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ANTIOXIDANT, ANTIMICROBIAL AND ANTITYROSINASE ACTIVITIES OF PHYTOCHEMICALS FROM THE LEAVES OF *GLOBIMETULA BRAUNII* (ENGLER) VAN TIEGH (LORANTHACEAE)

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ABSTRACT. Herbal preparations of *Globimetula braunii* leaves are used in tropical African countries for the treatment of several illnesses. A preliminary study of the leaf extracts of *Globimetula braunii* has confirmed the antioxidant, antibacterial, biochemical and toxicological potency of the plant. The leaf extracts were subjected to bioactivity guided fractionation that led to the isolation of the active compounds. Antioxidant, antityrosinase and antimicrobial inhibitory activities of the isolated compounds were evaluated. Structural elucidations of the isolated compounds were carried out spectroscopically, including the use of 1D/2D nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) spectroscopies and mass spectrometry. Results indicated that quercetin (**15**) isolated from ethyl acetate fraction exhibited the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) scavenging capacity and also showed the highest ferric reducing antioxidant potential (FRAP) equivalent value. Quercetin (**15**) and dodoneine (**14**) displayed the highest tyrosinase inhibition activity with IC_{50} value of 0.12±0.32 mM and 0.19±0.20 mM, respectively. All the isolated compounds showed weak to not active in the antimicrobial inhibition assay. The findings in this report have revealed the antioxidant and antityrosinase potential of *Globimetula braunii* and thus supporting their traditional uses.

KEY WORDS: *Globimetula braunii*, Antioxidant, Antityrosinase, Antimicrobial, Triterpenoid esters, Dodoneine

INTRODUCTION

Medicinal plants are composed of biologically active chemical constituents of diverse structural entities which have played a vital role as the main source of medicine in various ancient traditional system of medicine [1]. Explorations into the biological and pharmacological activities of medicinal plants throughout the past two centuries have produced compounds for the development of active therapeutic agents [2, 3]. Biologically active constituents from plants are significant and essential sources of novel drugs that may possibly lead to new and better treatments for many ailments [4]. Plants of the Loranthaceae family have been claimed to be widely used ethnomedicinally for the treatment of many diseases, including cardiovascular diseases [5], hepatic illness [6, 7], mental condition, sterility, urinogenital problems, skin diseases, malaria, epilepsy, infertility, stomach problems, and as laxative [8, 9]. *Globimetula braunii* (Engler) Van Tiegh is a parasitic plant of the Loranthaceae family native to central tropical region of Africa. In Nigeria, *G. braunii* are said to be effective against cholera, hypertension, diabetes, blood purifier, cancer, gastro-intestinal tract diseases and wound infections [10-12]. Our previous phytochemical

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studies on *G. braunii* have successfully isolated several triterpenoid esters [13], lactones and flavonoids [14]. We also reported that, the ethyl acetate extract of *G. braunii* has the ability to protect mice at dose of 150 mg/kg against pentylenetetrazole induced seizure by 83.33% [15]. Considering the importance of the therapeutic uses of the species in the management of hepatic illness, malaria and skin diseases, it is clear that a wider range of findings of the biological activities are needed to be discovered for their pharmacological properties. This study is therefore designed to study the phytochemical constituents and bioactivities of *Globimetula braunii* that will provide additional information on these ethnomedicinal claims and significant opportunities are available to offer a vast starting point for the discovery of unprecedented novel bioactive compounds as well as promising therapeutic and pharmacological properties from various extracts of this species.

