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Cytotoxic, Antioxidant and Antimicrobial Effects of Root Bark Extracts of *Gardenia angustifolia* (Cape jasmine)

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Abstract

Background: Empirical knowledge on the cautious application, safety and efficacy of medicinal plants is limited especially in developing countries around the world.

Objective: This study is aimed at investigating the cytotoxic, antioxidant and antimicrobial potential of *Gardenia angustifolia* root bark with a comparative objective on ethyl acetate and methanol extracts.

Methods: Compound elucidation was done using Gas Chromatography-Mass Spectrometry (GC-MS) analysis and Thin Layer Chromatography (TLC). Cytotoxic evaluation was conducted using *Artemia salina* and Human Embryonic Kidney-293 (HEK-293) cell line. Antioxidant assay was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) models. The agar-well diffusion method was employed for antimicrobial assay.

Results: Both crude extracts constitute thirty-five (35) bioactive components each by GC-MS analysis. The extracts showed a non-toxic effect toward brine-shrimp and HEK-293 cell line at a moderate concentration and displayed significant antimicrobial effects on some strains of microorganisms. Furthermore, the *in vitro* antioxidant analysis conducted using the DPPH and FRAP models revealed a concentration dependent antioxidant activity of the extracts.

Conclusion: The findings indicate that ethyl acetate extract of *Gardenia angustifolia* root bark is a better radical and microbial growth inhibitor, attesting to its antioxidant nature and as a promising target in antimicrobial drug design.

Keywords: Cytotoxic, Antioxidant, Antimicrobial, *Gardenia angustifolia*, Chromatography

Introduction

The use of traditional herbal medicine in the treatment of ailments is prevalent in many countries around the world especially in Asia, Africa and South America. Scientific evidence has shown that active principles called phytochemicals are responsible for their acclaimed therapeutic potentials [1]. There is increased application of herbal medicine due to the post-administration adverse side effects and economic burden of conventional drugs, which when combined with other factors, could contribute to a relatively ineffective regimen. This necessitates a shift in demand for therapeutic agents of natural origin which are considered less toxic and more pharmacology potent against free radical incurring diseases [2].

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Reactive oxygen species (ROS) can cause multiple organ damage and cell death. Additionally, they are known to be associated with the genesis of numerous illnesses which include neurological disorders, diabetes and cancer. There are antioxidants present in the human biological system which help to counter the effects of these reactive oxygen species. Glutathione peroxidase, catalase and superoxide dismutase are some examples of free radical scavengers. There has been documented evidences of medicinal plants with the ability to upregulate the activities of these antioxidants in experimental animals [3]. Furthermore, the successful treatment of infectious diseases is being greatly impaired by the growing resistance of pathogens to conventional and available antibiotics. Therefore, alternative therapy is being sought for in natural products in order to tackle the challenge of antibiotic resistance [4].

Gardenia angustifolia (*G. angustifolia*) commonly known as cape jasmine is a flourishing, flowering plant that, similar to the coffee plant, belongs to a large family known as Rubiaceae. *Gardenia* genus is well distributed in Africa, Asia, Madagascar, Pacific Island and Australia. It is predominantly located in the central region of Nigeria, with approximately 200 identified species [5]. Traditional medicine uses multiple parts of *G. angustifolia* to treat a variety of illnesses. Diverse biological impacts, including antioxidant, antipyretic, anti-inflammatory, and hypoglycaemic effects have been noted. Despite the acclaimed success in its healing ability, there is lack of scientific validation [6]. A dearth of scientific consideration on the pharmacological action of the root bark segment and to a larger extent, the *G. angustifolia* plant as a whole, prompted the choice of the current study. Therefore, this study evaluates the cytotoxic, antioxidant and antimicrobial effects of methanol and ethyl acetate root bark extracts of *G. angustifolia*.

Materials and Methods

Chemicals: All the chemicals and solvents purchased were of analytical grade.

Collection and extraction of plant material

The roots of *G. angustifolia* were collected from a local farm in Abocho, Dekina Local Government Area of Kogi State, Nigeria. The roots were washed and cleaned. They were cut into small pieces, air-dried, ground to a fine powder and stored in an airtight container prior to extraction. The powdered sample (1kg) was extracted by cold maceration method as described by Abdullahi and Mainul [7]. The sample was soaked in methanol and ethyl acetate solvents separately for 72 hours. After 72 hours, the mixture was filtered and evaporated to dryness using a rotary evaporator at 40 ^{0}C. The crude extract was stored in the refrigerator at 4 ^{0}C and used for further analysis. The yield of the extract was calculated and expressed in percentage using the formula below:

$$\% \text{ Yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of powdered sample}} \text{ x } 100$$

Thin layer chromatography (TLC)

The method described by Melvin [8] was adopted. Aluminum packed silica for TLC was used for thin layer chromatography. Briefly, methanol and ethyl acetate extracts of *G. angustifolia* root bark were spotted at the origin which was 2cm from the edge of the TLC sheet. The chromatogram was developed in the suitable solvent mixture of Toluene: chloroform: Acetone (8:5:7). The TLC plate was air dried at room temperature and the spots were visualized by placing the TLC plates in an iodine chamber. The Retention factor (R_f) value was estimated using the following equation.

$$\text{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed using a GC (6890 N agilent technologies) coupled with mass selective detector (Agilent 5975B) and a GC capillary column (30m x 320µm x 0.25µm thickness). The retention time, peak area (corresponding to the proportions of each compound) and chromatogram of the compounds in the extracts were identified and classified by comparison in accordance to the standard compounds in the database of National Institute of Standards and Technology (NIST).

