

Phytochemistry and Antimicrobial Activities of crude and diluted leaves and roots extracts of *Uvaria chamae* on selected bacteria

ABSTRACT

Plant metabolites are antibacterial. Antibacterial properties of aqueous, methanolic and n-hexane extracts of crude and diluted leaves and roots extracts of *Uvaria chamae* were studied to demonstrate its potential as achemotherapeutic agent. Test organisms include: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi* and *Proteus vulgaris*. Fresh tender leaves and roots of *Uvaria chamae* were collected, air-dried, grounded and soaked in methanol, n-hexane and aqueous solvents. Phytochemistry was done using standard procedures, antimicrobial activity was determined using agar-well diffusion method (punch hole) and MIC determined. Qualitative phytochemical analysis confirmed the presence of alkaloids, tannins, terpenoids, glycosides, saponins and flavonoids. Antimicrobial analysis of the plant parts using disc-diffusion method showed ten (10mg/ml) MIC concentration for aqueous extract of the leaves showed no activity, while root decoction inhibited only *Salmonella typhi* (14.00±0.47mm). Methanolic crude extracts of the leaves (UML_c) (400µg) exhibited antibacterial effects on all tested organisms, higher for *Pseudomonas aeruginosa* (23.67± 0.27mm) than gentamycin (10µg) control (21.33± 0.72) mm, while its 10⁻¹ (40mg) dilution was active against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Only crude n-hexane leaf extract (UNL_c) was active for *Pseudomonas aeruginosa* (13.00 ± 0.47mm). Crude methanolic extract of the roots UMR_c (400µg) was active against all tested bacteria and clearance for *Proteus vulgaris* (23.33±0.54mm) was greater than that in both controls (ciprofloxacin (5 µg) (22.33±0.72 mm) and (gentamycin 10µg) (16.67±0.72mm). UNR_c had activity against *S. aureus*, *B. subtilis* and *P. aeruginosa* (25.00 ± 0.47 mm, 22.00± mm, and 12.67 ± 0.27mm), while its 10⁻¹ dilution (40mg) had effects on *Bacillus subtilis* and *Staphylococcus aureus* (11.00± 0.82mm, 13 ± 0.47mm) only. The 10⁻² (4µg) dilutions of (UMR and UNR) had no activity. Comparison of inhibition size between UML (20.33± 0.27mm) and UMR (23.33± 0.54mm) against *Proteus vulgaris* was significantly greater than inhibition size of gentamycin (10µg) (16.67± 0.72) p<0.05. Activity of UML against *Pseudomonas aeruginosa* (23.67± 0.27mm) was comparable to both ciprofloxacin (25.33± 1.15mm) and gentamycin (21.33± 0.72mm) p>0.05. Aqueous extracts of *Uvaria chamae* roots could be modified and harnessed for eradicating *Salmonella typhi*, UML_c and UMR for *Proteus vulgaris* and UML for *Pseudomonas aeruginosa*. *Uvaria chamae* crude and diluted extracts of both parts are potential future antibiotics.

Keywords: *Uvaria chamae*, Gram-positive bacteria, Gram-negative bacteria, Antimicrobial, Phytochemistry.

1. INTRODUCTION

The use and search for antimicrobial drugs and dietary supplements derived from natural sources have accelerated in recent years. Scientists are combing the earth for biological reactive compounds from natural sources in attempts to derive new sources of useful drugs against infectious diseases. Sofowora, (1993) defined a medical plant as a plant widely employed in traditional medicine in West-Africa. *Uvaria chamae* belongs to the family Annonaceae and grows in free-growing forests, and dry coastal areas. It is distributed from Senegal to Zaire. Related species occur in most forest regions in the continent (Iwu, 1993).

Commonly called “Finger-root” or “Bush banana fruit” in English, this climbing shrub or small bushy tree grows up to 5m high in folk medicine. It is known as “Mmimi-ohia” in Igbo, bush Dennettia (Singha); “KasKaifi” in Hausa; “Eruju” or “Okoaja” in Yoruba; “Nkarikalkot” in Efik; “Awuloko or Ayiloko” by Igala tribes; and “Akotompo” by the Fula-fainte of Ghana (Omale *et al.*, 2013; Olumese *et al.*, 2016).

