complex formation molecules); and  $\text{Abs}_{\text{sample}}$  = absorbance of a tested sample

#### 2.4.4 Total Antioxidant Capacity (TAC)

This was carried out by the phosphomolybdenum method, according to the procedure described by Prieto *et al.* [25]. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in triplicate. The absorbance of the reaction mixture was determined at 695 nm, after cooling to room temperature. The total antioxidant capacity of each sample was expressed as the number of gram equivalent of ascorbic acid (AAE). The calibration curve was prepared by mixing ascorbic acid (1000, 500, 250, 125, 62.5 and 31.25  $\mu\text{g}/\text{mL}$ ) with methanol.

### 2.5 Antimicrobial Susceptibility Test

#### Micro-broth Dilution Method

This was carried out according to the method of Clinical and Laboratory Standards Institute [26]. The microorganisms were identified using their morphological characteristics and standard biochemical tests. The reference strains were *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus subtilis* NCTC 8236, MRSA ATCC 29213, and *Candida albicans* ATCC 24433. Bacteria were maintained on nutrient broth and fungi on Sabouraud Dextrose broth at 4°C, and sub-cultured regularly. The extracts and fractions were tested at 40, 20, 10, 5, 2.5 and 1.25 mg/mL concentrations, and were compared with Ciprofloxacin (0.4 mg/mL) and Ketoconazole (0.1 mg/mL) as standard antibacterial and antifungal drugs respectively. The negative controls were 50% aqueous methanol and 1% dimethylsulfoxide (DMSO). Micro-plates containing inoculi were incubated at 37°C for 24 hr. for bacteria strains and at 25°C for 72 hr. for fungal strains. The minimum inhibitory concentration (MIC) of extracts and fractions against the microorganism was recorded. Likewise, the minimum bactericidal concentration (MBC) against the bacteria and minimum fungicidal concentration (MFC) against the fungi determined. The extract/fraction that demonstrated the lowest MIC and broad-spectrum antimicrobial activity was recorded as the most active.

### 2.6 Statistical Analysis

All data obtained from the antioxidant tests were expressed as mean  $\pm$  standard error of mean (S.E.M). The data were analyzed using a One-way Analysis of Variance (ANOVA). This was followed by Tukey's multiple comparison test down the columns, using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, USA). Data with alphabets in superscripts are considered significant at  $P = .05$ , while data with the same alphabet in superscript are considered comparable at  $P > .05$ .

### 2.7 Fractionation of the Extract of *G. braunii* (GB) Leaf

The extract of GB leaf was fractionated using solvent-partitioning method [27]. A 50.0 g of the extract was dissolved in 150 mL of 20 % ethanol in distilled water in a 1.0 L separating

funnel. The suspension was successively partitioned with *n*-hexane (3x400 mL; 7.1 % yield), dichloromethane (3x400 mL; 28.5 % yield), ethylacetate (5x400 mL; 11.7 % yield) and *n*-butanol (2x250 mL; 23.6 % yield). The aqueous fraction left over was 24.9 % yield.

### 3. RESULTS AND DISCUSSION

#### 3.1 Antioxidant Activity of Plant Extracts and Fractions

The results (Figure 1 and Table 1) showed that the extract of *Globimetula braunii* (GB) leaf ranked the best antioxidant activity (AOX) among the plant extracts. It demonstrated a significantly ( $P = .05$ ) lower inhibitory concentration ( $IC_{50}$ ) of  $31.21 \pm 1.11 \mu\text{g/mL}$  than each of the other plant extract. Also, the extract of GB leaf exhibited the highest FRAP and TAC antioxidant activities, at  $109.30 \pm 0.76 \text{ mg AAE/g}$  and  $178.15 \pm 3.54 \text{ mg AAE/g}$  respectively. This showed that  $\approx 109 \text{ mg}$  and  $\approx 178 \text{ mg}$  of ascorbic acid in FRAP and TAC respectively, exhibited comparable antioxidant activities with 1 g of GB leaf extract. However, in the FIC assay, the AOX of the extract of GB stem was twice more active ( $IC_{50} = 137.97 \pm 5.60 \mu\text{g/mL}$ ) than GB leaf extract ( $281.10 \pm 12.09 \mu\text{g/mL}$ ).