Brine shrimp cytotoxicity assay

This was conducted using hatched brine shrimp (*Artemia salina*) larvae as described by Arogba [9]. 1g each of the crude extracts was dissolved in 50mL stock solution from which serial dilutions were made. Following incubation, 10 brine shrimp larvae were counted and transferred to different vials using a pasteur pipette by optimizing volume to 5mL with artificial sea water. A constant volume of 500µL of decreasing varied concentrations (2000, 1000, 500, 250, 125µg/mL) of a sample type or the reference potassium dichromate was added to the vials containing the shrimps. After 24 h, the dead larvae were counted for the determination of percentage lethality. The lethality inflicted by the extracts is defined by the absence of controlled motion of the Nauplii for a duration of 30 seconds.

The percentage lethality of the shrimp for each concentration was calculated as:

$$\% \text{ Lethality} = \frac{\text{Number of dead shrimps}}{\text{Number of surviving shrimps in control}} \text{ x } 100$$

The LC_{50} value of the extract was determined from linear regression curve of percentage lethality plotted against Log (concentration) as described by Asonugha *et al.* [10].

In vitro cytotoxicity assay on embryonic kidney (HEK293) cells

The cytotoxic effect of the crude extracts of *G. angustifolia* root bark was evaluated using 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) based assay. MTT was incubated on HEK 293 cell line at a seeding density of approximately 3×10^4 cells/well, using a 96-well micro plates reader. Assessment was in accordance to the method described by Mosmann [11] with slight modification. Concentration of the plant samples ranging from 25 µg/mL to 200 µg/mL and butylated hydroxytoluene (BHT) 100 µg/mL was incubated with HEK 293 cell line under the experimental condition comprising of 5% CO_2 at 37 °C for an observation period of 48 h. Previous medium was cautiously removed, while addition of the new medium containing 100 µg/mL, MTT solution in PBS to the wells of the micro plates reader before final incubation. The solution containing MTT was incubated simultaneously with the cells at 37 °C for a period of 4 h. 200 µL of dimethylsulfoxide (DMSO) was added to the well to dissolve salt (formazan) while removing the solution containing MTT and the medium. Absorbance was read at a wavelength of 570 nm on 96 microplate reader. Percentage cell viability was calculated using the formula below:

$$\% \, Cell \; viability = \frac{Absorbance \; of \; sample}{Absorbance \; of \; control} \times 100$$

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay

The assay was conducted according to the method described by Blois [12]. 1mL of 0.1mM solution of DPPH in methanol was mixed with 5µL of the extracts each at different concentrations (31.25 – 1000µg/mL). The absorbance was measured against a blank at 517nm using spectrophotometer.

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay

Ferric reduction effect of *G. angustifolia* root extracts was evaluated following the procedure outlined by Benzie and Strain [13] with slight modification. At low pH, in the presence of tripyridyltriazine, ferric-tripyridyltriazine (Fe^{3+} - TPTZ) complex is reduced to ferrous (Fe^{2+} - TPTZ) form with the formation of an intense blue colour with a characteristic absorption peak at 593nm. Ascorbic acid was used as a standard compound to create a calibration curve. Results were expressed in µg ascorbic acid equivalents (AAE)/mL of extract using the equation Y = 0.0013x + 0.0649 (Figure 7).

Determination of antimicrobial activity of root bark extracts of G. angustifolia

The antibacterial and antifungal activity of the extracts were determined using the agar-well diffusion method as described by Russell and Fur [14]; Irobi *et al*. [15]. The reference bacteria were subcultured into nutrient broth while the fungal isolates were cultured on the malt extract agar medium. The antimicrobial effect of the extracts were determined by the zones of inhibition procedure using streptomycin and nystatin as reference drugs for the antibacterial and antifungal studies, respectively. The minimum inhibitory, bactericidal and fungicidal concentrations (MIC, MBC and MFC) of the extracts were also estimated comparatively.

Statistical Analysis

Results were expressed as mean ± SEM of triplicate values. The charts were obtained and IC_{50} values were determined from the dose-response curve using Graph Pad Prism Windows 10.2.0 (392) (Graph Pad Software, Inc) programme.

Results

Extraction yield

The methanol and ethyl acetate extract of *G. angustifolia* root-bark has a percentage estimation of 14.3% and 9.6%, respectively. The methanol extract presents moderately high yields compared to the ethyl acetate extract (Table 1).

Table 1:- Solvent extract yield and physical characteristics of *G. angustifolia* root bark extracts.

Extract	Texture	Color	Percentage (%)
Methanol	Sticky solid	Dark brown	14.3
Ethyl acetate	Semi solid	Light brown	9.6

TLC and Chemical composition

The ethyl acetate extract of *G. angustifolia* root-bark showed the presence of six (6) bands while the methanol extract revealed the presence of four (4) bands. The R_f values 0.75, 0.61, 0.51, 0.44, 0.33 and 0.18 were estimated for ethyl acetate crude extract and 0.86, 0.80, 0.59 and 0.47 corresponds to methanol crude extract (Figure 1). Figures 2 & 3 elucidate the total ion chromatogram of the extracts. Furthermore, a total of thirty-five (35) compounds each was elucidated by the GC-MS analysis (Tables 2 & 3).

