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Antimicrobial Effect of *Geophila obvallata* (Schumach) Didr. Leaf Ectracts Against Pathogenic Microbes

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Abstract

The increase in synthetic drug resistance by pathogenic microbes has led to the development of plant-based antimicrobial drugs that are more reliable and non-lethal to human health at increased dosage. The antibacterial and antifungal potential of *Geophila obvallata* extracts were tested on clinical isolates (*Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes, Cryptococcus neoformans, Candida albicans* and *Aspergillus fumigatus*) using standard techniques. The zones of inhibition were shown to increase with increasing concentrations of the extracts. Inhibition was higher in Gram positive bacteria (9.10 to 31.00mm in 40mg/mL concentration) than Gram negative bacteria (3.50 to 27.00mm in 40mg/mL concentration), while the fungal isolates had the least zones of inhibition (2.83 to 25.00mm in 40mg/ml concentration). The minimum inhibitory concentrations (MIC) were lowest in the methanol extract than aqueous extract. Simillarly, MIC for bacteria (*Bacillus subtilis*) and fungi (*Aspergillus fumigatus*) were 0.3 and 2.0mg/mL respectively. Methanol extract had higher antibacterial and antifungal effect than aqueous extract. Ciprofloxacin, used as control for bacteria had the highest inhibitory activity (33.67mm) when compared to that of the highest concentration of plant extracts administered. Also, ketoconazole gave the highest zones of inhibition (32.33mm) on the fungi isolates compared to those of the extracts. The performance of the methanol extract of 40mg/mL of *Geophila obvallata* in the inhibition of *Bacillus subtilis* was not significantly different from that of Ciprofloxacin. The findings in this study therefore validate the antimicrobial effect of *Geophila obvallata* leaf extracts as well as its possible application in medicine.

1. Introduction

The increasing occurrence of multi-drug resistant strains of infectious bacteria and fungi is a major threat to public health worldwide. The distribution of these resistant strains in the population has rendered most antibiotics and major last – resort drugs ineffective in combating infections or diseases **(Mandal *et al.*, 2009; Bhatia and Narain, 2010)**. Microbial resistance to antimicrobial agents increases the chance of hospitalization and in most cases death **(Winstanley, 1997)**. In recent times, the indiscriminate use of antibiotics in most developing and developed countries of the world has led to reports of drug resistance to pathogenic microbes in humans **(Gupta *et al.*, 2017)**. As a result of this rapid spread and development of resistance microbial strains, the need for the discovery of novel antimicrobial agents that are effective alternatives to synthetic and hazardous chemical drugs cannot be over-emphasised. This has led to the exploration of plants and plant based products as novel remedies in the treatment of disease **(Basualdo *et al.*, 2007; Mandal *et al.*, 2010)**. Plant based antimicrobials (extracts) have been recognised as valuable alternatives in the treatment of most microbial infections compared to synthetic antibiotics due to their stability, non-lethality and efficacy **(Iwu *et al.*, 1999; Alam *et al.*, 2009)**. Currently, more than 30% of the antimicrobial agents produced in modern pharmacology are obtained directly or indirectly from plants and their extracts **(Murugesan *et al.*, 2011)**. The World Health Organization (WHO) has affirmed medicinal plants as the best source of drugs **(WHO, 2002)**.

Geophila obvallata (GO) (Schumach.) Didr. is a perennial, carpet forming, creeping herb usually wooden at the base **(Reeve, 1997)**. It possesses broadly ovate, dark green, hairless leaves and roots 6-9 meters long. It is mostly found in shady places under the cover of trees with few white flowered clusters **(Flora *et al.*, 2010)**. This plant species belongs to the taxonomic family *Rubiaceae* **(Robbrecht and Manen, 2006)** where there are over twenty (20) different species of which GO is the most commonly found in West tropical rainforests **(Owu *et al.*, 2012)**. Previous studies have shown that this plant possesses antimicrobial potentials. This is as a result of the rich secondary metabolites such as tannins, alkaloids, phenols, and flavonoids biosynthesised by the plant **(Iserhienrhien and Okolie, 2018)**. In West tropical Africa (Nigeria), the leaves of this neglected and greatly under- exploited plant has an array of health benefits

