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

A. O. Oriola¹,²*, A. J. Aladesanmi², E. O. Akinkunmi³ and I. J. Olawuni⁴

¹Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
²Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
³Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
⁴Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors’ contributions

This was a collaborative research among all authors. Authors AOO and AJA designed the study. Author AOO carried out the phytochemistry aspect of the work, performed the antioxidant tests under the supervision of author IJO. Author EOA performed the antimicrobial test. Author AOO performed the statistical analysis, wrote the first draft of the manuscript, managed the literature searches and managed the analyses of the study under the supervision of authors EOA and AJA. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i330219
Editor(s):
(1) Dr. Sechene Stanley Gololo, Sefako Makgatho Health Sciences University, South Africa.
(2) Professor, Marcello Iriti, Milan State University, Italy.
Reviewers:
(1) Iwona Rybakowska, Medical University of Gdansk, Poland.
(2) S. Danish Kadir, Bangladesh.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55052

Received 19 December 2019
Accepted 26 February 2020
Published 27 February 2020

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 ethnmedicine 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-H₂O (8:2). The extracts were subjected to in vitro antioxidant tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), Fe²⁺ 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

*Corresponding author: E-mail: aooriola@oauife.edu.ng;
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. \(O_2^-\), \(OH^+\), \(HO_2^-\), \(LOO^-\)) and reactive nitrogen species (RNS, such as \(NO\), \(ONOO^-\), \(NO_2^-\), \(N_2O_3\)), 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 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 “áfómọ 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.

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.

Keywords: Hemi-parasitic plant; Globimetula braunii; Tapinanthus bangwensis; Tapinanthus globiferus; antioxidant; antimicrobial.

Globimetula braunii (A. Rich) Tiegh, 

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 “áfómọ 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.

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 ((Fe$^{3+}$) to ferrous ion (Fe$^{2+}$). The formation of Pearl’s Prussian blue at 593 nm indicates the formation of 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 aforementioned 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), FeCl$_3$.6H$_2$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 Leucaena leucocephala (Caesalpinioideae) 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$_2$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:

$$\% \text{ Inhibition of Sample} = \frac{\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Control}}} \times 100$$

where,

- $\text{Abs}_{\text{Sample}} =$ Absorbance of Plant Extract or Fraction, and
- $\text{Abs}_{\text{Control}} =$ Absorbance of negative control

For each sample, the concentration which inhibited the DPPH radical solution by 50% was taken as the IC$$_{50}$$ 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
The absorbance of the solution was determined spectrophotometrically at 593 nm, exactly 10 minutes after mixing. To standardize 50 mL of the standard, FeSO₄·7H₂O, 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 FeCl₂·4H₂O and 5 mM ferrozine were diluted 20 times. An aliquot (1 mL) of different concentrations of extract was mixed with 1 mL FeCl₂·4H₂O 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–Fe²⁺ 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 FeCl₂ 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 µg/mL) with methanol.

2.5 Antimicrobial Susceptibility Test

2.5.1 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 ± 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 (Fig. 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 31.2±1.11 µg/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±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 more active ($IC_{50} = 137.97±6.60$ µg/mL) than GB leaf extract ($281.10±12.09$ µg/mL).

The results of partition fractions (Table 3) showed that purification enhanced the antioxidant activity of the extract of GB leaf. The ethylacetate fraction (EOAc) demonstrated the highest AOX among the partition fractions. Its DPPH free radical scavenging property ($IC_{50} = 8.58±1.39$ µg/mL) was significantly better ($P = .05$) than $L$-ascorbic acid ($IC_{50} = 11.84±1.61$ µg/mL), but was comparable with quercetin ($IC_{50} = 7.72±0.88$ µg/mL). The AOX of the EOAc 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 EOAc fraction exhibited equivalent activities with $L$-ascorbic acid at 178 mg and 485 mg respectively. The FIC ability of the EOAc fraction was also highest among the fractions with an $IC_{50}$ of 154.87±6.54 µg/mL. However, this activity was fifteen-times lower than EDTA, the positive control. On the whole, the EOAc 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 bioactivities 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 IC₅₀±SEM (µM) | FRAP±SEM (mgAAE/g) | TAC±SEM (mgAAE/g) | Fe²⁺ chelating ability IC₅₀±SEM (µM) |
|-------------|------------------|--------------------|-------------------|-------------------------------|
| TB Leaf     | 62.96±0.96b       | 100.19±0.34c       | 115.36±3.48a      | 291.82±6.71c                  |
| TB Stem     | 33.15±0.58d       | 106.12±0.49d       | 162.65±2.86d      | 275.71±11.35c                 |
| TG Leaf     | 60.42±7.78b       | 92.66±1.23b        | 150.97±4.68c      | 514.53±6.89d                  |
| TG Stem     | 46.23±1.08b       | 57.53±0.28a        | 129.03±1.20b      | 301.13±20.10b                 |
| GB Leaf     | 31.21±1.11c       | 109.30±0.76a       | 178.15±3.54a      | 281.10±12.09c                 |
| GB Stem     | 42.77±0.26b       | 105.95±0.11d       | 116.68±3.92a      | 137.97±5.60b                  |
| L-ascorbic acid | 13.10±0.06b | NA                 | NA                | NA                           |
| Quercetin  | 8.19±0.31a       | NA                 | NA                | NA                           |
| EDTA        | NA                | NA                 | NA                | 13.21±2.56a                  |

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, IC₅₀- 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.