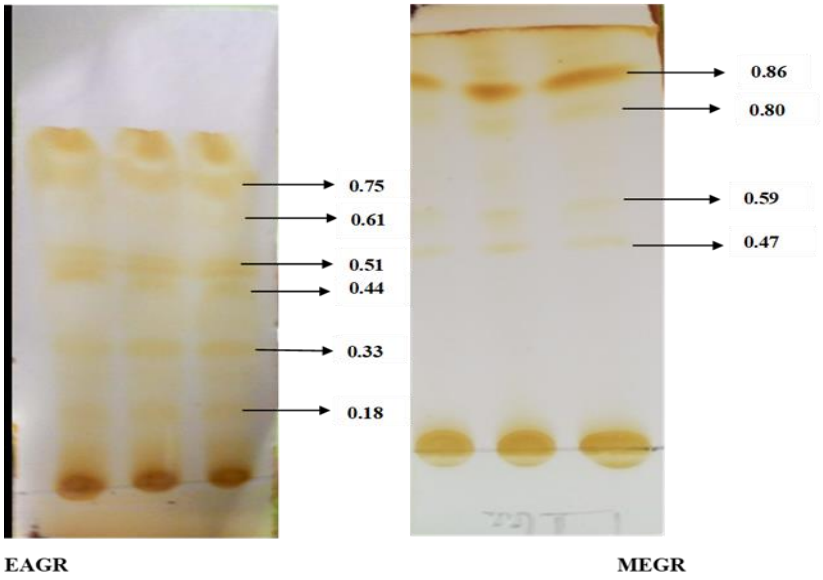

Figure 1:- Retention factor (R_f) values of Ethyl Acetate Extract of *G. angustifolia* Root Bark (EAGR) and Methanol Extract of *G. angustifolia* Root Bark (MEGR) with various bands of separation after treatment in iodine vapor.

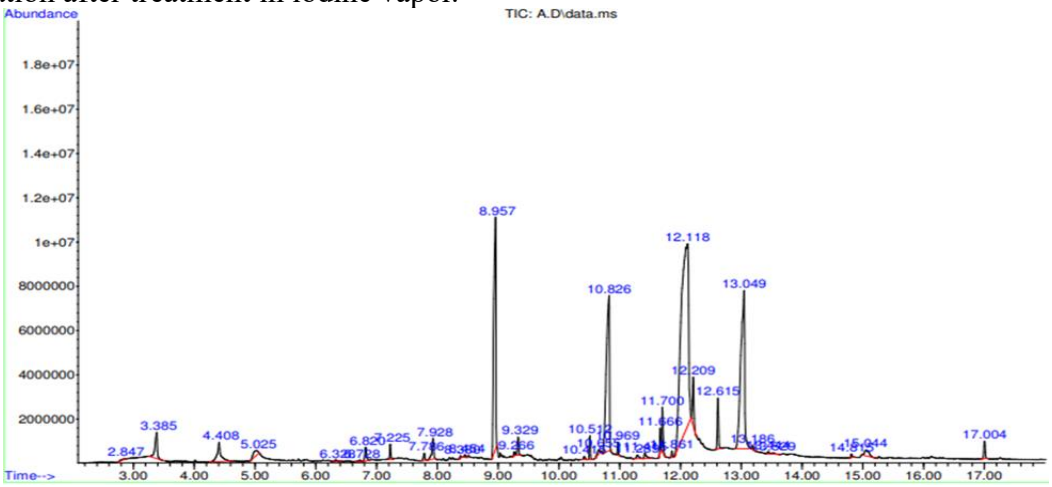

Figure 2:- Total ion chromatogram (TIC) of ethyl acetate extract of *G. angustifolia* root bark.

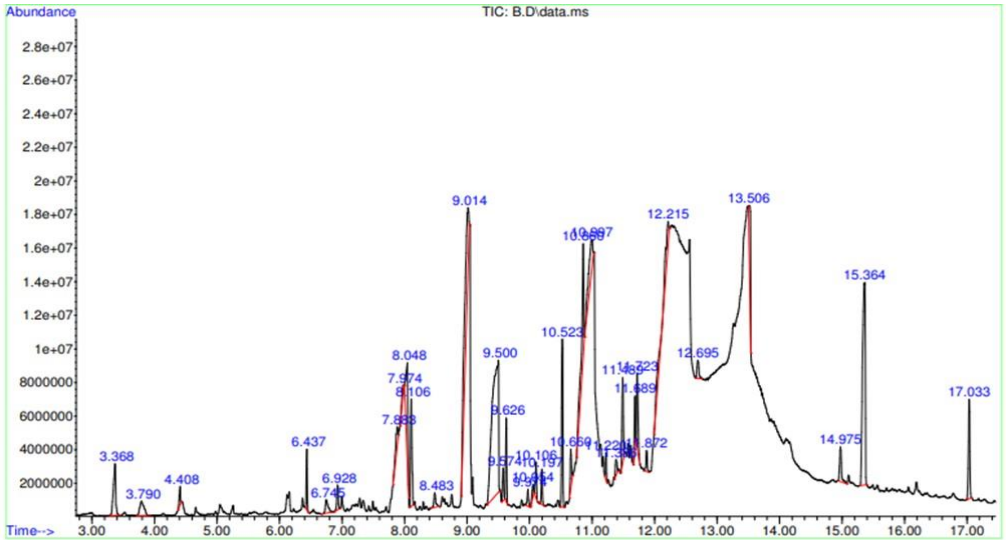

Figure 3:- Total ion chromatogram (TIC) of methanol extract of *G. angustifolia* root bark.