EXPERIMENTAL

General experimental procedures

All chemicals and reagents used were of analytical grade. *n*-Hexane, dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), ethyl acetate (EtOAc), acetone and methanol (MeOH) were used as the solvent system in chromatography method. Silica gel 60 F_{254} pre-coated aluminum sheet (0.20 mm, Merck Kieselgel) was used for thin layer chromatography (TLC) analysis. Vacuum liquid chromatography (VLC) was carried out using Merck SiO_2 (230-400 mesh). Column chromatography (CC) was performed on Merck SiO_2 (70-230 mesh) and Sephadex LH-20, while 1 mm thin glass plate of Merck SiO_2 60 F_{254} (20 × 20 cm) were used for preparative thin layer chromatography (PTLC). The ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at ambient temperature. Deuterated chloroform ($CDCl_3$) and acetone (CD_3COCD_3) obtained from Sigma Aldrich were used for the NMR analysis. IR spectra were recorded with a Perkins Elmer series 1600 spectrophotometer. The melting points were determined on a Leica Gallen III Kofler micro melting point apparatus equipped with microscope and were uncorrected. Ultraviolet (UV) spectra were measured with a Shimadzu UV 1601PC spectrophotometer. HRAPCI and ASAP-TOF-MS were performed using micro TOF-Q II and Xevo G2-S ASAP instruments respectively. Butylated hydroxyanisole (BHA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma Aldrich. Ascorbic acid (AA) was purchased from Goodrich Chemical Enterprise (GCE), while sodium acetate trihydrate, ferric sulfate, 2,4,6-tripyridyl-s-triazine (TPTZ) and potassium persulfate were purchased from Fluka. Absorbance was measured using the microplate reader EPOCH (BioTek). *Gram*-positive bacteria, i.e. *Staphylococcus aureus* (ATCC29737) and *Bacillus subtilis* (ATCC6633) and *Gram*-negative bacteria, i.e. *Escherichia coli* (ATCC10536) and *Pseudomonas aeruginosa* (ATCC9027) together with fungal strain *Aspergillus niger* (ATCC16888) were obtained from Saintifik Sdn. Bhd. (Malaysia). Nutrient agar (NA), Sabouraud dextrose agar (SDA), nutrient broth (NB), Sabouraud dextrose broth (SDB), streptomycin sulfate and nystatin were obtained from Oxoid (Italy). Kojic acid, *L*-tyrosin-3,4-dihydroxyl-*L*-phenylalanine (*L*-DOPA) and mushroom tyrosinase were purchased from Sigma Aldrich.

Collection and identification of plant material

The leaves of *Globimetula braunii* growing on *Piliostigma thonningii* plant were collected from Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria in October 2014. The plant was authenticated by Mr. U.S. Gallah and a voucher specimen (No 9016) for *G. braunii* and (No 7151) for *P. thonningii* were deposited at the herbarium of Biological Science Department, Ahmadu Bello University, Zaria, Nigeria.

