Original Research Article

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

ABSTRACT

The study evaluated the antioxidant and antimicrobial activities of some hemi-parasitic West African plants (mistletoes) implicated as remedy for microbial infections in the Nigerian ethnomedicine. They comprised *Tapinanthus bangwensis* (TB), *Tapinanthus globiferus* (TG) and *Globimetula braunii* (GB) parasitizing the trees of *Citrus paradisii, Ficus sur* and *Leucena leucocephala* respectively. This was with a view to determining the most active plant extract and partition fraction. The leaf and stem of each plant was separately air-dried, powdered and macerated in ethanol-H_20 (8:2). The extracts were subjected to *in vitro* antioxidant tests using a 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), Fe^{2+} chelating ability (FIC) and total antioxidant capacity (TAC) assays. The antioxidant activity was compared with *L*-ascorbic acid and quercetin as positive controls, while ethylenediaminetetraacetic acid (EDTA) was the positive control used in the FIC assay. The antimicrobial test was carried out using microbroth dilution method against reference strains of methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 29213, *Bacillus subtilis* NCTC 8236, *Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC 10145, and *Candida albicans* ATCC 24433 with ciprofloxacin and ketoconazole as standards. The minimum inhibitory concentration (MIC) of the extracts and fractions were determined. The most active GB leaf extract was fractionated to obtain *n*-hexane, dichloromethane, ethylacetate, *n*-butanol and aqueous fractions, and were evaluated for the bio-activities. The result showed that the extract of GB leaf demonstrated the best bioactivities with DPPH-IC_{50} value of 31.21±1.11 µg/mL, FRAP value of 109.30±0.76 mg AAE/g, TAC value of 178.15±3.54 mgAAE/g and MIC of 5.0 – 10.0 mg/mL. The Ethylacetate fraction of GB leaf demonstrated the highest bioactivities. It was four-fold, three-fold and two-fold activities better than the mother extract in the DPPH, TAC, and antimicrobial studies respectively. The EtOAc fraction and quercetin were comparable ($P > 0.05$) in DPPH antioxidant activity, and also exhibited broad-spectrum-antimicrobial activity with MIC of 1.25 mg/mL against MRSA and *Candida albicans.* In conclusion, the extract of *G. braunii* leaf demonstrated considerable antioxidant and antimicrobial activities. The bioactivities resides in the moderately polar ethylacetate fraction. The study therefore 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. $O_2^{\bullet-}$, $OH^{\bullet}$, $HO_2^{\bullet}$, $LOO^{\bullet}$) and reactive nitrogen species (RNS, such as

$^{\bullet}NO$, $ONOO^{-}$, $^{\bullet}NO_2$, $^{\bullet}N_2O_3$), 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 methicillin-resistance *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 "àfòmó oníìsánó" 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.

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), $FeCl_3.6H_2O$, sodium phosphate, ammonium molybdate, ethylenediaminetetraacetic acid (EDTA), 70149 Nutrient Broth No 3 and S3306 Sabouraud Dextrose (Sigma-Aldrich Co., St. Louis, MO 63103 USA). Plant materials were grinded on a Christy Grinding machine (UK), concentration of extract was carried on a Heidolph RT Rotavapor (Buchi, Germany), and absorbance of test samples was determined on a CamSpec M 107 Spectrophotometer (Spectronic Camspec Ltd, UK). 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 paradisii* (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_20 (8:2), at room temperature for 72 hr. They were filtered, concentrated *in vacuo* at 50 °C. 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.* [20]. 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:

$$\%\ Inhibition\ of\ Sample = \left[\frac{Abs_{Sample} - Abs_{Control}}{Abs_{Control}}\right] x\ 100$$

where, $Abs_{Sample} = Absorbance\ of\ Plant\ Extract\ or\ Fraction,$ and

$$Abs_{Control} = Absorbance\ of\ negative\ control$$

For each sample, the concentration which inhibited the DPPH radical solution by 50% was taken as the IC_{50} value.

2.4.2 Ferric Reducing Ability of Plasma (FRAP) Assay

This was carried out according to the method of Benzie and Strain [21], 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 $3H_2O$ 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 $FeCl_3.6H_2O$ (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, $FeSO_4.7H_2O$, 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 [22]. Solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine were diluted 20 times. An aliquot (1 mL) of different concentrations of extract was mixed with 1mL $FeCl_2 \cdot 4H_2O$. 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 at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated by using the formula:

$$\%\ Chelating\ abilty\ of\ Sample = \left[\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}}\right] x\ 100$$