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(folklore) translating from its use by the locals in the treatment of sores from guinea worm infections, diarrhea, infertility and hypertension. The plant leaves have been screened for phytochemicals and reported to possess antioxidant activity **(Iserhienrhien and Okolie, 2018)**. Previous toxicological studies by **Iserhienrhien and Okolie, (2020)** revealed that the hydro-methanolic extract of this plant is non-lethal at a dose of 100 mg/kg. Also, the antifungal **(Portillo *et al.*, 2001**), antibacterial (**Rao *et al.*, 2017**) and anticholinesterase (**Dash and Sahoo, 2017**) effects have also been studied in the leaves of a similar genus, *Geophila repens*. However, there is paucity in scientific data regarding the efficacy of the plant as an antimicrobial agent. Considering the vast potential of this plant, this study investigated the antimicrobial effect of *Geophila obvallata* (Schumach) Didr. leaf extracts against some medically important bacteria and fungi.

2. Material and methods

2.1 Plant collection

Geophila obvallata leaves were obtained from the *Gelegele* forest and identified by Dr. H.A. Akinnibosun (taxonomist), at the life science Department, University of Benin, Nigeria, with voucher number UBHa 0312 assigned to it.

2.2 Plant extraction

The plant leaves were manually detached, cleaned and air-dried for 30 days. Dried plant parts were grinded using an electric mill GM 150 (Retch, Germany) until a smooth texture was obtained, and was later weighed and packaged. The pulverized leaves (200g) were extracted in the soxhlet extractor using methanol (70%) (1:10 w/v) **(Iserhienrhien *et al.*, 2018)** followed by homogenization and continuous agitation for 3 days. Whatman's filter paper (No. 1) was used to separate the homogenate from the filtrate, after which it was concentrated using a rotary vacuum evaporator R-250V (Buchi, UK) until the methanol evaporated at 40°C **(Edeoga *et al.*, 2005)**. The same procedure was adopted for the aqueous extract. Both extracts were stored at 4°C for analysis. The methanol and aqueous yield (%) were 4.85 and 2.57 respectively, calculated using the formula below:

$$\textbf{\textit{Percentage Yield}}\ (\%) \frac{\textbf{\textit{= Dry weight of extract}}}{\textbf{\textit{Dry weight of plant material}}} \textbf{\textit{x}}\ \mathbf{100}$$

2.3 Preliminary phytochemical profiling

The bioactive constituents present in the methanol and aqueous extracts of *Geophila obvallata* were previously screened for flavonoids, polyphenols, alkaloids, tannins, saponins, steroids, terpenoids and cardiac glycosides **(Iserhienrhien and Okolie, 2018)**, according to the methods described by **Sofowora, (1993).**

2.4 Microbial culture

A total of 9 clinical isolates were obtained from stock cultures sourced from the Department of Pharmaceutical Microbiology laboratory, Faculty of Pharmacy, University of Benin, Nigeria. The referenced microorganisms include: *S. aureus*, *K. pneumoniae, P. aeruginosa, E. coli*, *B. subtilis*, *S. pyogenes, C. neoformans, C. albicans* and *A. fumigatus.* Prior to use, the test microorganisms were authenticated and sub-cultured from stock into sterile nutrient broth (for bacteria) and Sabouraud Dextrose broth (for fungi) and incubated overnight at 38^{o}C for 24hours for bacteria and at room temperature (25±2^{o}C) for 72hours for fungi. After incubation, overnight broth culture was adjusted to 0.5 McFarland standard to give an inoculum size of approximately 1.5 x10^{8} cfu/mL and a further one in hundred serial dilution (1:100) using normal saline solution to yield approximately 10^{6} cfu/mL.