Extraction and isolation

The dried, powdered leaves (3.3 kg) were extracted using a cold extraction method with solvents of different polarity starting with *n*-hexane, CH_2Cl_2, EtOAc and MeOH for 72 h to yield black gummy residues after solvent evaporation. A partial fraction of *n*-hexane extract (GBPTH, 60 g) was subjected to silica gel VLC (10 × 10 cm) using a gradient of *n*-hexane:$CHCl_3$:EtOAc:MeOH to afford 22 fractions. The fractions were pooled together based on TLC profile to seven major fractions (GBPTH 1-7). Fractions of GBPTH 1-3 were subjected to column chromatography (CC) over silica gel and eluted with *n*-hexane:EtO_2 to give lupeol palmitate (**8**). GBPTH 4 was purified by CC to afford globrauneine A (**1**), globrauneine C (**3**), globrauneine D (**4**) and lupeol (**7**). Fractions 35 to 79 obtained from CC of GBPTH 4 were combined and concentrated before subjected to preparative TLC to afford globrauneine E (**5**). Pooled fractions of GBPTH 5-6 were subjected to CC to give the globrauneine F (**6**). A partial fraction of CH_2Cl_2 extract (GBPTD, 45 g) was subjected to silica gel VLC (10 × 10 cm) using a gradient of *n*-hexane:EtOAc:MeOH to afford 13 fractions (GBPTD 1-13). Column chromatographic purifications of the combined fractions GBPTD 3-12 afforded globrauneine B (**2**), lup-20(29)-en-3β,15α-diol (**9**), friedelin (**10**), β-sitosterol (**11**) and octacosanoic acid (**12**). The MeOH extract (120 g) was suspended in distilled water, filtered with cotton wool and partitioned successively with *n*-hexane, $CHCl_3$, EtOAc and MeOH to obtain *n*-hexane (5.6 g, 1.64%), $CHCl_3$ (3.8 g, 1.11%), EtOAc (19.5 g, 5.70%), MeOH (34.7 g, 10.10%) fractions, and the residual aqueous fraction (AF, 16.8 g, 4.9%). A partial fraction of the EtOAc (GBPTEF, 19.5 g) was subjected to silica gel VLC (10 × 10 cm) using $CHCl_3$:EtOAc:MeOH as eluents to give five major fractions indicated as GBPTEF 1-5. GBPTEF 1-3 were further purified by CC over silica gel to yield (1*R*,5*S*,7*S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one (**13**), dodoneine (**14**) and quercetin (**15**). Then GBPTEF 4 was purified by CC to afford (+)-catechin (**16**). The MeOH fraction (GBPTMF, 45 g) was subjected to VLC (10.0 × 10.0 cm) and eluted with $CHCl_3$:EtOAc:MeOH to afford five major fractions (GBPTM 1-5). Further purification of fractions 3-5 by CC over silica gel and Sephadex LH-20 yielded quercitrin (**17**) and rutin (**18**) while further purification with PTLC yielded avicularin (**19**).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was carried out on the isolated compounds according to the procedure described by Ja'afar *et al.* [16] with slight modifications. The DPPH reagent (100 µL) was added to the sample (100 µL) with concentrations ranging from 100-1000 mg/L prepared in methanol. The reaction mixture was allowed to incubate for 30 min in the dark at room temperature and the absorbance were measured at 517 nm. The DPPH methanol solution was used as negative control, while butylated hydroxyanisole (BHA) and Trolox were used as the positive controls. The percentage inhibition of DPPH (%) was calculated using the following formula.

$$\% \text{ Inhibition} = [(A_{\text{blank DPPH}} - [A_{\text{sample}} - A_{\text{blank sample}}])/A_{\text{blank DPPH}}] \times 100$$

where $A_{\text{blank DPPH}}$ is the absorbance of DPPH reagent with methanol and A_{sample} is the absorbance of the test compound with DPPH reagent. The IC_{50} value, defined as the concentration of each sample required to give 50% of the absorbance was calculated from the results. All tests and analyses were run in triplets and averaged.

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) activity

The ABTS assay was evaluated according to the technique described by Zou *et al.* [17] with slight modifications. Potassium persulfate and ABTS were suspended in distilled water to obtain a concentration of 4.9 mM and 7 mM, respectively. An equivalent amount of these two solutions were mixed and stored for 12 to 16 hours at room temperature. The ABTS solution was diluted

with distilled water to attain an absorbance of 0.7 at 734 nm. Then, 190 µL of ABTS solution and 10 µL of sample solution were added to 96-well plates and the absorbance was recorded after 30 min incubation in dark at room temperature. The percentage of antioxidant activity was calculated using the following formula:

$$\% \text{ ABST Scavenging} = [(A_{\text{blank ABTS}} - A_{\text{sample}})/A_{\text{blank ABTS}}] \times 100$$

$$*A_{\text{sample}} = A_{\text{ABTS + Sample}}$$

where $A_{\text{blank ABTS}}$ is the absorbance of ABTS solution with methanol and A_{Sample} is the absorbance of the tested samples with ABTS solution. All tests and analyses were run in triplets and averaged.

Ferric reducing antioxidant potential (FRAP) activity

The analysis was performed according to the method described by Channarong *et al*. [18] and Shahwar *et al*. [19] with minor modifications. The FRAP reagent contained 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solutions in a ratio 10:1:1. The reaction mixture consisting of 5 µL of test sample, 15 µL of methanol and 150 µL of FRAP reagent were added to the 96-well plates. The reaction mixture was incubated at 37 °C for 10 min and then the absorbance was recorded at 573 nm. $FeSO_4.7H_2O$ solution (0.1-1.0 mM) was used to obtain calibration curves of standard antioxidants.