The results of partition fractions (Table 3) showed that purification enhanced the antioxidant activity of the extract of GB leaf. The ethylacetate fraction (EtOAc) demonstrated the highest AOX among the partition fractions. Its DPPH free radical scavenging property ( $IC_{50} = 8.58 \pm 1.39 \mu\text{g/mL}$ ) was significantly better ( $P = .05$ ) than *L*-ascorbic acid ( $IC_{50} = 11.84 \pm 1.61 \mu\text{g/mL}$ ), but was comparable with quercetin ( $IC_{50} = 7.72 \pm 0.88 \mu\text{g/mL}$ ). The AOX of the EtOAc fraction was highest in the FRAP and TAC assays. An evaluation of the AOX of fractions in the FRAP and TAC assays showed that 1 g of EtOAc fraction exhibited equivalent activities with *L*-ascorbic acid at 178 mg and 485 mg respectively. The FIC ability of the EtOAc fraction was also highest among the fractions with an  $IC_{50}$  of  $154.87 \pm 6.54 \mu\text{g/mL}$ . However, this activity was fifteen-times lower than EDTA, the positive control. On the whole, the EtOAc fraction exhibited: quadruple DPPH-AOX; triple TAC-AOX; and double FIC-AOX each when compared with the extract of GB leaf.

The extract and fraction of GB leaf exhibited AOX via hydrogen atom transfer (HAT) as in the DPPH assay, and single electron transfer (SET) as in the TAC, FRAP and FIC assays [20, 28]. The research findings corroborated the work of Ja'afar *et al.* [29] which reported the DPPH-AOX of the methanol extract of *G. braunii*. Some compounds such as quercetin, rutin and avicularin have been reported from the ethylacetate-soluble and methanol-soluble extracts of *G. braunii* leaf [30]. While the *in vivo* antioxidant activity of the ethylacetate fraction of the plant has also been reported [11], hence this study has established the *in vitro* antioxidant activity of the extract of GB leaf and its ethylacetate fraction by HAT and SET mechanisms of action.

#### 3.2 Antimicrobial Activity of Plant Extracts and Fractions

The antimicrobial analysis of the plants showed that the extracts of *G. braunii* (GB) leaf was susceptible to most of the reference strains of pathogenic microorganisms at an MIC range of 5.0 – 20.0 mg/mL and broad-spectrum antimicrobial activity. GB leaf extract exhibited the least MIC of 5.0 mg/mL against MRSA, a potentially dangerous type of bacteria and a major nosocomial pathogen that causes severe morbidity and mortality worldwide with high resistance to many commercially available antibiotics [31,32]. The ethanol extract of *G. braunii* leaf was therefore selected, thus, justified its fractionation.

An evaluation of the antimicrobial activity of the partition fraction showed that purification enhanced the activity of the extract (Table 4), a scenario which was also observed in the antioxidant activity of the fractions. Therefore, it can be deduced that the study established a direct relationship between antioxidant and antimicrobial activities of the fractions. [Direct link](#)

between antioxidant and some biological activities of natural products such as antimicrobial, hemolytic and cytotoxic activities has been established [33].

The ethylacetate fraction exhibited broad-spectrum antimicrobial activity. It demonstrated activity against MRSA and *C. albicans* at 1.25 mg/mL when compared with ciprofloxacin (0.25 mg/mL) and ketoconazole (0.10 mg/mL). *C. albicans* is the most prevalent cause of fungal infections in humans worldwide, ranging from skin infections to life-threatening systemic infections [34]. The bioactivity of this plant against *C. albicans* is in consonance with the report of antifungal activities of some African mistletoes such as *Viscum album* and *Phragmanthera capitata* [35,36]. The bio-activities of the EtOAc and *n*-BuOH fractions of *G. braunii* leaf against *C. albicans* is reported here for the first time.