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 = .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 (n=3)
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 | MBC | MIC | MBC | MIC | MBC | MBC | MBC | 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 | - | - | - |
| 50% MeOH      | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Standards     | 0.2 | 0.4 | 0.2 | 0.4 | 0.2 | 0.2 | 0.2 | 0.4 | 0.1 | 0.1 | - | - | - |

*Standards are positive controls such as Ciprofloxacin against bacteria and Ketoconazole against fungi.*

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

| Test sample | DPPH IC50±SEM (µg/mL) | FRAP±SEM (mgAAE/g) | TAC±SEM (mgAAE/g) | Fe²⁺ chelating ability IC50±SEM (µg/mL) |
|-------------|------------------------|--------------------|--------------------|----------------------------------------|
| GB Leaf     | 32.0±1.19±a            | 111.4±1.42±a       | 175.7±5.27±a       | 278.7±15.71±c                          |
| n-Hex       | 75.8±5.08±b            | 172.8±1.12±b       | ND                 | 576.4±12.31±b                          |
| DCM         | 98.0±6.40±c            | 102.6±10.25±b      | 245.45±7.24±b      | 353.9±21.89±d                          |
| EtOAc       | 8.5±1.39±d             | 178.6±2.04±d       | 485.81±50.41±d     | 154.87±6.54±e                          |
| n-BuOH      | 16.0±0.52±e            | 175.38±0.97±d      | 283.83±23.01±c     | 298.7±32.51±f                          |
| Aqueous     | 86.97±24.74±f          | 49.94±18.23±a      | ND                 | ND                                     |
| L-ascorbic acid | 11.84±1.61±g        | -                  | -                  | -                                      |
| Quercetin   | 7.72±0.88±a            | -                  | -                  | -                                      |
| EDTA        | -                      | -                  | -                  | 10.83±1.90±a                          |

*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, IC50= 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 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 10145 | B. subtilis NCTC 8236 | MRSA ATCC 29213 | C. albicans ATCC 24433 |
|--------------|--------------------|---------------------------|-----------------------|-----------------|-----------------------|
|              | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | 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 | 0.25 | 0.10 | -   | -   |

*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 of *G. braunii* leaf as a remedy for microbial infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

Authors thank Mr. I. I. Ogunlowo and Mr. Adedoyin, both staff of the Department of Pharmacognosy, Faculty of Pharmacy, O.A.U., Ile-Ife, for the assistance rendered in the plant collection process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Halliwell B, Gutteridge JMC. Antioxidants and free radicals in human health. Free Radicals in Biology and Medicine. 1995;18:125-126.
2. Gutteridge JMC, Halliwell B. Free radicals and antioxidants in the year 2000: A historical look to the future. Annals of the New York Academy of Sciences. 2000;899:136-147.
3. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. International Journal of Biomedical Sciences. 2008;4(2):89-96.
4. World Health Organization. Fact sheets: The top 10 causes of death. In: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva World Health Organization; 2018. (Accessed 15 June 2019)
5. Li B, Webster TJ. Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopaedic infections. J. Ortho. Res. 2018;36(1):22-32.
6. Aukema JE. Vectors, viscin and viscaceae: mistletoes as parasites, mutualists and resources. Front Ecol. Environ. 2003;1(3):212-219.
7. Calder DM. Mistletoes in focus: An introduction. In: Calder M. and Bernhardt P. The Biology of Mistletoes. Eds., Academic Press. San Diego, CA, 1983;1-18.
8. Burkhill HM. Useful plants of West Tropical Africa. Edition 2. Families J-L. Royal Botanic Gardens. Kew. 1985;3:548-560.
9. Tizhe TD, Alonge SO, Aliyu RE. Antibacterial activity of *Globimetula braunii* sourced from five different host trees in Samaru, Zaria, Nigeria. Int. J. Curr. Sci. 2015;18:117-123.
10. Adesina SK, Illoh HC, Johnny II, Jacobs IE. African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: An update. Afr J Tradit Complement Altern; 2013.
11. Okpuzor J, Ogbunugafor HA, Kareem GK. Antioxidative properties of ethyl acetate fraction of *Globimetula braunii* in normal albino rats. Journal of Biological Sciences. 2009;9(5):470-473.
12. Cook JA, Van der Jagt DJ, Dasgupta A, Mounkaila G, Grew RS, Blackwell W, Grew RH. Use of the Trolox assay to estimate the antioxidant content of seventeen edible wild plants of Niger. Life Sci. 1998;63(2):105-110.
13. Ndukwe IG, Amupitan JO, Ashonibare OE. Phytochemical analysis and antimicrobial activity screening of the crude extracts from the aerial parts of *Tapinanthus globiferus*. Nig. J. Chem. Res. 2001;6:43-46.
14. Patrick-Iwuanyanwu KC, Onyeike EN, Wegwu MO. Anti-inflammatory effect of crude methanolic extract and fractions of African mistletoe *Tapinanthus bangwensis* (Engl. & K. Krause) on wistar albino rats. Der Pharmacia Lettre. 2010;2(6):76-83.
15. Erukainure OL, Abowane JA, Adefegha AS, Egwuche RU, Fafunso MA. Antilipemic and hypcholesteremic activities of Globimetula braunii in rats. Exp. Toxicol. Pathol. 2011;63(7-8):657-661.