Anti-tyrosinase inhibition activity

Antityrosinase inhibitory activity was measured according to the technique described by Likhitwitayawuid *et al.* [20] with minor modification. The assay mixtures consisting of 40 µL of test sample (0.1 mg/mL), 40 µL of mushroom tyrosinase enzyme (100 units/mL) and 40 µL of *L*-DOPA solution (2.5 mM) in 80 µL of sodium phosphate buffer (100 mM, pH 6.8) was added to a 96-well plate. The assay mixture (200 µL) was incubated at 37 °C for 10 min, and the absorbance was measured at 475 nm. The absorbance of similar mixture with DMSO instead of the sample was used as negative control, and kojic acid was used as positive control. The percentage inhibition of tyrosinase activity was calculated as the following:

$$\text{Inhibition (\%)} = [\text{A (blank)} - \text{A (tyrosinase + sample)}]/\text{A (blank)} \times 100$$

Broth microdilution assay

Minimum inhibition concentration (MIC) and minimum bacterial/fungal concentration (MBC/MFC) values were observed by the broth micro dilution methods described by Gulluce *et al*. [21] and Murray *et al*. [22]. The MIC test was evaluated in a sterile 96-well microliter plate. Each compound (3.6 mg) was separately dissolved in MeOH (2.0 mL) to obtain a sample stock solution with concentration 1800 µg/mL. Two wells were represented in each plate for positive and negative controls. Then the wells were loaded with Sterile NB/SDB (100 µL) which were used as diluents to each micro plate well from B to H followed by the addition of samples stock (100 µL) in the first two rows (A and B). Two-fold serial dilution of the mixture of sterile broth and stock samples (100 µL) in row B was transferred to each well in order to obtain a concentration range from 1800 to 14.13 µg/mL. Then the wells were reloaded with bacterial (100 µL) and fungal (100 µL) suspension given a final volume of 200 µg/mL. The microplates were incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for fungi. Microbial growth was indicated by the presence of turbidity and a pellet at the base of the well. MIC values were determined as the lowest concentration of the samples without visible signs of microorganism growth. Streptomycin sulfate and nystatin were employed as positive controls for bacteria and fungi, respectively. The (MBC/MFC) values were determined by pipetting 10 µL of the clear well

containing mixture of samples and inoculums and spreading gently on the surface of the NA (5 mL) for bacteria and SDA (5 mL) for fungi in a petri dish (90 × 15 mm) using a glass rod. The plates were wrapped and incubated upside down at 37 °C for 24 h (bacteria) and 30 °C for 48 h (fungi). The MBC/MFC was determined as the lowest concentration of the test sample at which 99% of the bacteria/fungi colony were killed after the incubation period.

Statistical analysis

The statistical analysis was performed using SPSS for Windows (version 21). The data were analyzed using one-way ANOVA and a post hoc t-test for multiple comparison. Three replicates for each tested sample were used with data presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Chromatographic separations of the leave extracts of *Globimetula braunii* growing on *P. thonningii* led to the isolation of 19 known compounds (Figure 1). The chemical structures of the compounds have been identified on the basis of spectral analysis (NMR, FTIR and MS) and comparison with reported data as globrauneine A-F **1-6** [13], lupeol **7** [23], lupeol palmitate **8** [24], lup-20(29)-en-3β,15α-diol **9** [25], friedelin **10** [26], sitosterol **11** [27], octacosanoic acid **12** [28], (1*R*,5*S*,7*S*)-7-[2-(4-hydroxy phenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one **13** [29], (*R*)-6-[(*S*)-2-hydroxy-4-(4-hydroxyphenyl)butyl]-5,6-dihydropyran-2-one (dodoneine) **14** [14], quercetin **15** [30], (+)-catechin **16** [31], quercitrin **17** [32], rutin **18** [33] and avicularin **19** [34]. The *in vitro* biological assays of the leaf extracts and isolated compounds of *Globimetula braunii* were performed based on antioxidant, antityrosinase and antimicrobial activities as summarized in Table 1. This work is the first report of the antioxidant, antityrosinase and antimicrobial activities from the phytochemical point of view of *Globimetula braunii*. The antioxidant study was evaluated by measuring the quantity of total antioxidant capacity (TAC) using DPPH, ABTS and FRAP techniques. All the tested triterpenes were inactive and did not scavenge free antioxidant radicals even at the highest concentration tested (1000 µg/mL). Flavonoids and phenolics are well known to be essential antioxidant constituents that are obtained from natural plants [35]. The number and position of the hydroxyl groups on the aromatic ring and the type of substituents in the compound have a substantial effect on the antioxidant activity [36]. Quercetin **15 (**IC_{50} value 0.82 µg/mL) exhibited strong antioxidant activity comparable with those of quercitrin **17 (**IC_{50} value (22.48 µg/mL) and rutin **18** (IC_{50} value (28.53 µg/mL).