where $Abs_{control}$ = absorbance of control (the control contains $FeCl_2$ and ferrozine, complex formation molecules); and Abs_{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.* [23]. 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). 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 µg/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 [24]. 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, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 29213, and *Candida albicans* ATCC 24433. Bacteria were maintained on nutrient broth and fungi on Sabouraud Dextrose broth at 4^{o}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^{o}C for 24 hr. for bacteria strains and at 25^{0}C for 72 hr. for fungal strains. The minimum concentration of extracts and fractions that inhibited the growth of each microorganism was taken as the MIC, while the minimum concentration of extract that killed the bacteria and fungi were MBC and MFC respectively. 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 ± 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 [25]. 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 DPPH assay was based on the spectrophotometric determination of the free radical scavenging capacity of antioxidants in the extracts and fractions towards the DPPH free radical by decrease in absorbance. Therefore, the test provided 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 power of antioxidants (FRAP) in the plants was a measure of their free radical reducing abilities, that is, ability to reduce ferric ion ((Fe^{3+}) to ferrous ion (Fe^{2+}). The formation of Pearl's Prussian blue, measured at 593 nm

indicated the formation of Fe^{2+}. The evaluation of the total antioxidant capacity (TAC) became necessary as this is one important tool used for the determination of the additive antioxidant properties of natural products, especially medicinal plants [26].

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±1.11µM than each of the other plant extract. The findings from this study corroborated the work of Ja'afar *et al*. [27] which reported the DPPH-AOX of the methanol extract of *G. braunii*. The study also showed that GB leaf extract exhibited the highest FRAP and TAC antioxidant activities, at 109.30±0.76 mg AAE/g and 178.15±3.54 mg AAE/g respectively. This showed that ≈109 mg and ≈178 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 higher than the that of GB leaf. The latter exhibited a 50 % Fe^{2+} chelating ability at a concentration of 281 µM; an activity that was significantly lower (P= .05) when compared with GB stem extract with an IC_{50} value of 137.97 µM. The mechanisms of action of the most active GB leaf extract were: hydrogen atom transfer (HAT) which is characteristic of the DPPH assay; and single electron transfer (SET) exhibited by the FRAP and TAC assays [28].

The results of partition fractions (Table 3) showed concentration-enhanced-antioxidant activity. The ethylacetate fraction (EtOAc) proved to be the best among the partition fractions. Its DPPH free radical scavenging property (IC_{50} = 8.58 µM) was significantly better (P = .05) than *L*-ascorbic acid (IC_{50} = 11.84 µM), but was comparable in activity with quercetin (IC_{50} = 8.18 µM), a flavonoid that is widely distributed in nature and which has also been reported in this plant [29]. An evaluation of the AOX in the FRAP and TAC assays showed that they demonstrated equal activities with 1 g of EtOAc at 178 mg and 485 mg respectively. The FIC ability of the EtOAc fraction was significantly better than each of the other fractions. However, its activity was fifteen-times lower than EDTA. On the whole, the EtOAc fraction exhibited: quadruple DPPH-AOX; triple TAC-AOX; and double FIC-AOX each when compared with the mother extract. Some Compounds such as quercetin, rutin and avicularin have been reported from the ethylacetate-soluble and methanol-soluble extracts of *G. braunii* leaf [29]. While the *in vivo* antioxidant activity of the ethylacetate fraction of the plant has also been reported [11], hence this study has established the GB leaf plant extract and its ethylacetate fraction for *in vitro* antioxidant activity via 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 a minimum inhibitory concentration (MIC) range of 5.0 – 20.0 mg/mL and broad-spectrum antimicrobial activity. GB leaf extract gave the least MIC of 5.0 mg/mL against methicillin-resistant *Staphylococcus aureus* (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 [30,31]. 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 purification-enhanced-activity (Table 4), a scenario which was observed in the antioxidant activity. Therefore, it can be deduced that the study established a direct relationship between antioxidant and antimicrobial activities of the fractions. Direct-link between antioxidant activity and some biological activities of natural products such as antimicrobial, hemolytic and cytotoxic activities have been established [32].

The ethylacetate fraction (EtOAc) exhibited broad-spectrum-antimicrobial activity. It demonstrated susceptibility against MRSA and *Candida albicans* at 1.25 mg/mL, 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 [33]. 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* [34,35]. 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 IC_{50}±SEM (µM)	FRAP±SEM (mgAAE/g)	TAC±SEM (mgAAE/g)	Fe^{2+} Chelating Ability IC_{50}±SEM (µM)
TB Leaf	$62.96±0.96^{g}$	$100.19±0.34^{c}$	$115.36±3.48^{a}$	$291.82±6.71^{c}$
TB Stem	$33.15±0.58^{d}$	$106.12±0.49^{d}$	$162.65±2.86^{d}$	$275.71±11.35^{c}$
TG Leaf	$60.42±7.78^{g}$	$92.66±1.23^{b}$	$150.97±4.68^{c}$	$514.53±6.89^{d}$
TG Stem	$46.23±1.08^{f}$	$57.53±0.28^{a}$	$129.03±1.20^{b}$	$301.13±20.10^{c}$
GB Leaf	$31.21±1.11^{c}$	$109.30±0.76^{e}$	$178.15±3.54^{e}$	$281.10±12.09^{c}$
GB Stem	$42.77±0.26^{e}$	$105.95±0.11^{d}$	$116.68±3.92^{a}$	$137.97±5.60^{b}$
L-ascorbic acid	$13.10 ±0.06^{b}$	NA	NA	NA
Quercetin	$8.19±0.31^{a}$	NA	NA	NA
EDTA	NA	NA	NA	$13.21±2.56^{a}$