The main uses of *Uvaria chamae* in Nigeria and other West African countries are for the remedy for jaundice and intermittent fever. The root bark is used for respiratory catarrh and for dysentery. The increasing rate at which microorganisms resist modern antibiotics in recent times has further fuelled the need to carry out research in our local Medicinal plant to come up with new and more active antimicrobial agents that would be effective and affordable in the treatment of microbial infections.

In the light of modern medicine, *Uvaria chamae* plant parts and extracts has been proved by several researchers to be quite efficacious. Abu *et al.* (2020) showed that several researches on the biological contents and activities of secondary metabolites accumulated in *Uvaria chamae* and its plant parts in the light of modern medicine are truly biologically active and validates their claims in traditional system of medicine. Extensive literature on anti-inflammatory activity of *Uvaria* species showed that its extracts and isolates exhibited significant anti-inflammatory activity through various established mechanism of action (Jalil *et al.*, 2020). It was noted that 6,7-di-O-Methyl-baicalein, fexuvarol B, chrysin,(-)-zeyleanol, 6-hydroxy5,7-dimethoxy-favone, and pinocembrin were the most potent anti-inflammatory compounds and had comparable IC50 with positive controls. The aqueous extract of *Uvaria chamae* on multi-resistant strains of *Salmonella enterica* showed that the plant is good for the development of an improved traditional medicine for the management of salmonellosis (Legba *et al.*, 2020). Popoola *et al.* (2019) showed the anti-oxidative, antimutagenic, and DNA-damaging activities of *Uvaria chamae* root in herbal treatment of cancer. In Ogbu *et al.* (2020) histological results on biochemical and toxicological effects of its ethanolic leaf extract showed no abnormality in the kidneys and mild peri-portal lymphocytic infiltration of the liver proving ethanolic extract of leaves of *Uvaria chamae* had no serious deleterious effects on Wistar albino rats and may be safely used in traditional medical practice.

Notwithstanding that several plant parts contain active metabolites, each plant part may contain higher proportions than others. This fact is important in choosing the plant parts to use as well as the solvent extract that will be more beneficial in extracting higher concentration of required phytochemical yield. In a study by Obasi and Obasi (2021), *Uvaria chamae* stem was noted to have appreciable amounts of medicinally important phytochemicals, with solubility differing in different solvents.

Several multidrug-resistant gram -positive bacteria were highly sensitive to ethanol extracts of *Uvaria chamae* roots and the activity was more extensive than that from conventional broad-spectrum antibiotics. The ion mobility mass spectrometry analysis revealed the presence of ten chalcone and dihydrochalcone chemical structures responsible for the antimicrobial activity of *Uvaria chamae* ethanol extracts confirming and justifying the medical properties of these roots used as a traditional medicine.

The aim of the work therefore is to derive the phytochemical constituents and access the antibacterial properties of aqueous, methanolic and n-hexane crude and dilutions of extracts of the leaves and roots of *Uvaria chamae* on some selected clinical bacterial organisms.

2. MATERIALS AND METHODS

2.1 Bacterial isolates:

Six clinical isolates confirmed to be these organisms were obtained from the Medical Pathology and Microbiology Department of University Teaching Hospital, University of Nigeria, Nsukka, Nigeria. They include: *Staphylococcus aureus* and *Bacillus subtilis* for Gram positive and *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus vulgaris* for Gram negative bacteria. All were collected in slants in McCartney bottles containing Mueller - Hinton agar and appropriately stored until needed.

2.2 Collection of Plant Materials:

Fresh *Uvaria chamae* leaves and roots were harvested from a forest in Nodu Village Okpuno, Awka in Awka-South Local Government Area in Anambra State, and identified and authenticated at Botany Department of University of Nigeria, Nsukka.

2.3 Preparation of Plant Extract:

The leaves of *Uvaria chamae* were dried under the shade for 7 days, separately grounded to a fine powder with a mechanical grinder and pulverized. The resulting powder was weighed, 20g collected and extracted using water, methanol and n-hexane as described by Eloff (1991) after soaking in 100ml needed solvent at room temperature for 72 hours, shaking regularly. Soxhlet extractor was used for extraction according to (Evans, 1989). The dry roots were washed, dried, grounded into fine powder and weighed. Extraction was done as for the leaves as above. The extract was then filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness at 35°C using evaporating dish. Extracts obtained were stored at 2-4°C until needed.