Table 2:- Detected compounds from GC-MS evaluation of ethyl acetate extract of *G. angustifolia* root bark

Peak no	Retention time (min)	Area (%)	Name of compound	Molecular formula
1	2.847	0.12	Erythritol	$C_4H_{10}O_4$
2	3.385	1.68	Pantolactone	$C_6H_{10}O_3$
3	4.408	1.86	Benzoic acid	$C_7H_6O_2$
4	5.025	0.74	5 – Hydroxymethylfurfural	$C_6H_6O_3$
5	6.382	0.11	Succinic acid, tridec-2-yn-1-yl 2- methylbutyl ester	$C_{22}H_{38}O_4$
6	6.728	0.09	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$
7	6.820	0.67	2,1,3-Benzothiadiazole	$C_5H_4N_2S$
8	7.225	0.46	3-pyridinecarboxylic acid, 5-(1-methoxyethyl)-, methyl ester	$C_{10}H_{13}NO_3$
9	7.785	0.26	Dodecanoic acid	$C_{12}H_{24}O_2$
10	7.928	1.04	2,6 – Dimethylisonicotinic acid thioamide	$C_8H_{10}N_2S$
11	8.380	0.09	2-Furanmethanamine	C_5H_7NO
12	8.454	0.10	2,6 -Octadienal, 3, 7-dimethyl-,(E)	$C_{10}H_{16}O$
13	**8.957**	**13.48**	**Phenanthrene, 9,10-dihydro-**	**$C_{14}H_{12}$**
14	9.266	0.15	4-((1E) – 3-Hydroxy-1-propenyl) – 2-Methoxyphenol	$C_{10}H_{12}O_3$
15	9.329	0.56	Tetradecanoic acid	$C_{14}H_{28}O_2$
16	10.415	0.13	2-Furanmethanamine	C_5H_7NO
17	10.512	0.64	Hexadecanoic acid, methyl ester	$C17H_{34}O_2$
18	10.655	0.30	Palmitoleic acid	$C_{16}H_{30}O_2$
19	**10.826**	**13.36**	**n-Hexadecanoic acid**	**$C_{16}H_{32}O_2$**
20	10.969	0.34	Ethyl tridecanoate	$C_{15}H_{30}O_2$
21	11.289	0.25	9 – Hexadecenoic acid, methyl ester, (Z) -	$C_{17}H_{32}O_2$
22	11.415	0.33	Heptadecanoic acid	$C_{17}H_{34}O_2$
23	11.666	0.66	9,12-Octadecadienoic acid, methyl ester	$C_{22}H_{44}O_4Si$
24	11.700	1.41	9 – Octadecenoic acid (Z) -, methyl ester	$C_{19}H_{36}O_2$
25	11.861	0.19	Heptadecanoic acid, 16 – methyl-, methyl ester	$C_{19}H_{38}O_2$
26	**12.118**	**38.80**	**Oleic acid**	**$C_{18}H_{34}O_2$**
27	12.209	1.86	Octadecanoic acid	$C_{18}H_{36}O_2$
28	12.615	1.77	1,3 -Cyclododecadiene, (E,Z) -	$C_{12}H_{20}$
29	**13.049**	**16.69**	**Gamolenic acid**	**$C_{18}H_{30}O_2$**
30	13.186	0.16	Tricyclo [4.2.1.1 (2,5)] decan-9-one	$C_{10}H_{14}O$
31	13.444	0.10	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$
32	13.529	0.09	Gamolenic acid	$C_{18}H_{30}O_2$
33	14.815	0.13	Ethanone, 2-hydroxy-1,2 -bis (4-methoxyphenyl)-	$C_{16}H_{16}O_4$
34	15.044	0.73	1H-Cyclopenta[a]pentalene-7-ol, decahydro-3,3,4,7a-tetramethyl-,acetate	$C_{17}H_{28}O_2$
35	17.004	0.67	Squalene	$C_{30}H_{50}$

Table 3:- Detected compounds from GC-MS evaluation of methanol extract of *G. angustifolia* root bark

Peak no	Retention Time (min)	Area (%)	Name of compound	Molecular formula
1	3.368	2.66	2-(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (+/-.)-	$C_6H_{10}O_3$
2	3.790	1.36	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$
3	4.408	0.60	Benzamine, 4-methoxy-	C_7H_9NO
4	6.437	1.29	Tetradecane	$C_{14}H_{30}$
5	6.745	0.80	2,4,6-Octatrienoic acid	$C_8H_{10}O_2$
6	6.928	0.76	Cyclohexane, 4-methyl-1-(1-methylethenyl)-	$C_{10}H_{16}$
7	7.883	1.40	Dodecanoic acid	$C_{12}H_{24}O_2$
8	7.974	0.62	Dodecanoic acid	$C_{12}H_{24}O_2$
9	8.048	3.72	Dodecanoic acid	$C_{12}H_{24}O_2$
10	8.105	3.37	Hexadecane	$C_{16}H_{34}$
11	8.483	0.61	Cyclopentanol, 1-(1-methylene-2-propenyl)-	$C_9H_{14}O$
12	**9.014**	**9.75**	**2-Oxodamantane-1-carboxylic acid, methyl ester**	**$C_{12}H_{16}O_3$**
13	**9.500**	**19.28**	**Tetradecanoic acid**	**$C_{14}H_{28}O_2$**
14	9.574	0.84	1-octadecene	$C_{18}H_{36}$
15	9.626	1.91	Octadecane	$C_{18}H_{38}$
16	9.974	0.47	2-pentadecanone, 6,10, 14-trimethyl	$C_{18}H_{36}O$
17	10.054	0.48	1,5-Decadiyne	$C_{10}H_{14}$
18	10.106	0.90	Pentadecanoic acid	$C_{15}H_{30}O_2$
19	10.197	0.86	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$
20	10.523	4.94	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$
21	10.660	1.11	Pthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$
22	10.860	5.53	Dibutyl phthalate	$C_{16}H_{22}0_4$
23	10.997	3.84	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
24	11.220	0.50	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$
25	11.386	0.60	Cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$
26	11.489	2.47	Phthalic acid, butyl cycloheptyl ester	$C_{19}H_{26}O_4$
27	11.689	1.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$
28	11.723	2.23	9-Octadecenoic acid, methyl ester, (E) -	$C_{19}H_{36}O_2$
29	11.872	0.62	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
30	12.215	5.06	9,12-Octadecadienoic acid (Z,Z)-Linoelaidic acid	$C_{18}H_{32}O_2$
31	12.695	0.75	9,12-Octadecadienoic acid (Z,Z)-Linoelaidic acid	$C_{18}H_{32}O_2$
32	13.506	2.49	1,3-Cyclododecadiene, (E,Z)-	$C_{12}H_{20}$
33	14.975	1.40	Ethanone, 2-hydroxy-1,2-bis 94-	$C_{16}H_{16}O_4$
34	**15.364**	**12.40**	**Disooctyl phthalate**	**$C_{24}H_{38}O_4$**
35	17.033	3.06	Squalene	$C_{30}H_{50}$