**Table 1. Antioxidant activity of plant extracts**

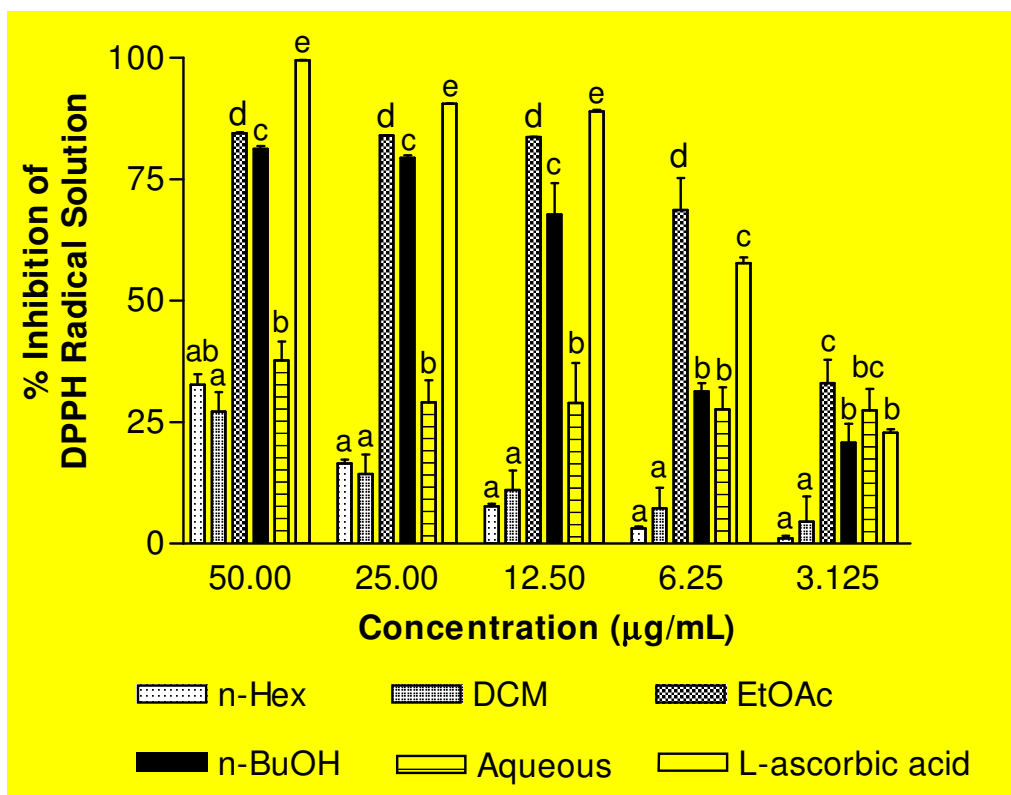
Test Samples	DPPH	FRAP±SEM	TAC±SEM	Fe <sup>2+</sup> Chelating Ability
	IC <sub>50</sub> ±SEM (µM)	(mgAAE/g)	(mgAAE/g)	IC <sub>50</sub> ±SEM (µM)
TB Leaf	62.96±0.96 <sup>g</sup>	100.19±0.34 <sup>c</sup>	115.36±3.48 <sup>a</sup>	291.82±6.71 <sup>c</sup>
TB Stem	33.15±0.58 <sup>d</sup>	106.12±0.49 <sup>d</sup>	162.65±2.86 <sup>d</sup>	275.71±11.35 <sup>c</sup>
TG Leaf	60.42±7.78 <sup>g</sup>	92.66±1.23 <sup>b</sup>	150.97±4.68 <sup>c</sup>	514.53±6.89 <sup>d</sup>
TG Stem	46.23±1.08 <sup>f</sup>	57.53±0.28 <sup>a</sup>	129.03±1.20 <sup>b</sup>	301.13±20.10 <sup>c</sup>
GB Leaf	31.21±1.11 <sup>c</sup>	109.30±0.76 <sup>e</sup>	178.15±3.54 <sup>e</sup>	281.10±12.09 <sup>c</sup>
GB Stem	42.77±0.26 <sup>e</sup>	105.95±0.11 <sup>d</sup>	116.68±3.92 <sup>a</sup>	137.97±5.60 <sup>b</sup>
L-ascorbic acid	13.10 ±0.06 <sup>b</sup>	NA	NA	NA
Quercetin	8.19±0.31 <sup>a</sup>	NA	NA	NA
EDTA	NA	NA	NA	13.21±2.56 <sup>a</sup>