2.5 Antimicrobial assay of plant extracts

Antimicrobial susceptibility test was carried out using agar-well diffusion method **(Vinothkumar *et al.*, 2010)**. Wells of 7 mm in diameter were made into previously seeded Mueller Hinton / Sabouraud agar plates using a flamed (sterile) cork borer. Prior to seeding, the turbidity of the overnight broth culture of each isolates were adjusted to match 0.5 McFarland turbidity standard and diluted (1:100) to give approximately 1x10^{6} cfu/mL microbial suspension. Sterile swab sticks were then dipped into the standardized microbial suspension and gently streaked on the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. This was achieved by rotating the plate 90 degrees followed by 45 degrees with continuous streaking, and finally by streaking round the diameter of the agar. The 7 mm wells were filled with different volumes of the stock concentration of the extracts corresponding to 40, 30, 20 and 10 mg/mL concentrations. The same quantity of sterilized distill water, standard drugs (1 µg/mL of Ciprofloxacin for bacteria plates and 10 µg/mL of Ketoconazole for fungal plates) served as controls setup for the experiment. The plates were left to stand for 1 hour on a work-bench to allow diffusion of the extracts before incubating bacterial plates overnight at 37ºC and the fungal plates at room temperature (25±2^{o}C) for 72 hours. The diameter of clear zone was observed and measured in mm (millimeters). The experiments including controls were done in triplicates and the mean zones of inhibitions calculated.

2.6 GC–MS analysis of methanol extract

The analysis was done with GCMS-QP2010SE (Shimadzu, Japan). Helium served as carrier gas at a column flow rate of 3.22mL/min with a split ratio of 5:1. 1 µl of sample was injected into the column at an injection temperature of 250.00 °C and detection temperature was 290 °C. The oven starts at 60 °C while the hold time is for 2 mins, this is maintained at 10 °C /min to 290 °C without holding. Total running time was 21 mins. The detector was a flame ionization detector (FID). Detected peaks were compared with NIST 11s, library to verify the names and molecular weights of the samples constituents.

2.7 Data analyses

Data collected during this study were analyzed using MINITAB 16 and represented as mean ± standard deviation. Fishers Pairwise Comparison (FPC) of ANOVA at a significance level of $P < 0.05$ was used to test the variability in susceptibility of the microorganisms towards the extractives.

3. Results

The *in vitro* experiment conducted showed that the best antimicrobial effect on *K. pneumonia*, *P. aeruginosa*, *E. coli*, *S. aureus* and *S. pyogenes* were seen in the methanol (30 and 40 mg/mL) extract group when compared with the aqueous extract group. The zones of inhibition on culture plate of the isolates at 30 and 40 mg/mL of the methanol extract were measured as 25.67 and 27.00 mm, respectively for *K. pneumonia*, 21.33 and 24.00 mm, respectively for *P. aeruginosa*, 25.67 and 26.00 mm, respectively for *E. coli*, 24.33 and 27.33 mm, respectively for *S. aureus*, 27.67 and 29.33 mm, respectively for *S. pyogenes* (**Table 1**). Indicating that both concentrations of the methanol extracts of *Geophila obvallata* performed very well in limiting the growth