Cytotoxicity assay

The cytotoxic assay depicts a concentration dependent pattern of toxicity to the brine-shrimps with LC_{50} values 897.4 and 532.1µg/mL corresponding to the methanol and ethyl

acetate crude extracts against the standard, $K_2Cr_2O_7$ toxicity (LC_{50} = 291.7µg/mL) (Table 4). Similarly, the crude extracts, methanol and ethyl acetate, displayed a moderate cytotoxic action at low concentration and a high percentage lethality at concentration greater than 200µg/mL to the HEK-293 cell line (Figure 4).

Table 4:- Cytotoxic effect of methanol and ethyl acetate extracts of *G. angustifolia* root bark on brine shrimps (*Artemia salina*)

	% Lethality		
Conc. (µg/mL)	**MEGR**	**EAGR**	**$K_2Cr_2O_7$ (%)**
2000	90	90	100
1000	40	90	100
500	20	30	80
250	10	20	50
125	10	10	10
LC_{50}	897.4[c]	532.1[b]	291.7[a]

MEGR – Methanol Extract of *G. angustifolia* root-bark, EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark

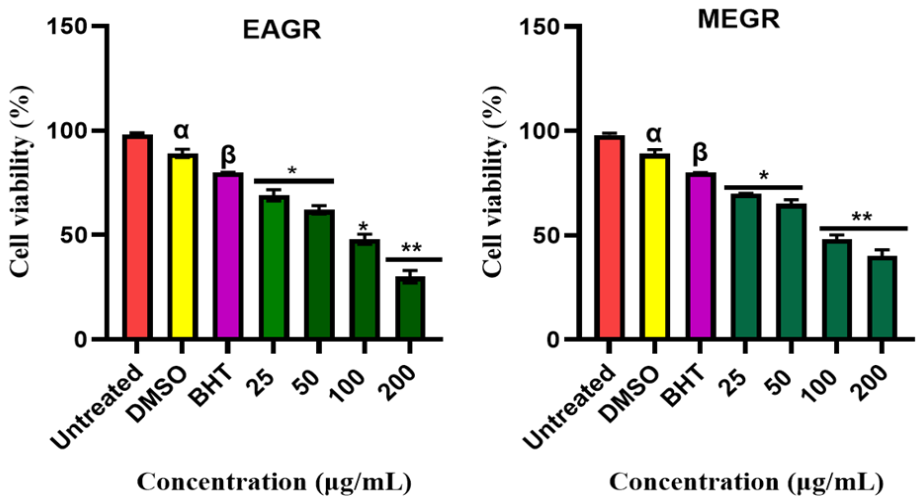

Figure 4:- Cytotoxic effect of methanol and ethyl acetate extracts of *G. angustifolia* root-bark on HEK293 cells using MTT assay. Data are expressed as mean ± SEM, n = 3.
MEGR – Methanol Extract of *G. angustifolia* root-bark; EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark, DMSO – Dimethyl sulfoxide; BHT – Butylated hydroxytoluene.

In vitro Antioxidant activity

Complementing the existing data, the ethyl acetate and methanol crude extracts displayed high free radical inhibitory effects on DPPH with IC_{50} values of 14.53 and 55.80µg/mL, respectively, against the synthetic reference antioxidant compound, ascorbic acid (IC_{50} = 7.91µg/mL) (Figure 5). Following the analysis of antioxidant activity using FRAP method, the free radical scavenging activity of ethyl acetate crude extract is higher than the methanol crude extract of *G. angustifolia root* bark. (Figure 6).

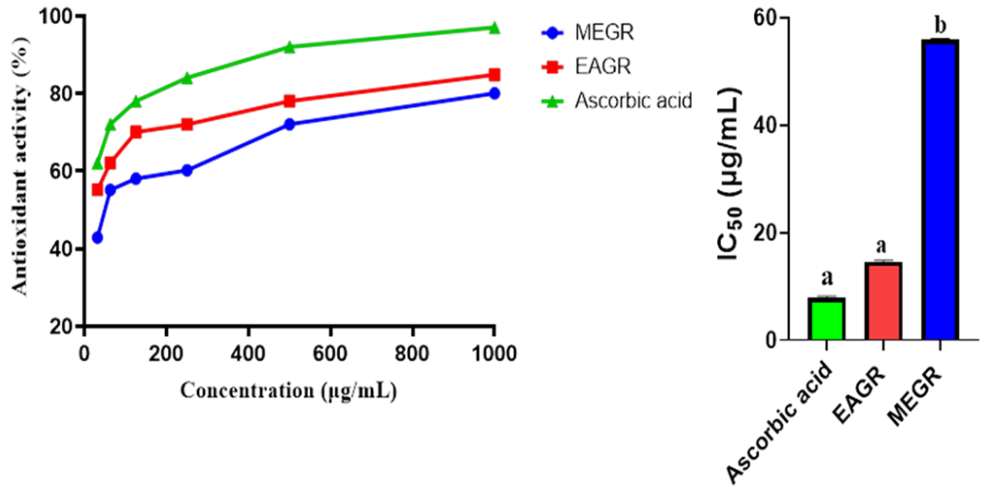

Figure 5:- DPPH radical scavenging ability of Ascorbic acid, Methanol and Ethyl acetate extracts of *G. angustifolia* root bark.