2.4 Phytochemical screening of the crude extracts:

Phytochemistry of the plant materials were done to ascertain the presence of bioactive components in the leaves and roots extracts using the method of Evans (1989), the phytochemicals tested for include; alkaloids, saponins, flavonoids, glycosides, terpenoids, tannins, acidity, lipid and oil.

2.5 Preparation of dilutions of the extracts:

After preparing crude extracts, crude concentrated aqueous, n-hexane soluble and methanolic portions of the extracts were prepared to contain 10mg/ml (10,000µg/ml). One hundred (100mg) of the concentrated aqueous extracts was dissolved in 10ml of normal saline to obtain a stock of 10mg/ml. One hundred (100mg) of the metabolic extracts (leaves / roots) and 100 mg of n-hexane extracts (roots) were separately dissolved in 10ml of dimethylsulphoxide (DMSO).

The n-hexane extracts of the leaves were dissolved in 10 ml of 3% tween 85 to obtain a stock of 10mg/ml. The non-polar (solvent) extracts were diluted up to 1: 100 (10^{-2}) so that for each non-polar extract, a crude (stock) and two dilutions (1:10 10^{-1} : 1:100 (10^{-2})) was obtained.

2.6 Collection of bacterial strains for antibacterial screening/ susceptibility screening

Pure clinical strains of the test bacterial strains were collected from medical pathology and Microbiology Department of University Teaching Hospital, University of Nigeria, Nsukka, Nigeria. These include: *Staphylococcus aureus* and *Bacillus subtilis* for gram positive and *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus vulgaris* for gram negative bacteria.

A known concentration of extract in milligram (10mg/ml) of each extract was reconstituted in sterile distilled water. Each bacterial suspension was cultured in peptone water for 24 hours and of 0.1ml of a 10^7 c.f.u cell/ml suspension of each test organism compared with a McFarland's suspension was used as inoculum size for all bacteria.

2.7 Susceptibility testing:

Agar-gel diffusion method (punch-hole method) was used as described by (Levian,1980). The inoculum comprised of a 1 in 100 dilution of the overnight broth culture of the test organism. Twenty (20) ml of sterile molten agar Mueller-Hinton agar (Oxoid, USA) was poured into each of the plates and seeded to allow homogenous setting.

Four (4) wells of 7mm diameter were bored on each petri dish using a sterile glass cork-borer, each appropriately labeled for crude extract (400µl crude) or dilution of crude extract (10^{-1} (40µl) and 10^{-2} (4µl)). For crude extract testing, testing was done in duplicates using two petri dishes for each organism, whereas two other petri dishes had eight wells punched into them respectively, one for 1 in 10 dilutions while the other for 1 in 100 dilution of extracts respectively. The petri dishes were labelled appropriately with each organism. Each hole was filled with two drops (0.1 ml) of each plant extracts (leaves and root) of both stock solutions (crude) and dilutions of crude (10^{-1} and 10^{-2}) suspensions, and 0.1ml of a 10^7 cell/ml suspension of each test organism (compared with a McFarland's standard culture) was dropped at the center of the appropriate labelled plates.

For the aqueous extracts, five wells were punched per plate for each organism and all processes repeated in triplicate. On

each plate, (10µg) Gentamycin and (5µg) Ciprofloxacin (Liophilchem, Italy) antibiotic discs were used as positive drug control while methanol was used as negative control. Two (2) drops of both stock solutions (crude extracts) and dilution were used for the agar gel diffusion test. The plates were incubated for 24 h at 37°C and the diameter of inhibition zones measured in milliliter and results noted. Each experiment was done in duplicates in aseptic conditions.

2.8 Statistical Analysis

Data generated from this study was analyzed using statistical package for social sciences (SPSS version 20.0) window 19. The results were presented in tables and expressed as mean± standard deviation. ANOVA was used to compare means, and values were considered significant at $p < 0.05$.