This could be associated with flavonols containing a catechol group in ring B that are highly active and potent than the corresponding flavonol glycosides because of the presence of a 3-hydroxyl group on the saturated ring C adjacent to a carbonyl group [37]. The presence of 2,3-double bond in conjugation with the 4-carbonyl group in ring C will also increase the potential of the scavenging activities of the compounds [37]. The results were consistent with the antioxidant activity of the leaf extracts of *Globimetula braunii* growing on *P. thonningii* and *Parkia biglobosa* where a strong antioxidant activity was reported for EtOAc and MeOH extracts [16]. The weak antioxidant activity of the triterpenoids is probably due to lack of high hydrogen donating capacity to act as free radicals [38].

The tyrosinase inhibition activity of the leaf extracts and some isolated compounds from *Globimetula braunii* were studied using *L*-DOPA as substrate. Each sample was assayed at different concentrations and their relative activities were expressed as percentage inhibition and IC_{50} values. Kojic acid was used as positive control. The ethyl acetate fraction (GBPTEF) showed the highest percentage inhibition of 72.58% with IC_{50} values of 0.14±0.67 mM. Quercetin **15**, dodoneine **14**, and (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl) ethyl]-2,6-dioxabicyclo[3.3.1]-nonan-3-one **13** showed potent antityrosinase inhibition activities with percent inhibition of 78.09%, 70.42%, and 67.62% (IC_{50} values of 0.12±0.32 mM, 0.19±0.20 mM and 0.25±0.56 mM), respectively.

However, all other compounds exhibited moderate to weak antityrosinase activity compared to kojic acid (I% = 86.24%, IC_{50} = 0.09±0.31 mM). The strong tyrosinase inhibition activity of quercetin **15** was due to the presence of the hydroxyl group at position 3 which played a vital role in chelating the copper in the active center of the tyrosinase [39]. This result is consistent with previous study which showed that quercetin **15** exhibited a significant tyrosinase inhibition activity with IC_{50} values of 0.07 mM (Kojic acid, IC_{50} = 0.014 mM) [31]. Flavonoid without 3-hydroxyl or α-hydroxy-keto groups may not be essential for the tyrosinase activity as several compounds such as luteolin and glabridin that have no 3-hydroxyl or α-hydroxy-keto groups but exhibited tyrosinase inhibitory activity. As such, the presence of hydroxyl groups in dodoneine **14** and (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]-nonan-3-one **13** may be essential for eliciting tyrosinase inhibitory activity and perhaps the reason for their high percent inhibition. The number and position of the hydroxyl group is crucial since it acts as a hyrogen-bonding donor and may favourably interact with the tyrosinase enzyme, thus inhibit the reaction [39].