**Footnote:** n=3, Data are expressed as Mean ± S.E.M = Mean values ± Standard error of means of three experiments, GB- *Globimetula braunii*, TB- *Tapinanthus bangwensis*, TG- *Tapinanthus globiferus*, NA- Not applicable, EDTA- ethylenediaminetetraacetic acid, mgAAE/g- milligram of ascorbic acid equivalent per gram of sample, IC<sub>50</sub>- concentration of sample that caused 50% inhibition of free radical. Data with different alphabets in superscript are significantly different at P = .05 down each column, while those with same alphabets are comparable at P>0.05

**Table 2. Antimicrobial activity of plant extracts**

Plant Extract	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>B. subtilis</i>		MRSA		<i>C. albicans</i>	
	ATCC 25923		ATCC 10145		NCTC 8236		ATCC 29213		ATCC 24433	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
TB Leaf	40.0				40.0		5.0	40.0		
TB Stem	40.0		40.0		20.0				40.0	
TG Leaf					40.0					
TG Stem			40.0							
GB Leaf	40.0		40.0		10.0	40.0	5.0	20.0	10.0	20.0
GB Stem							10.0	20.0	40.0	
50 % MeOH										
Standards	0.2	0.4	0.2	0.4	0.2	0.2	0.2	0.4	0.1	0.1

**Footnote:** Standards are positive controls such as Ciprofloxacin against bacteria and Ketoconazole against fungi, GB- *Globimetula braunii*, TB- *Tapinanthus bangwensis*, TG- *Tapinanthus globiferus*, MIC- minimum inhibitory concentration, MBC- minimum bactericidal concentration, MFC- minimum fungicidal concentration, (-) microorganism not susceptible >40 mg/mL. Standards: Ciprofloxacin against bacteria and Ketoconazole against fungi. The highlighted values show the spectrum of antimicrobial activity.



**Fig. 1. Antioxidant Activity of fractions of *G. braunii* leaf against the stable (DPPH) free radical solution monitored at 517 nm wavelength**

Test drugs: *n-Hex*- *n*-hexane, *DCM*- dichloromethane, *EtOAc*- ethylacetate, *n-BuOH*- *n*-Butanol, data represented as bars with different alphabets in superscript are significantly different at  $P = 0.05$ , while those with same alphabets are comparable at  $P > 0.05$ . bars on the graph are expressed as Mean  $\pm$  S.E.M = Mean values  $\pm$  Standard error of means of three experiments ( $n=3$ )