3. Results

The results were expressed where appropriate as mean ± standard error of mean using the students' t-test at 95% confidence unit. Diameters of clearance were represented in millimeter (mm). The result of the phytochemical analysis of the plant root and leaves extracts and the antimicrobial activities of the extracts in the test organisms are given in tables I and 2, 3 and 4.

In table 1, the **result of the phytochemicals in the *Uvaria chamae* plant parts extracts** showed that six phytochemical constituents were obtained from both roots and leaves parts extracts each (tannins, flavonoids, terpenoids, alkaloids, glycosides (anthracene derivatives), saponins). More of tannins, flavonoids and terpenoids were in both plant parts, varying in quantity when compared between both. Both plant parts were non-acidic and had no fats and oil. Tannins, flavonoids and glycosides were more from roots compared with leaves.

Table 2 showed that the effects of crude aqueous extracts of *Uvaria chamae* leaves and roots on growth of test organisms showed that all crude aqueous extracts of both plant parts (leaves and roots) were resistant to all test organisms (7mm), except the crude aqueous root extract (400µg) which was susceptible to *Salmonella typhi* (14.00±0.47).

In table 3, the effect of methanolic and n-hexane extracts of leaves of *Uvaria chamae* on the growth of test organisms showed that UML_c (400µg) extracts had the highest activity against all tested organisms and extracts. Highest clearance among all bacteria was observed in *B. subtilis* (24.33±0.54) when compared with the controls, ciprofloxacin (5µg) (33.67 ± 0.27) and gentamycin (10µg) (24.67 ± 0.72).

The concentration of 10⁻¹ (40µg) concentration had activity only for *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (11.33 ± 0.54), which recorded the lowest activity in all. There was no activity at all observed for UML_c 10⁻¹ (4µg) and in UNL_c 10⁻¹ (40µg) and its corresponding 10⁻² (4µg) concentrations respectively. UNL_c (400 µg) had activity only for *Pseudomonas aeruginosa*.

In table 4, the effect of methanolic and n-hexane extracts of roots of *Uvaria chamae* on the bacteria species showed that crude methanolic extracts of the roots of *Uvaria chamae* (400µg) had greater activity than other concentrations, clearing all bacteria tested and was highest for *Proteus vulgaris* (23.33 ± 0.54) when compared with controls; Ciprofloxacin (5µg) (33.67 ± 0.27) and gentamycin (10µg) (24.67 ± 0.72).

Lowest clearance was observed in UMR 10⁻¹ (40µg) concentration against *Staphylococcus aureus* (9.67 ± 0.27) among all tested organisms. No activity was observed with the extract for *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. UMR and UNR 10⁻² (4µg) extract was both not active against any bacteria tested.

Table 5 explained the comparison between the diameter of zones of inhibition of different organisms by crude extracts and control drugs. For root extracts, crude n-hexane extract of *Uvaria chamae* root (400µg) showed the highest clearance among all plant extract parts. This activity was for *Staphylococcus aureus* (25.00 ± 0.47), significantly differing with the control ciprofloxacin (5µg) (29.00±1.25) $p < 0.05$. Zones of clearance for UML and UMR also differed significantly in clearance in both controls gentamycin 10µg and ciprofloxacin (5µg). Crude UMR extract also showed highest clearance for *Proteus vulgaris* (23.33 ± 0.54), differing significantly with gentamycin control drug (16.67 ± 0.72) ($p < 0.05$).

For leaf extracts, the crude methanolic extract of *Uvaria chamae* leaf (400µg) showed the highest clearance among all plant extract parts and in *Bacillus subtilis* (24.33 ± 0.72), significantly differing from the control, Ciprofloxacin (5µg) (33.67 ± 0.27) $p < 0.05$. Also in the same bacteria, UMR and UNR showed a significant difference in clearance between their zones of inhibition and in both control drugs ($p < 0.05$).

For *Pseudomonas aeruginosa*, highest clearance was UML (23.67 ± 0.27) $p < 0.05$, but with significant difference between both controls in UMR only. *Proteus vulgaris* demonstrated more sensitivity to UMR (23.33 ± 0.54), $p < 0.05$ between it and gentamycin and also in UML.

UML crude extracts were more active to *E.coli* (15.33 ± 0.27) and *Salmonella typhi* (16.33 ± 0.54), with a significant difference between both control drugs and UML as well as UMR extracts respectively in both organisms.