and metabolic activities of the isolates compared to the aqueous extract and the control setup for the experiment (DH_2O). Ciprofloxacin (CIP) a synthetic drug, performed better than all other groups (Table 1). However, the performance of the methanol extract at 40 mg/mL of *Geophila obvallata* in the inhibition of the microbe *B. subtilis* was not significantly different ($P > 0.05$) from that of the synthetic drug Ciprofloxacin. The methanol extract of *Geophila obvallata* had the best antifungal effect on all the fungal isolates (*C. neoformans*, *C. albicans*, and *A. fumigatus*), especially at 40 mg/mL of the administered treatment when compared with other groups. The group treated with 40 mg/mL methanol extract significantly ($P < 0.05$) inhibited the radial mycelial growth of *C. albicans* and *A. fumigatus* when compared with the aqueous extract group. Also, the group treated with 40 mg/mL of the methanol extract produced similar effects on the pathogen *C. albicans* (31.67 mm clear zone of inhibition) and *A. fumigatus* (30.15 mm clear zone of inhibition) just like that of the synthetic drug (Ketoconazole), other treatment concentrations and water extracted botanicals had appreciable control of the fungal isolates (**Table 2**).

Table 1 Inhibition zone diameter of *Geophila obvallata* extracts against test bacterial isolates

	Zones of Inhibition (mm)									
	Aqueous extracts				Methanol extracts					
Organisms	**10mg/ml**	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**10 mg/ml**	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**CIP(1µg/ml)**	**DH_2O**
K. pneumoniae	6.00±1.00^{f}	11.33±1.53^{e}	15.67±2.52^{d}	19.67±1.53^{c}	10.17±1.76^{e}	20.00±2.00^{c}	25.67±1.16^{b}	27.00±1.73^{b}	33.00±1.00^{a}	0.00±0.00^{g}
P. aeruginosa	3.50±1.50^{g}	6.00±1.80^{fg}	10.33±2.52^{de}	13.33±2.52^{cd}	8.00±2.00^{ef}	16.67±2.08^{c}	21.33±2.52^{b}	24.00±2.00^{b}	31.00±1.73^{a}	0.00±0.00^{h}
E. coli	8.00±1.00^{f}	11.33±0.58^{e}	17.33±1.53^{d}	20.67±0.58^{c}	11.33±0.58^{e}	21.33±1.53^{c}	25.67±1.16^{b}	26.00±1.00^{b}	32.33±3.22^{a}	0.00±0.00^{g}
S. aureus	9.17±1.04^{e}	11.33±2.31^{e}	17.00±2.00^{d}	20.67±1.53^{c}	11.00±1.00^{e}	21.00±1.73^{c}	24.33±3.06^{b}	27.33±0.58^{b}	32.67±2.52^{a}	0.00±0.00^{f}
B. subtilis	10.67±0.58^{e}	18.33±1.53^{d}	19.67±1.53^{d}	23.00±2.00^{c}	13.33±3.06^{e}	25.00±1.73^{c}	29.00±1.00^{b}	31.00±1.00^{a}	33.67±2.08^{a}	0.00±0.00^{f}
S. pyogenes	9.67±1.53^{f}	15.00±1.00^{e}	19.00±1.73^{d}	21.67±2.08^{c}	11.67±2.08^{f}	23.33±1.53^{c}	27.67±0.58^{b}	29.33±1.53^{b}	32.33±0.58^{a}	0.00±0.00^{g}

Means with the same alphabets across the row are not significantly different ($P > 0.05$) using Fishers Pairwise Comparison (FPC).
Data collected are represented as mean ± standard deviation

Table 2 Inhibition zone diameter of *Geophila obvallata* extracts against test fungal isolates

	Zones of Inhibition (mm)								
	Aqueous extracts				Methanol extracts				
Organisms	**10mg/ml**	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**10 mg/ml**	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**KET (10µg/ml)**
C. neoformans	3.83±1.60^{h}	7.83±1.26^{gh}	11.67±1.53^{ef}	12.00±2.00^{e}	8.50±1.80^{fg}	18.00±2.65^{d}	21.33±1.53^{c}	25.00±2.00^{b}	32.33±1.53^{a}
C. albicans	2.83±1.26^{g}	5.00±3.04^{fg}	9.67±2.08^{de}	10.67±0.58^{d}	7.50±2.29^{ef}	15.00±1.00^{c}	21.33±1.53^{b}	31.67±1.53^{a}	31.70±1.53^{a}
A. fumigatus	3.83±0.76^{g}	7.83±1.26^{f}	11.67±1.53^{e}	12.00±2.00^{e}	8.17±0.76^{f}	15.67±1.16^{d}	21.67±2.89^{c}	30.15±1.16^{a}	32.00±1.73^{a}