**Table 3. Antioxidant activity of partition fractions of *G. braunii* leaf**

Test Sample	DPPH $IC_{50} \pm SEM$ ( $\mu g/mL$ )	FRAP $\pm SEM$ (mgAAE/g)	TAC $\pm SEM$ (mgAAE/g)	Fe <sup>2+</sup> Chelating Ability $IC_{50} \pm SEM$ ( $\mu g/mL$ )
GB Leaf	32.01 $\pm$ 1.19 <sup>d</sup>	111.43 $\pm$ 1.42 <sup>b</sup>	175.73 $\pm$ 5.27 <sup>a</sup>	278.76 $\pm$ 15.71 <sup>c</sup>
<i>n-Hex</i>	75.89 $\pm$ 5.05 <sup>e</sup>	172.88 $\pm$ 1.12 <sup>c</sup>	ND	576.41 $\pm$ 12.31 <sup>e</sup>
<i>DCM</i>	98.06 $\pm$ 6.40 <sup>f</sup>	102.66 $\pm$ 10.25 <sup>b</sup>	245.45 $\pm$ 7.24 <sup>b</sup>	353.95 $\pm$ 21.89 <sup>d</sup>
<i>EtOAc</i>	8.58 $\pm$ 1.39 <sup>a</sup>	178.64 $\pm$ 2.04 <sup>e</sup>	485.81 $\pm$ 50.41 <sup>d</sup>	154.87 $\pm$ 6.54 <sup>b</sup>
<i>n-BuOH</i>	16.06 $\pm$ 0.52 <sup>c</sup>	175.38 $\pm$ 0.97 <sup>d</sup>	283.83 $\pm$ 23.01 <sup>c</sup>	298.79 $\pm$ 32.51 <sup>c</sup>
Aqueous	86.97 $\pm$ 24.74 <sup>e,f</sup>	49.94 $\pm$ 18.23 <sup>a</sup>	ND	ND
<i>L</i> -ascorbic acid	11.84 $\pm$ 1.61 <sup>b</sup>	-	-	-
Quercetin	7.72 $\pm$ 0.88 <sup>a</sup>	-	-	-
EDTA	-	-	-	10.83 $\pm$ 1.90 <sup>a</sup>

**Footnote:**  $n=3$ , GB Leaf- *G. braunii* leaf extract, *n-Hex*- *n*-hexane, *DCM*- dichloromethane, *EtOAc*- ethylacetate, *n-BuOH*- *n*-Butanol, EDTA- ethylenediamine tetraacetic acid, (-) not applicable, ND- not determined due to very low bioactivity,  $IC_{50}$ - Concentration of sample that caused 50 % inhibition of free radical, mgAAE/g- milligram of ascorbic acid equivalent per gram of sample. Data with different alphabets in superscript each column, while those with same alphabets are comparable at  $P > 0.05$

**Table 4. Antimicrobial activity of partition fractions of *G. braunii* leaf**

Test Samples	<i>E. coli</i>		<i>P. aeruginosa</i>		<i>B. subtilis</i>		MRSA		<i>C. albicans</i>	
	ATCC 25923		ATCC		NCTC 8236		ATCC 29213		ATCC 24433	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Extract					10.00		5.00		10.00	20.00
<i>n</i> -Hex										
DCM	2.50	5.00	5.00	10.00	1.25	2.50	2.50	5.00	2.50	5.00
EtOAc	2.50	5.00	5.00	10.00	0.63	1.25	1.25	2.50	1.25	2.50
<i>n</i> -BuOH	10.00	20.00	2.50	10.00	10.00	20.00	2.50	5.00	1.25	2.50
Aqueous					20.00				20.00	
50% MeOH										
1% DMSO										
Standards	0.25	0.25	0.25	0.50	0.25	0.25	0.25	0.25		0.10

**Key and Footnote:** Standards are positive controls such as Ciprofloxacin and Ketoconazole against bacteria and fungi respectively, MIC: minimum inhibitory concentration, MBC- minimum bactericidal concentration, MFC- minimum fungicidal concentration, Standards: Ciprofloxacin against bacteria and Ketoconazole against fungi; *n*-Hex: *n*-hexane; DCM: dichloromethane; EtOAc: ethylacetate; *n*-BuOH: *n*-butanol; MeOH: methanol; DMSO: dimethylsulfoxide; (-) microorganism not susceptible at > 20 mg/mL.

#### 4. CONCLUSION

The hydro-ethanol extract obtained from the leaf of *G. braunii* demonstrated considerable antioxidant and antimicrobial activities. Purification of this extract enhanced the bioactivities. The moderately polar ethylacetate fraction exhibited the highest activities among the fractions; hence, validates the folkloric use *G. braunii* leaf as a remedy for microbial infections.

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