Table I: Phytochemicals present in the *Uvaria chamae* plant parts extracts

Phytochemical constituents	Roots	Leaves
Alkaloids	++	+++
Glycosides	++ Anthracene derivatives	+
Saponins	+	+
Acidity	-	-
Lipid and oil	-	-
Tannins	+++	++
Flavonoids	+++	++
Terpenoids	+++	+++

Key:

+ = Present

++= Moderately present

+++ = highly present abundant

Table 2: Effects of crude aqueous extracts of *Uvaria chamae* leaves and roots on growth of test organisms.

Plant extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Proteus vulgaris</i>
UCLc (400µg)	7mm	7mm	7mm	7mm	7mm	7mm
UCRc (400µg)	7mm	7mm	7mm	7mm	14.00 ± 0.47	7mm

NB: Zones of inhibition of 7mm means no inhibitory effects. KEYS: UCL_c(*Uvaria* crude aqueous leave), UCR_c(*Uvaria* crude aqueous root).

Table 3: Effects of methanolic and n-hexane extracts of leaves of *Uvaria chamae* on the growth of test organisms.

Extract	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Proteus vulgaris</i>
UMLc (400µg)	24.33 ± 0.54	14.00 ± 0.94	15.33 ± 0.27	23.67 ± 0.27	16.33 ± 0.54	20.33 ± 0.27
10 ⁻¹ (40µg)	13.33 ± 0.54	11.33 ± 0.54	7	13.00 ± 0.47	7	7
10 ⁻² (4µg)	7	7	7	7	7	7
UNLc (400µg)	7	7	7	13.00 ± 0.47	7	7
10 ⁻¹ (40µg)	7	7	7	7	7	7
10 ⁻² (4µg)	7	7	7	7	7	7

Ciprofloxacin (5µg)	33.67 ± 0.27	29.00 ± 1.25	29.33 ± 0.54	25.33 ± 1.15	32.00 ± 0.47	22.33 ± 0.72
Gentamycin (10µg)	24.67 ± 0.72	26.00 ± 0.47	19.67 ± 0.72	21.33 ± 0.72	23.00 ± 0.82	16.67 ± 0.72

Table 4: Effects of methanolic and N-Hexane extracts of roots of *Uvaria chamae* on the bacteria species

Extracts	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhi</i>	<i>Proteus vulgaris</i>
UMRc (400µg)	16.67±1.19	13.67± 0.54	14.67± 0.27	21.67± 0.72	13.67±0.27	23.33 ± 0.54
10 ⁻¹ (40µg)	9.67± 0.77	9.67± 0.27	7	7	7	12.33 ± 0.27
10 ⁻² (4µg)	7	7	7	7	7	7
UNRc (400µg)	22± 0.00	25.00 ± 0.47	7	12.67 ± 0.27	7	7
10 ⁻¹ (40µg)	11.00 ± 0.82	13.00 ± 0.47	7	7	7	7
10 ⁻² (4µg)	7	7	7	7	7	7
Ciprofloxacin (5µg)	33.67 ± 0.27	29.00 ± 0.125	29.33 ± 0.54	25.33 ± 1.15	32.00 ± 0.47	22.33 ± 0.72
Gentamycin (10µg)	24.67 ± 0.72	26.00 ± 0.47	19.67 ± 0.72	21.33 ± 0.72	23.00 ± 0.82	16.67 ± 0.72

Keys:

UMRc = Methanol extracts of crude root extract

UNRc = N-Hexane extracts of crude root extract

Table 5: Comparison between the diameter of zones of inhibition of different organisms by crude extracts and control drugs.