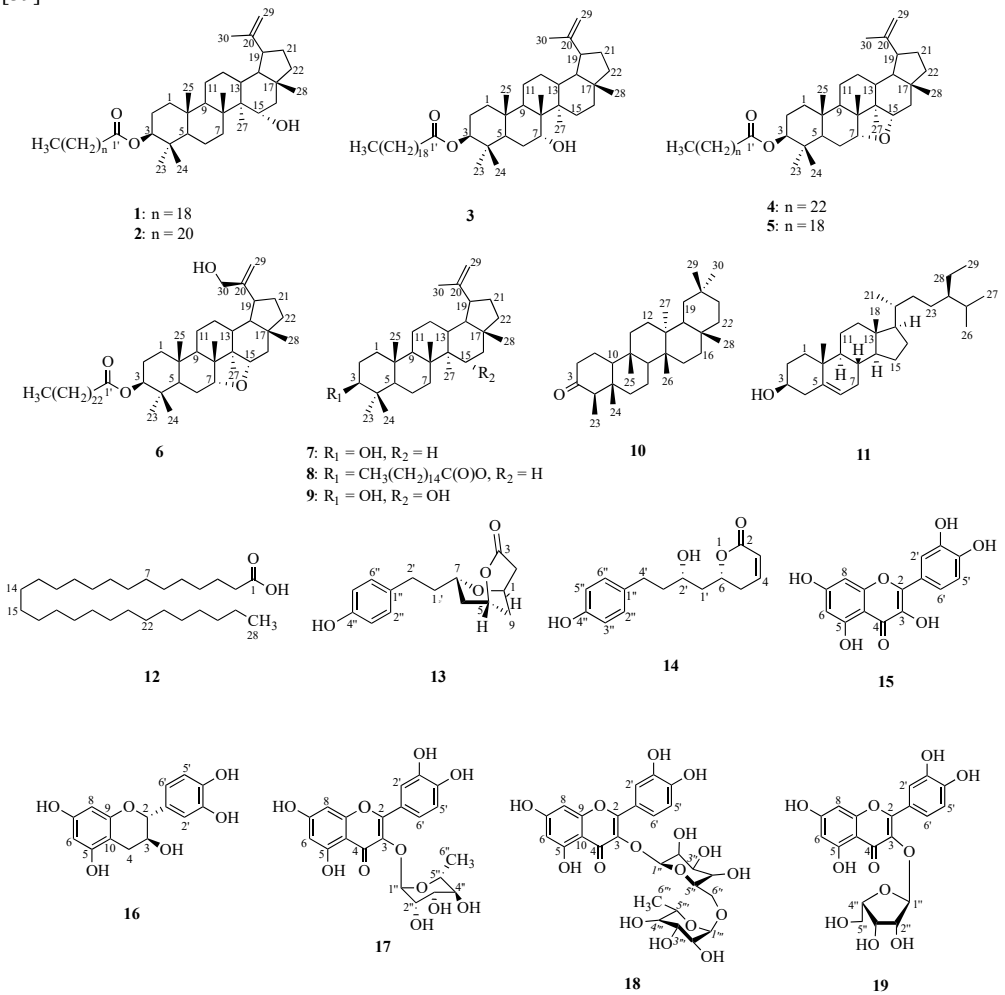

Figure 1. Structure of compounds **1–19**.

Table 1. Free radical scavenging (DPPH, $ABTS^{\bullet+}$ and FRAP) and tyrosinase inhibitory activities of leaf extracts and isolated compounds of *G. braunii*.

Extracts/fraction compounds	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	mM FRAP equivalent to $FeSO_4.7H_2O$ (1.0 mM)	TIA Inhibition (I%) at 100 µg/mL	TIA IC_{50} (mM)
Extracts					
GBPTH	R	R	R	39.59	ND
GBPTD	R	R	R	42.02	ND
GBPTEF	R	R	R	72.58	0.14±0.67
GBPTMF	R	R	R	68.57	0.24±0.12
Pure compounds					
(1)	>1000	NT	NT	38.56	ND
(2)	>1000	NT	NT	37.64	ND
(3)	>1000	NT	NT	31.87	ND
(4)	>1000	NT	NT	32.89	ND
(5)	>1000	NT	NT	29.76	ND
(6)	>1000	NT	NT	42.23	ND
(7)	>1000	NT	NT	52.77	0.44±0.30
(8)	>1000	NT	NT	19.60	ND
(9)	>1000	NT	NT	NT	NT
(10)	>1000	NT	NT	NT	NT
(11)	>1000	NT	NT	50.00	0.53±0.70
(12)	>1000	NT	NT	NT	NT
(13)	184.0	622.70	1.13±0.07	67.62	0.25±0.56
(14)	128.6	603.70	1.67±0.02	70.42	0.19±0.20
(15)	0.82	312.01	3.94±0.49	78.09	0.12±0.32
(16)	8.49	513.50	3.44±0.04	31.53	ND
(17)	22.48	410.90	3.74±0.07	49.82	ND
(18)	28.53	513.50	3.44±0.02	61.99	0.37±0.56
(19)	57.98	529.60	2.03±0.20	59.09	0.40±0.76
Standard					
BHA	11.30	317.70	5.69±0.42	NA	NA
Trolox	0.90	354.01	NT	NA	NA
AA	NT	NT	5.06±0.07	NA	NA
Kojic Acid	NA	NA	NA	86.24	0.09±0.31