MEGR – Methanol Extract of *G. angustifolia* root-bark, EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark

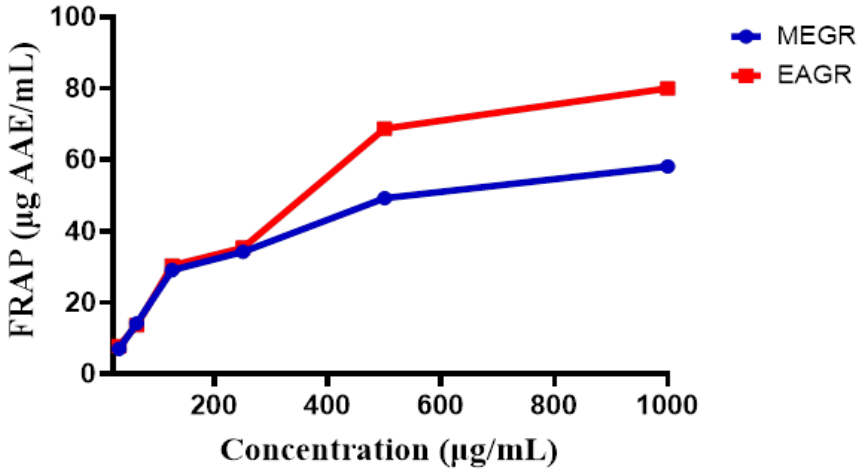

Figure 6:- Ferric reducing antioxidant power (FRAP) activities of methanol and ethyl acetate extracts of *G. angustifolia* root bark.

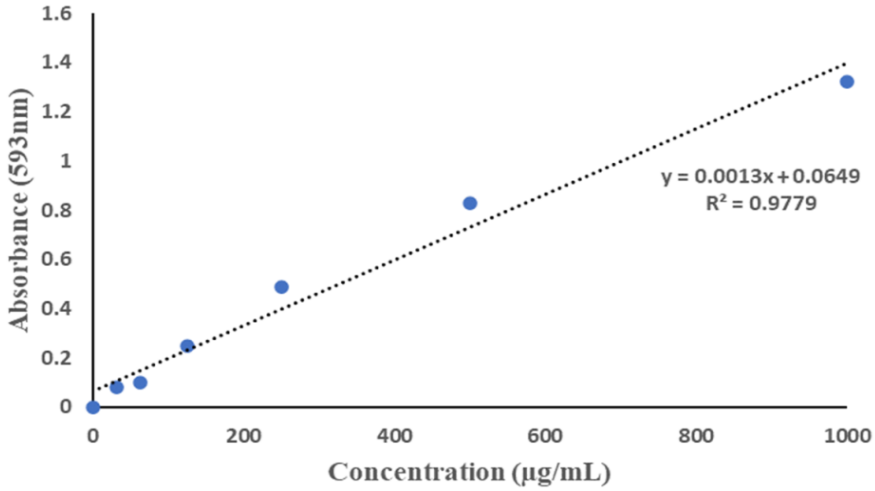

Figure 7:- The calibration curve of Ascorbic acid

Antimicrobial activity

The crude extracts showed growth inhibitory action against selected reference strains of bacteria and fungi isolates. The ethyl acetate extracts showed its inhibitory effect against four bacterial strains which are *Clostridium sporongenes* (NCIB 532) (10.00 ± 0.03mm), *Bacillus cereus* (NCIB 532) (11.75 ±0.13mm), *Bacillus polymyxa* (NCIB 87) (12.25 ± 0.11mm) and *Morganella morganii* (NTCT 235) (10.50 ±0.01mm). Out of eight (8) bacteria strains tested, resistance to the methanol crude extract was observed in six (6) strains while two (2) were minimally inhibited. This includes *Clostridium sporongenes* (NCIB 532) (14.00 ± 0.12mm) and *Bacillus polymyxa* (NCIB 87) (15.50 ± 0.22mm) (Table 5). The inhibitory capacity of ethyl acetate crude extract was high with a wide range of bacteriocidal and bacteriostatic effects when compared to the methanol crude extract. It also had a sharp margin to the reference standard antibiotic (streptomycin) under the condition of investigation. The extracts in reference to the standard antifungal showed degree of growth inhibition on some fungi organisms. Ethyl acetate crude extract inhibited *Candida albicans* (NCIMB 15203), *Candida pseudotropicalis* (ATCC 8619) and *Saccharomyces cerevisiae* (ATCC 9763) (17.50 ± 0.15mm, 16.50 ± 0.23mm and 12.00 ±0.02mm) respectively. On the other hand, methanol crude extract inhibited two (2) fungi strains *Aspergillus flavus* (ATCC 9643) and *Penicillium chrysogenum* (ATCC 10106) (28.50 ± 0.50mm and 19.00 ± 0.00mm) respectively (Table 7). A minimum inhibitory concentration and fungi concentration for both extracts ranged between 50 – 100 mg/mL, depicting an index of their antifungal actions (Table 6 & 8).

Table 5: Antibacterial activity of methanol and ethyl acetate extracts of *G. angustifolia* root bark.