Footnote: *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, AAE/g- ascorbic acid equivalent per gramme, 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, EDTA- ethylenediaminetetraacetic acid*

Table 2. Antimicrobial activity of plant extracts

Plant Extract	*E. coli* ATCC 25923		*P. aeruginosa* ATCC 10145		*B. subtilis* NCTC 8236		*MRSA* ATCC 29213		*C. albicans* ATCC 24433	
	MIC	MBC	MIC	MBC	MIC (mg/mL)	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 (highlighted purple)* against bacteria and Ketoconazole (*highlighted in orange*) *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.*

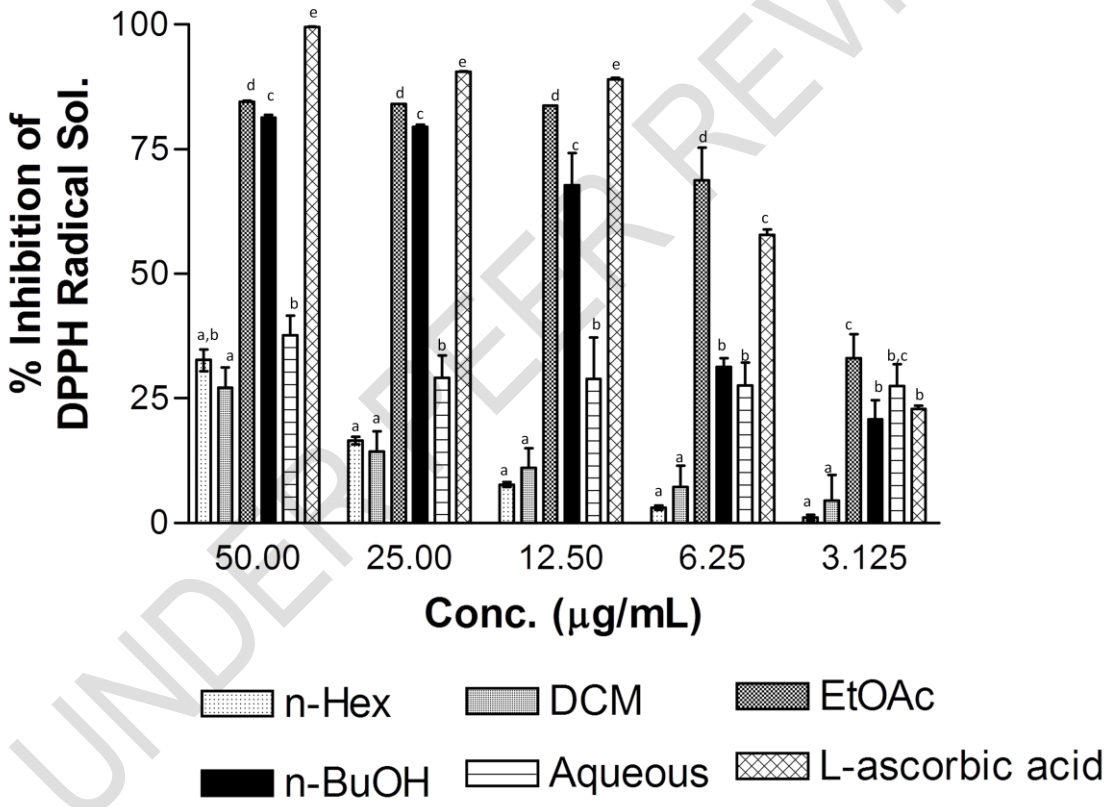

Fig. 1. Antioxidant Activity of fractions of *G. braunii* leaf against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical solution at 517 nm wavelength

Test drugs: 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 ± S.E.M = Mean values ± Standard error of means of three experiments

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

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

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 that caused 50 % inhibition of free radical, mgAAE/g: amount (in mg) of ascorbic acid that will give an equal antioxidant activity as that of 1 g of each fraction. 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 4. Antimicrobial activity of partition fractions of *G. braunii* leaf

Test Samples	*E. coli* ATCC 25923		*P. aeruginosa* ATCC		*B. subtilis* NCTC 8236		*MRSA* ATCC 29213		*C. albicans* ATCC 24433	
	MIC	MBC	MIC	MBC	MIC (mg/mL)	MBC	MIC	MBC	MIC	MFC
Extract	-	-	-	-	10.00	-	5.00	-	10.00	20.00
n-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
n-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* against bacteria (highlighted in purple) and Ketoconazole against fungi (highlighted in pink), *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. The highlighted values show the spectrum of antimicrobial activity.*

4. CONCLUSION

The hydro-ethanol extract obtained from the leaf of *G. braunii* parasitizing *L. leucocephala* tree, demonstrated considerable antioxidant and antimicrobial activities. Purification of the plant extract enhanced both bioactivities, which resided in the moderately polar ethylacetate

fraction. The study therefore validates the folkloric use *G. braunii* leaf as a remedy for microbial infections.

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