Means with the same alphabets across the row are not significantly different ($P > 0.05$) using Fishers Pairwise Comparison (FPC).
Data collected are represented as mean ± standard deviation

The GC-MS results revealed twenty-six peaks of phytocompounds present in the methanol extract. Some of these include: Phenols (0.12%), Oxalic acid, isobutyl nonyl ester (0.08%), N-(1H-Tetrazol-5-yl) benzamide (0.08%), cis-Undec-4-enal, Hexadecanoic acid, methyl ester (0.35%), 1-Heptadec-1-ynyl-cyclopentanol (0.06%), Undecanoic acid, 10-methyl-, methyl ester (0.21%), Hydrofol Acid, 12-methyl-, methyl ester (0.31%), 8-Hexadecenal, 14-methyl-, (Z)-(0.07%), Dimethoxybicyclo (0.98%), 8-Hexadecenal, 14-methyl-, (Z)-(0.21%), 11-Oxa-dispiro, methyl ester, (Z)- (0.36%), Hexadecanoic acid, methyl ester (11.06%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (%),11-Octadecenoic acid, methyl ester (13.16%), Methyl stearate (31.49%), cis-Vaccenic acid (4.18%), Oleic Acid (2.87%), 9,12-Octadecadienoic acid (Z,Z)- (4.18%), methyl ester (2.87%)(, 7-Hexadecenoic acid (2.45%), methyl ester, (Z)- (2.17%), Methyl 9-eicosenoate (3.25%), N-(1H-Tetraazol-5-yl)benzamide, (2.16%), cis-9-Hexadecenal (2.16%), Methyl 16-hydroxy-hexadecanoate (3.31%)respectively (Figure 1; Table 3). Table 3 shows the bioactivities of the reported phyto-compounds.