All values are expressed as mean ± SD for three replicates experiment. IC_{50} is defined as the concentration sufficient to obtain 50% of the maximum scavenging capacity; R = Reported, ND = Not determined, NT = Not tested, NA = Not applicable, TIA = tyrosinase inhibitory activity. Globrauneine A (**1**), Globrauneine B (**2**), Globrauneine C (**3**), Globrauneine D (**4**), Globrauneine E (**5**), Globrauneine F (**6**), Lupeol (**7**), Lupeol palmitate (**8**), lup-20(29)-en-3β,15α-diol (**9**), Friedelin (**10**), Sitosterol (**11**), Octacosanoic acid (**12**), (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl) ethyl]-2,6 dioxabicyclo [3.3.1]-nonan-3-one (**13**), Dodoneine (**14**), Quercetin (**15**), (+)-Catechin (**16**), Quercitrin (**17**), Rutin (**18**), Avicularin (**19**), GBPTH = *n*-Hexane extract, GBPTD = Dichloromethane extract, GBPTEF = ethyl acetate fraction, GBPTMF = methanol fraction.

The leaf extracts and nineteen phytochemicals were tested for their potential antimicrobial activity. All the extracts displayed weak to inactive inhibition against all the tested *Gram*-positive, *Gram*-negative and fungal strains. Quercetin **15** showed moderate inhibition against *Pseudomonas aeruginosa* (MIC and MBC value 250 µg/mL). Furthermore, (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]-nonan-3-one **13** and dodoneine **14** have shown a moderate activity against *Escherichia coli* with MIC value 250 µg/mL. All the other tested phytochemicals showed weak to inactive inhibition against all the selected bacterial and fungal strains tested.

Table 2. Antibacterial and Antifungal Activities of the Leaf of *G. braunii.*

Compounds/ microbes	*Gram*-positive bacteria				*Gram*-negative bacteria				Fungi	
	B.s		*S.a*		*E.c*		*P.a*		*A.n*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
GBPTH	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000
(1)	1000	>1000	1000	1000	1000	1000	1000	>1000	>1000	>1000
(2)	500	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
(3)	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000
(4)	1000	500	500	1000	500	500	1000	1000	1000	<1000
(5)	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
(6)	1000	>1000	500	1000	1000	1000	>1000	>1000	>1000	1000
(7)	500	1000	500	1000	>1000	>1000	>1000	>1000	>1000	>1000
(8)	500	1000	1000	>1000	1000	>1000	>1000	>1000	>1000	>1000
GBPTD	500	500	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000
(9)	1000	>1000	1000	1000	1000	1000	1000	>1000	>1000	>1000
(10)	1000	>1000	>1000	>1000	500	1000	>1000	>1000	>1000	>1000
(11)	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000
(12)	1000	>1000	1000	1000	1000	1000	1000	>1000	>1000	>1000
GBPTM	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	500	>1000
(13)	500	1000	500	1000	250	500	>1000	>1000	500	1000
(14)	500	500	500	1000	250	500	>1000	>1000	1000	>1000
(15)	500	1000	>1000	>1000	>1000	>1000	250	250	>1000	>1000
(16)	500	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000
(17)	500	1000	500	1000	500	500	1000	1000	1000	1000
(18)	500	500	500	1000	500	500	1000	1000	1000	<1000
(19)	500	1000	>1000	>1000	500	1000	>1000	>1000	1000	>1000
Streptomycin Sulphate	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	-	-
Nystatin	-	-	-	-	-	-	-	-	15.6	15.6