Control Organism	Crude Extract (400µg)	Zone of Inhibition (mm) mean ± SEM	Gentamycin 10µg x= 26± 0.67	Ciprofloxacin (5µg) x = 29.00±1.25
			P – value	P – value
<i>Staphylococcus aureus</i>	UML	14.00 ± 0.94	P <0.05	P <0.05
	UMR	13.67 ± 0.54	P <0.05	P <0.05
	UNR	25.00 ± 0.47	P >0.05	P <0.05
			(x = 24.67 ± 0.72)	(x = 33.67 ± 0.27)
<i>Bacillus subtilis</i>	UML	24.33 ± 0.72	P >0.05	P <0.05
	UMR	16.67 ± 1.19	P <0.05	P <0.05
	UNR	22.00 ± 0.00	P <0.05	P <0.05
			(x ² = 21.33 ± 0.72)	(x ² = 25.33 ± 1.15)
<i>Pseudomonas aeruginosa</i>	UML	23.67 ± 0.27	P >0.05	P >0.05
	UMR	21.67 ± 0.72	P >0.05	P >0.05
	UNR	12.67 ± 0.27	P <0.05	P <0.05
			(x = 16.67 ± 0.72)	(x = 22.33 ± 0.72)
<i>Proteus vulgaris</i>	UML	20.33 ± 0.27	P <0.05	P >0.05
	UMR	23.33 ± 0.54	P <0.05	P >0.05
			(x = 19.67 ± 0.72)	(x = 29.33 ± 0.54)
<i>E.coli</i>	UML	15.33 ± 0.27	P <0.05	P <0.05
	UMR	14.67 ± 0.27	P <0.05	P <0.05
			(x = 23.00 ± 0.82)	(x = 32.00 ± 0.47)
<i>Salmonella typhi</i>	UML	16.33 ± 0.54	P <0.05	P <0.05
	UMR	13.67 ± 0.27	P <0.05	P <0.05

DISCUSSION

In this era of antibiotic-resistant “superbugs”, the need for developing new antibiotic agents is very important especially when they are natural and have few side effects. Plant extracts have attractive sources for new drugs since they contain plant-derived phytochemicals containing antimicrobial properties which are active against pathogens.

The result of the phytochemical analysis of the leaves and roots of *Uvaria chamae* indicated the presence of (tannins, flavonoids, terpenoids, Alkaloids, Glycosides (anthracene derivatives), saponins). Root extracts contained more of tannins, flavonoids and terpenoids and the leaves more of alkaloids and terpenoids. Both plant parts had no lipid and oil and were not acidic.

Variations in phytochemical constituents in *Uvaria chamae* root was also recorded by (Donatus and Iroabuchi, 2009). They observed that antimicrobial effects of the extracts may be attributed to a single or combined effect of these bioactive constituents.

The water extracts of the plant parts (crude extracts and dilutions) tested were low on antibacterial activities except for *Salmonella typhi* for which the aqueous extract of *Uvaria chamae* root produced inhibitions of 14.00 ± 0.47 at (400 μ g) concentration in table I. The poor activity seen with the water extracts is not so surprising since nearly all the identified components from the plants (phytochemicals) active against micro-organisms are aromatic or saturated organic compounds and were obtained through an initial ethanol or methanol extraction (Cowan, 1999).

In the present work, methanolic extracts appeared to be more superior in clearing the bacterial species tested. This was shown in the present study by its higher clearance rate than with other solvent extracts. Methanol and its high polarity dissolve a vast number of phytochemicals compounds with easy extract ability of polar primary and secondary metabolites and is cheaper (Abubakar and Haque, 2020). It has good polarity with almost zero dielectric constant and penetrates the cell content well making it a better solvent. The use of methanol in solvent extraction for antimicrobial studied is very much superior to the use of n-hexane for the same purpose in the present work. This agrees with the work of Rabe and Von Staden (1997) who demonstrated that the highest antimicrobial activity was found in methanol extracts of the leaf studied and also the work of (Unaeye *et al.*, 2019).

Methanolic extracts of *Uvaria chamae* leaf and roots were sensitive to all tested organisms. This proves it can penetrate the cells of all plant parts, bringing out their secondary metabolites and its suitability as an herbal extracting solvent. The highest activity observed in UMLC(400 μ g) (crude leaf extract) against all tested organisms compared to all the other extracts with highest clearance observed with *B. subtilis* (24.33 ± 0.72) when compared with the controls, Ciprofloxacin (5 μ g) (33.67 ± 0.27) and Gentamycin (10 μ g) (24.67 ± 0.72 mm) (table 3) also supports the explanation above confirming its potential as a possible antibiotic drug source. Even when compared with the zone of inhibition of control drugs in table 5, it was significantly comparable with Gentamycin (10 μ g) ($x = 33.67 \pm 0.27$ mm) in clearing *B. subtilis* ($p < 0.05$). Also for the same bacteria, UMR and UNR showed a significantly comparable clearance in their zones of inhibition and between both control drugs. Superiority of methanol solvent as a good extracting fluid for these plant part could be as a result of differences in their polarity.