Test organism	Zone of Inhibition (mm)		
	MEGR (100mg/mL)	EAGR (100mg/mL)	Streptomycin (30µg/mL)
Gram-positive			
Clostridium sporongenes (NCIB 532)	14.00 ± 0.12[b]	10.00 ± 0.03[a]	12.50 ± 0.01[b]
Bacillus cereus (NCIB 532)	_	11.75 ± 0.13[a]	13.00 ± 0.02[a]
Bacillus polymyxa (NCIB 87)	15.50 ± 0.22[c]	12.25 ± 0.11[a]	14.50 ± 0.10[b]
Gram-negative			
Morganella morganii (NTCT 235)	_	10.50 ± 0.01[a]	13.50 ± 0.02[a]
Serratia marcescens (NCIB 1377)	_	_	_
Klebsiella pneumoniae (NCIB 418)	_	_	_
Proteus vulgaris (NCIB 67)	_	_	_
Salmonella typhimurium (ATCC 14028)	_	_	13.50 0.12

= No inhibition, MEGR – Methanol Extract of *G. angustifolia* root-bark, EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark. Values are mean ± SEM of three replicates.

Table 6:- Expression of Antibacterial activity as MIC and MBC of methanol and ethyl acetate extracts of *G. angustifolia* root bark.

Test organism	MEGR (100mg/mL)		EAGR (100mg/mL)		Streptomycin (30µg/mL)	
Gram Positive Bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Clostridium sporongenes (NCIB 532)	50	100	50	> 100	3.75	3.75
Bacillus cereus (NCIB 532)	_	_	50	50	3.75	7.30
Bacillus polymyxa (NCIB 87)	50	50	50	> 100	3.75	3.75
Gram Negative Bacteria						
Morganella morganii (NTCT 235)	_	_	50	100	3.75	3.75
Serratia marcescens (NCIB 1377)	_	_	_	_	_	_
Klebsiella pneumoniae (NCIB 418)	_	_	_	_	_	_
Proteus vulgaris (NCIB 67)	_	_	_	_	_	_
Salmonella typhimurium (ATCC 14028)	_	_	_	_	7.5	15

- = no Inhibition, MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MEGR – Methanol Extract of *G. angustifolia* root-bark, EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark.

Table 7:- Antifungal activity of methanol and ethyl acetate extracts of *G. angustifolia* root bark

Fungi	Zone of Inhibition (mm)		
	MEGR (100mg/mL)	EAGR (100mg/mL)	Nystatin (4µg/mL)
Aspergillus flavus (ATCC 9643)	28.50 ± 0.50^{a}	_	20.00 ± 0.00^{b}
Candida albicans (NCIMB 15203)	_	17.50 ± 0.15^{b}	28.00 ± 0.02^{a}
Candida pseudotropicalis (ATCC 8619)	_	16.50 ± 0.23^{b}	26.50 ± 0.01^{a}
Penicillium chrysogenum (ATCC 10106)	19.00 ± 0.00^{b}	_	22.50 ± 0.05^{a}
Saccharomyces cerevisiae (ATCC 9763)	_	12.00 ± 0.02^{b}	20.00 ± 0.00^{a}

- = no Inhibition, MEGR – Methanol Extract of *G. angustifolia* root-bark, EAGR – Ethyl acetate Extract of *G. angustifolia* root-bark.

Table 8:- Expression of Antifungal activity as MIC and MFC of methanol and ethyl acetate extracts of *G. angustifolia* root-bark.

Test organism	MEGR (100mg/mL)		EAGR (100 mg/mL)		Nystatin (4 µg/mL)	
Fungi	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus flavus (ATCC 9643)	50	50	_	_	1	2
Candida albicans (NCIMB 15203)	_	_	100	> 100	0.25	1
Candida pseudotropicalis (ATCC 8619)	_	_	100	> 100	0.5	2
Penicillium chrysogenum (ATCC 10106)	50	100	_	_	0.5	1
Saccharomyces cerevisiae (ATCC 9763)	_	_	50	> 100	0.25	1

- = No inhibition, MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration,

Discussion

Plant-based agents with therapeutic effects against invading microbes constitutes an interesting research arena aiming at stalling the increasing impacts of infectious diseases and identifying a novel target of various plants [16]. In this study, 14.3% and 9.6% yields of ethyl acetate and methanol crude extracts justify a differential composition arising from polarity of the chosen solvents (ethyl acetate and methanol) having affinity toward different phytochemicals. Affinity of the solvents to the phytochemicals may translate to the appearance of six and four bands on the TLC spotted ethyl acetate and methanol crude extracts, respectively. The most abundant compound in the ethyl acetate and methanol extracts are oleic acid (38.80%) and tetradecanoic acid (19.28%), respectively. Oleic acid has been previously reported to have antibacterial, hypoglycemic, anti-inflammatory and antioxidant properties [17]. Tetradecanoic acid has also been reported to have antifungal, antiviral and antiparasitic properties [18].

Brine shrimp cytotoxicity assay have always been considered as a rapid, simple pharmacological assay for cytotoxicity assessment. The result of this research shows that percentage lethality increases with increase in concentration of the extracts. This result is in agreement with the study conducted by Agbodjento *et al*. [19] which indicated increase in percentage lethality with increasing concentration of *Gardenia ternifolia* root extract. Our study further indicates that the methanol extract of *G. angustifolia* root bark has a higher LC_{50} (897.4µg/mL) and therefore is less toxic than the ethyl acetate extract of *G. angustifolia* root bark with a lower LC_{50} (532.1µg/mL). Plants with a recorded LC_{50} values lower than 1000µg/mL are classified as cytotoxic [20]. Therefore, the crude extracts of *G. angustifolia* root bark has the potential of use as cytotoxic drugs at higher doses.