All values were expressed as mean ± SD for three replicates experiment. Globrauneine A **(1)**, Globrauneine B **(2)**, Globrauneine C **(3)**, Globrauneine D **(4)**, Globrauneine E **(5)**, Globrauneine F **(6)**, Lupeol **(7)**, Lupeol palmitate **(8)**, lup-20(29)-en-3β,15α-diol **(9)**, Friedelin **(10)**, Sitosterol **(11)**, Octacosanoic acid **(12)**, (1*R*,5*S*,7*S*)-[2-(4-hydroxyphenyl) ethyl]-2,6 dioxabicyclo [3.3.1]-nonan-3-one **(13)**, Dodoneine **(14)**, Quercetin **(15)**, (+)-Catechin **(16)**, Quercitrin **(17)**, Rutin **(18)**, Avicularin **(19)**, GBPTH = *n*-Hexane extract, GBPTD = Dichloromethane extract, GBPTMF = methanol fraction.

The result of the antimicrobial activity of the extracts is in contrast with the previous findings of the antibacterial assays carried out on aqueous and EtOH extracts of *G. braunii* growing on the trunk of *Eucalyptus* against five bacterial strains (*Pseudomonas aeruginosa*, *Kleibsiella aeruginosa*, *Escherichia coli, Staphylococcus aureus,* and *Proteus spp*). The results showed that except for *Staphylococus aureus*, both the aqueous and ethanol extracts inhibited a pronounced antibacterial activity [40]. Also, the extracts of *G. braunii* parasitizing on other host plants (*T. catappa*, *K. senegalensis*, and *C. grandis*) were effective against the organisms tested, especially the extract of *G. braunii* parasitizing on *K. senegalensis* [41]. Likewise, MeOH leaves extracts of Nigerian *G. oreophila* was also studied for antibacterial activity against six bacterial strains (*Escherichia coli*, *Klebsiella spp*, *Shigella spp*, *Salmonella typi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*). The result of the study demonstrated what can be considered as an effective source of antimicrobial agents with the highest activity on *Shigella spp* [42]. However, the antibacterial activity carried out on the aqueous leaf extract of Nigerian *G. braunii* growing on five different host plant against four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis, Salmonella typi* and *Escherichia coli*), showed the extracts of *G. braunii* parasitizing on *T.*

mantaly and *A. lebbeck* to be inactive against all the bacterial strain tested [43]. This result was in conformity with the data observed from the extracts and the compounds isolated. The justification was supported by the previous finding of the evaluation of antimicrobial activity of globimetulin A and B isolated from *Globimetula dinklagei* against four bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typi* and *Candida albicans*). The compounds were inactive against all the bacterial strain tested at concentrations of up to 1500 µg/mL [43]. Similarly, all the isolated phytochemicals were inactive against the fungal strain tested. The differences observed in the antimicrobial activity of *G. braunii* may be attributed to the morphogenetic and phenogenetical variations of plants harvested at the floral budding, vegetative, fresh fruiting, full flowering and mature fruiting stages which could affect the microbial activity of the plant [44]. Additionally, scientific evidences have also revealed that their compositions of medicinal activities are dependent on the species harvesting period and host plants [10, 45].

CONCLUSION

The present study is the first report on antioxidant, antityrosinase and antimicrobial activity of isolated compounds from the leaves of *Globimetula braunii*. Interestingly, the antioxidant activity of the isolated compounds of *G. braunii* correlated with that of the crude extracts, likewise the antityrosinase and antimicrobial activities. The information in this report has revealed the antioxidant and antityrosinase potential of *Globimetula braunii* and thus offer some logical proof supporting their conventional uses by distinct ethnic groups in Africa for treating skin disorder and as antioxidants. Further analyses should be conducted to study the individual constituents *in vivo* antioxidant activity and other significant biological activities to enhance the better understanding of the health effect of this medicinal plant and isolated compounds.

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