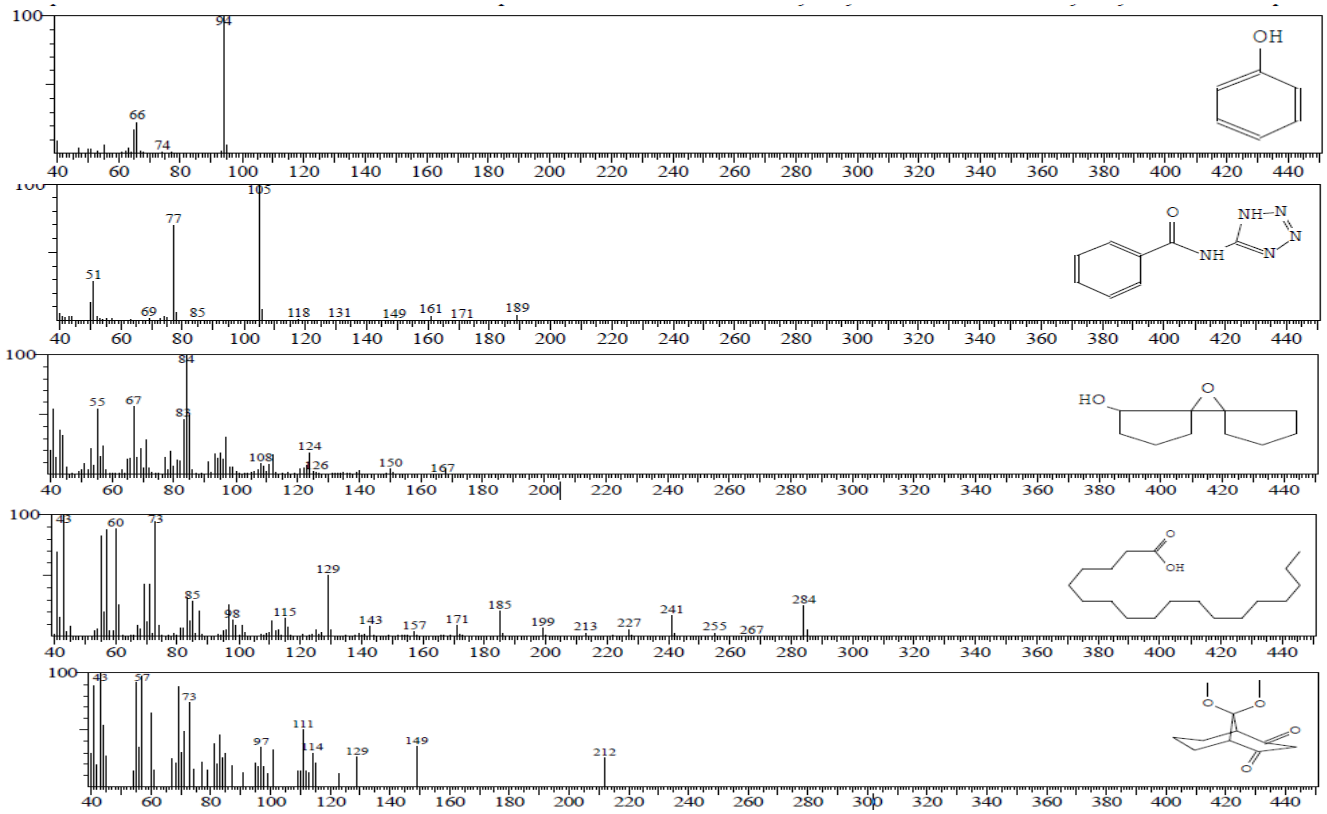

Figure 1 Mass spectra of some of the identified phytocompounds (phenol, N-(1H-Tetraazol-5-yl)benzamide, 11-Oxa-dispiro, Hydrofol Acid, Dimethoxybicyclo, respectively).

Table 3 Bioactivities of the reported phyto-compounds in the methanol extract of *Geophila obvallata*

S/N	Compounds	Bio-activity	References
1	Phenol	Antioxidant, Anticancer, Antimicrobial	**(Bakkalbasi and Mentesz, 2008)**
2	Oxalic acid, isobutyl nonyl ester	-	-
3	N-(1H-Tetrazol-5-yl)benzamide	Antipsychotic action	**(Roessner and Sabine, 2011)**
4	cis-Undec-4-enal	-	-
5	Undecanoic acid, 10-methyl-, methyl ester	Biomarker for facultative aerobes	**(Maribel *et al.*, 2007)**
6	1-Heptadec-1-ynyl-cyclopentanol	Antihelminthic, Ophthalmic action	**(Macedo *et al.*, 2019)**
7	Hydrofol Acid	Antimicrobial, antifungal	**(Carson *et al.*, 2006)**
8	8-Hexadecenal, 14-methyl-, (Z)-	Anticardiovascular, Antiviral	‘
9	2,4-Undecadienol	Fragrance agent	‘
10	8-Hexadecenal, 14-methyl-, (Z)-	Anticardiovascular	‘
11	Dimethoxybicyclo	Antimicrobial, Antiinflammatory	**(Rajeswari *et al.*, 2012)**
12	11-Oxa-dispiro	Antimicrobial, Antioxidant	**(Rajeswari *et al.*, 2012)**
13	7-Hexadecenoic acid, methyl ester, (Z)-	Antioxidant, hypocholesterolemic, nematicide	**(Moonjit and Himaja, 2014)**
14	N-(1H-Tetraazol-5-yl)benzamide	Antioxidant, antimicrobial	**(Moonjit and Himaja. 2014)**
15	n-Hexadecanoic acid	Antioxidant hypocholesterolemic, nematicide	**(Moonjit and Himaja, 2014)**
16	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anticardiovascular	‘
17	11-Octadecenoic acid, methyl ester	Anticardiovascular	‘
18	Methyl stearate	-	**(Ann *et al.,* 2013)**
19	cis-Vaccenic acid	Anticancer	**(Sales-Campo *et al.,* 2013)**
20	Oleic Acid	Anticardiovascular, Anti-inflammatory	**(Sales-Campo *et al.,* 2013)**
21	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Lubricant, Antiandrogenic and Flavor	**(Moonjit and Himaja, 2014)**
22	7-Hexadecenoic acid, methyl ester, (Z)-	Antiandrogenic and Flavor	**(Moonjit and Himaja, 2014)**
23	Methyl 9-eicosenoate	-	‘
24	Methyl 18-methylnonadecanoate	-	-
25	cis-9-Hexadecenal	Anti-inflammatory	**(Ann *et al.,* 2013)**
26	Methyl 16-hydroxy-hexadecanoate	Antioxidant	**(Sales-Campo *et al.,* 2013)**