Furthermore, cytotoxicity assay of the extracts was also conducted on HEK-293 cell line using the 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The MTT assay is a colorimetric assay that measures the metabolic activity of the cell which is based on the ability of NADP(H)-dependent oxidoreductase enzymes in active mitochondria of viable cells to cleave the tetrazolium ring of MTT leading to the formation of purple colored formazan. The cell viability is defined by the amount of formazan produced [21]. The result of this study revealed a dose-dependent cytotoxic effect of the methanol and ethyl acetate extracts of *G. angustifolia* root bark on HEK-293 cell line. The highest cytotoxic effects of the extracts are observed at the highest studied dose of 200 µg/mL with a lower cell viability observed with the ethyl acetate extract when compared with the methanol extract. Therefore, at higher doses the methanol and ethyl acetate extracts of *G. angustifolia* can serve as potential anticancer agents. The study conducted by Vindhya and Leelavathi [22] also indicated a dose-dependent cytotoxic effect of *Gardenia latifolia* and *Gardenia gummifera* on Michigan Cancer Foundation – 7 (MCF-7) cell line.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay is a simple and widely used technique to assess the antioxidant activity of plant extracts or food samples. In order to create a stable molecule, DPPH, a stable free radical, has the ability to take on an electron or a hydrogen radical [23]. The result of the DPPH radical scavenging assay indicates that the free radical scavenging ability of the extracts and standard (ascorbic acid) increases in a concentration dependent manner. The ethyl acetate extract of *G. angustifolia* root bark has a significantly higher antioxidant capacity with an IC_{50} of 14.53µg/mL when compared to the methanol extract of *G. angustifolia* root bark with an IC_{50} value of 55.8µg/mL. The antioxidant capacity of the ethyl acetate crude extract of *G. angustifolia* root bark is comparable to that of standard (ascorbic acid) with an IC_{50} value of 7.91 µg/mL.

In FRAP assay, ascorbic acid was used as the standard solution. Ascorbic acid scavenges free radicals and prevent chain reactions. The radical scavenging ability of ascorbic acid is due to the presence of its hydroxy group. The presence of polyhydroxy group is indicative of increase in antioxidant activity [24]. In this assay, the reduction power of the extracts is indicative of the ability of the extracts to convert Fe^{3+} to Fe^{2+}. The value of FRAP was expressed as µg equivalent ascorbic acid/mL (AAE). The result of this study revealed that the reduction power of the extracts was concentration dependent. The highest reducing power was observed in the highest concentration of

both extracts (1000µg/mL). However, the ethyl acetate crude extract of *G. angustifolia* root bark has higher reducing power when compared to the methanol extract of *G. angustifolia* root bark. Compounds with reducing power have the ability to stabilize free radicals by giving them electrons or hydrogen atoms to make them more stable. Therefore, these compounds may function as antioxidants [25]. Reactive oxygen species have been known to induce oxidative stress and are involved in the pathogenesis of various diseases by accelerating damage to the DNA and causing lipid peroxidation and carbonylation of proteins. Research has shown that various plants possess antioxidant activities which makes them promising therapeutic targets for diseases involving reactive oxygen species [26]. Furthermore, the antioxidant capacity of a plant could be attributed to the flavonoids and phenolics content of the plant [27].

The result of this study showed that the methanol and ethyl acetate extracts of *G. angustifolia* root bark have antibacterial and antifungal activity against some of the strains of microorganisms tested. However, the standard antibiotic streptomycin (30 µg/mL) and the standard antifungal Nystatin (4 µg/mL) exhibited inhibitory effect against a wider range of microorganisms when compared with the extracts of *G. angustifolia* root bark. From the result, the methanol extract exhibited stronger antibacterial effect against gram-positive bacteria but all the gram-negative bacteria strains tested were resistant to methanol extract. The ethyl acetate extract also exhibited stronger antibacterial effect against gram-positive bacteria and susceptibility was observed in only one (1) out of five (5) gram-negative bacteria tested (*Morganella morganii* NTCT 235). Ethyl acetate extract exhibited inhibitory effect on three (3) out of five (5) tested fungi while the methanol extract inhibited two (2) out of five (5) tested fungi. Therefore, the ethyl acetate extract has antimicrobial effect against more strains of microorganisms when compared to the methanol extract. This result is comparable with other research works which have revealed that in comparison to gram-positive bacteria, the gram-negative bacteria are less susceptible and more resistant to antibacterial agents [28][29]. The observed differences in the susceptibility of the different strains of microorganisms to the individual extracts can be attributed to the permeability of the inherent bioactive constituents to permeate the cell wall of the bacteria and the solubility of the bioactive constituents in individual solvents. The MIC of the extracts was lower than the MBC and MFC. This is indicative of a high antimicrobial capacity of the extracts [30].

Conclusion

This research indicates that the radical scavenging and microbial growth inhibitory activities of the methanol and ethyl acetate extracts of *G. angustifolia* root bark may be attributed to the presence of identified bioactive compounds in the extracts. This study further offers scientific explanation on the traditional use of *G. angustifolia* root bark as an antimicrobial agent. Specifically, the ethyl acetate crude extract of *G. angustifolia* root bark exhibits mild cytotoxicity, higher antioxidant and antimicrobial activity than the methanol extract of *G. angustifolia* root bark. Therefore, it can be considered a potential candidate for cytotoxic and antimicrobial microbial drug design.

Recommendation

In vivo studies to assess the safety and efficacy of the extracts on experimental animal models and structural elucidation of the bioactive compounds responsible for the bioactivity of the extracts are required for further studies.

Limitation

This experiment is devoid of life animals in its entirety.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical clearance

There is no ethical reference number in this research

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