16. Ekhaise FO, Kayode AM, Sylvester U. Evaluation of the methanolic extract of mistletoe (Tapinanthus bangwensis) leaves grown on orange trees for the phytochemical properties and its physiological effects on streptozotocin-induced diabetes mellitus in laboratory animals. Global J. Pure and Applied Sci. 2011;17(3).

17. Bassey P, Sowemimo A, Lasore O, Spies L, van de Venter M. Biological activities and nutritional value of Tapinanthus bangwensis leaves. African Journal of Biotechnology. 2012;11(73):13821–13826.

18. Oriola AO, Aladesanmi AJ, Arthur G. Anticancer activity of three African mistletoes. Nigerian Journal of Natural Products and Medicine. 2018;22:129-134.

19. Ja’afar MK, Jamil S, Basar N, Saker SD, Mohammed MG. Globrauneine A–F: Six new triterpenoid esters from the leaves of Globimetula braunii. Natural Product Research; 2019. DOI: 10.1080/14786419.2019.1586693

20. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, et al. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J. Nutr. 2003;133:2812–2819.

21. Tankeshwar A. Minimum inhibitory concentration (MIC): Broth dilution method - procedure and interpretation. In: Antibiotic resistance. MicrobeOnline. (Accessed 22 February, 2020) Available:http://www.microbeonline.com/min

22. Sanchez-Moreno C, Larrauri JA, Saura-Calixto FA. Procedure to measure the antiradical efficiency of polyphenols. Journal of Science Food and Agriculture. 1998;76:270-276.

23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “Antioxidant Power”: The FRAP assay. Analytical Biochemistry. 1996;239(1):70-76.

24. Singh N, Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. Food Chemistry. 2004;85(4):611-616.

25. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry. 1999;269:337-341.

26. Clinical and Laboratory Standards Institute CLSI. Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational Supplement M100 – S24. CLSI, Wayne, PA, USA; 2014.

27. Kupchan SM, Tsou G, Sigel CW. Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from Datisca glomerata. J. Org. Chem. 1973;38(7):1420-1.

28. Santos-Sánchez NM, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant compounds and their antioxidant mechanism; 2019. DOI: 10.5772/intechopen.85270

29. Ja’afar MK, Jamil S. and Basar N. Antioxidant activity of leaf extracts of Globimetula braunii (Engler) van Tiegh parasitizing on Piliostigma thonningii and Parkia biglobosa. Jurnal Teknologi. 2017;79(5):43-47.

30. Ja’afar MK, Jamil S, Basar N, Bakar MB, Sarker SD, Flanagan KJ, Sengé MO. Lactones and flavonoids isolated from the leaves of Globimetula braunii. Natural Products Communication. 2017;12(9):1455-1458.

31. Center for Disease Control and Prevention (CDC). MRSA Fact Sheet. (Accessed 10 February, 2020) Available:https://www.cdc.gov/mrsa/pdf/mrsa_consumerfactsheet_f.pdf

32. Haddadin AS, Fappiano SA, Lipsett PA. Meticillin resistant Staphylococcus aureus (MRSA) in the intensive care unit. Postgrad. Med. J. 2002;78:385-392.

33. de Fretas Araújo MG, Hilário F, Vilegas W, dos Santos LC, Brunetti IL, Sotomayor CE, Bauab TM. Correlation among antioxidant, antimicrobial, hemolytic and antiproliferative properties of Leiothrix spiralis leaves extract. Int. J. Mol. Sc. 2012;13(7):9260-9277.

34. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013;4(2):119-128.
35. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal. 2017;8. [Article ID: 9658598]

36. Akalazu JN, Ohazurike NC, Onuoha CI. Antifungal activity of *Viscum album* (African Mistletoe) leaf extract on *Dioscorea rotundata* (poir) (white yam) tuber rot disease. International Journal of Advances in Sciences, Engineering and Technology. 2016;4(2):189-192.

DOI: https://doi.org/10.1155/2017/9658598