4. Discussion

The rational use of medicinal plants in tackling multi-drug resistant strains of infectious bacteria and fungi is becoming invaluable. Currently, there are rising trends in the use of natural products (extractives) of plants as indispensable tools in combating the emergence of microbial resistance. The various bioactive compounds inherent in these plants can deploy numerous mechanisms in minimizing antimicrobial resistance thereby increasing their antibacterial or antifungal efficacy **(Galeane *et al*., 2017).**

Assessing the antibacterial and antifungal activities of the *Geophila obvallata* leaf extracts employed in this study suggests its potential use in the management of patients with multi-drug resistance to pathogenic microbes. The preliminary qualitative and quantitative phytochemical screening of the methanol and aqueous leaf extracts of *Geophila obvallata* revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, reducing sugars, steroids, tannins and terpenoids **(Iserhienrhien and Okolie, 2018).** These secondary metabolites are well recognised for their antioxidant, antifungal and antibacterial effects in line with previous findings from other studies **(Tan *et al*., 2008; Bianco *et al*., 2015; Kouadri, 2018)** and have been considered as a treatment regimen for numerous disease conditions.

In this study, the crude aqueous and methanol extracts of *Geophila obvallata* showed appreciable inhibitory activity against both gram negative bacteria (*K. pneumonia*, *P. aeruginosa*, *E. coli*) and gram positive bacteria (*S. aureus* and *S. pyogenes, B. subtilis*) especially at higher doses. However, the methanol extracts at a dose of (40 mg/mL) showed the best growth inhibitory performance, especially against the gram positive microbe *B. subtilis,* which was not significantly different from that of the control (Ciprofloxacin). The antibacterial effect highlighted in the crude methanol extract may be due to (1) the ability of the methanol solvent to permeate the cell walls of the plant thereby liberating bioactive constituents in the leaf such as flavonoids and polyphenolic compounds with antibacterial properties **(Mohammed and Omer, 2015)**, (2) a synergistic effect of the secondary metabolites present in the milieu at that dosage (**Dowe *et al*., 2016)**. This is in line with the findings of **Ho *et al*. (2010)** and **Vijayan *et al*. (2013)** that had similar results when they determined the antimicrobial effects of *Orthosiphon stamineus* methanol leaf extract. Also, it was generally observed that the gram positive bacteria were more susceptible to the inhibitory action of the methanol extract than the gram-negatives. This is probably due to the presence of inhibitory lipopolysaccharide components in the mycobacterial cell wall of the gram negatives, which limit the concentration and permeability of antibacterial agents. This finding correlates with the report of **Zeinab *et al*. (2020)**, that gram-positive bacteria are usually more susceptible to antimicrobial agents. According to the findings of **Kumar *et al.* (2011),** Gram negative bacteria are less susceptible to antimicrobial agents than Gram positives, as a result of the phospholipidic composition of their bacterial membrane with an exclusion limit of 600 Da.

Simillarly, the methanol extract also showed significant growth inhibitory properties against the radial mycelia growth of fungal isolates (*C. neoformans*, *C. albicans*, and *A. fumigatus*) especially at higher doses (40 mg/mL) compared to the aqueous extract. Also, the group treated with 40 mg/mL of the methanol extract produced similar effects like the synthetic drug (Ketoconazole) on the pathogens *C. albicans* (31.67 mm clear zone of inhibition) and *A. fumigatus* (30.15 mm clear zone of inhibition). This is in correspondence with previously published results by **Neharkar and Laware, (2013)** revealing the inhibitory effects of methanol extracts on fungal activity. This can also be linked to the high concentration of alkaloids reported in earlier research by **Iserhienrhien and Okolie, (2018)** on the qualitative phytochemicals present in *Geophila obvallata* methanol leaf extracts.

The MIC test was used to determine the lowest concentration of the extract that would inhibit the visible growth of the clinical isolates after overnight incubation **(Fabry *et al*., 1998)**. The crude aqueous and methanol extract both showed significant activity against gram-positive and gram-negative clinical isolates with MIC values ranging from 0.3 mg/mL to 10.00 mg/mL. However, the methanol extract had the best activity on 5 out of the 9 clinical isolates [*S. aureus*, *E. coli*, *B. subtilis*, *S. pyogenes* and *A. fumigatus*] with MICs ranging from 0.3-2 mg/mL compared to the aqueous extract. The most promising antimicrobial effect was observed against gram-positive bacteria [*S. aureus*, *B. subtilis* and *S. pyogenes*] compared with the gram-negatives. The methanol extract demonstrated the highest antibacterial activity against *B. subtilis* with a MIC of 0.3 mg/mL and the best antifungal activity against *A. fumigatus* with a MIC of 2 mg/mL. Potent antimicrobial agents are known to possess MIC values equal to or lower than 0.50 mg/ml **(Sartoratto *et al*., 2004).** This is a clear indication of exceptional antimicrobial activity **(Duarte *et al*., 2005)** and is in line with earlier antimicrobial susceptibility tests (agar-well diffusion method) carried out in this study. The differences in bacterial vulnerability observed in this study are in agreement with previous findings **(Wink, 2012)** and could be due to (1) diversity in microbial resistance and (2) phytochemical constituents (tannins, alkaloids, terpenoids and flavonoids) present in the active methanol extract with a broad spectrum of antimicrobial action.

The GC–MS chromatogram showed twenty-six peaks of phytocompounds present in the methanol extract with known biological activities while the others have not been linked to any known biological function. The mass spectra of these compounds were matched with the NIST database which helped to elucidate the corresponding peaks. Some of the bioactive compounds found in the extract and their roles according to literature include: phenols which are reported to possess antioxidant, anticancer and antimicrobial effects **(Bakkalbasi and Mentesz, 2008)**, N-(1H-Tetrazol-5-yl) benzamide which possess antipsychotic and anxiolytic action **(Roessner and Sabine, 2011),** Hydrofol Acid and 11-Oxa-dispiro, possess antioxidant, hypocholesterolemic effects (Moonjit and Himaja, 2014) while Dimethoxybicyclo is known for its antihelminthic, antimicrobial and ophthalmic action **(Macedo *et al*., 2019).**

5. Conclusion

The crude aqueous and methanol leaf extracts of *Geophila obvallata* showed exceptional concentration-dependent antibacterial and antifungal activity against selected clinical isolates. However, the methanol extract at a dose of 40 mg/mL showed the best antimicrobial effect against the tested isolates. These findings confirm the antimicrobial potency of the plant extract and give credence to the orthodox use of the medicinal plant as a suitable alternative to synthetic antimicrobial agents.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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