Several studies have validated the superiority of ethanolic extract of plant materials over other solvents (Kigigha *et al.*, 2015; Opoku and Akoto, 2015) in line with findings in this research in table 5, where leaf extracts of crude methanolic extract of *Uvaria chamae* leaf (400 μ g) had the highest clearance among all plant extract parts for *Bacillus subtilis* (24.33 ± 0.72). This confirms its potential broad-spectrum nature for spore bearers, Gram-positive and negative bacteria. At a concentration of 10^{-1} (40 μ g), it still had activity for *Bacillus subtilis* (13.33 ± 0.54 mm) *Pseudomonas aeruginosa* (13.00 ± 0.47 mm) and *Staphylococcus aureus* (11.33 ± 0.54 mm), with *Staphylococcus aureus* recording the lowest activity among all. Reasons could be as earlier stated, as well as variations in cell wall sizes. This herbal part therefore seems to penetrate gram-negative organisms more. In a study by Koudokpon *et al.* (2018), several multidrug-resistant gram-positive bacteria were highly sensitive to ethanolic extracts of *Uvaria chamae* roots and the activity was more extensive than that from conventional broad-spectrum antibiotics. The ion mobility mass spectrometry analysis revealed the presence of ten chalcone and dihydrochalcone chemical structures responsible for the antimicrobial activity of *Uvaria chamae* ethanol extracts confirming and justifying the medical properties of these roots used as a traditional medicine.

There was no activity at all observed for UMLC 10^{-1} (4 μ g) leaf extract dilution concentration and in *Uvarian*-hexane crude leaf extract, 10^{-1} (UNL_c) (40 μ g) and its corresponding 10^{-2} (4 μ g) diluted concentrations respectively (7mm). This could be attributed to the dose-response relationship (Eloff, 2019). UNL_c (400 μ g) had activity only for *Pseudomonas aeruginosa* (13.00 ± 0.47) in the research. Non-polarity of the solvent extract could be contributory to its low activity, though value obtained for *Pseudomonas* was significantly comparable with the control drug Gentamycin 10 μ g (21.33 ± 0.72) $p < 0.05$.

In table 4, UNR crude root extracts of the non-polar solvent were also potent and at 10^{-1} (40 μ g) dilution of the extract, though its potency as antimicrobial have been documented to contain saponins, alkaloids and hydrolysable tannins, it is not as much as that seen with methanolic extract. Its activity may be low for bacteria due to its variations in selectivity of solubility for antibiotic metabolites which is dependent on concentration or mass loss produced by some non-polar solvents due to their poor solubility. This was also observed by (Ogbuanu *et al.*, 2020). Okwuosa *et al.* (2012) noted its high activity against *Candida spp.* That means, selection of its activity could also be dependent on the microbial class involved. Crude methanolic extracts of the roots of *Uvaria chamae* (400 μ g) had greater activity than other root concentrations, clearing all bacteria tested and highest for *Proteus vulgaris* (23.33 \pm 0.54) compared with controls. Activity on several bacteria can be attributed to the high nutritive value and multi-metabolite contents in *Uvaria chamae* roots (Enin *et al.*, 2021).

Lowest clearance was observed in UMR 10^{-1} (40 μ g) concentration against *Staphylococcus aureus* (9.67 \pm 0.27) among all tested bacteria. Findings from this work contradicted that by Inyang and Fischer (2017) who found no inhibition zone on *Staphylococcus aureus* with ethanoic extracts of *Uvaria chamae* root at 25mg/dl, 50mg/dl, 100 and 200mg /dl concentrations. Differences in phytochemical constituents as well as factors that affect in-vitro testing of herbal plants could be contributory. However, no activity was observed with the UMR 10^{-1} (40 μ g) extract on *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. UMR and UNR 10^{-2} (4 μ g) dilutions of root concentrations extracts were both inactive against all bacteria tested (7mm) maybe because of lowered potency of the extracts.

With regards to plant parts, while comparing between the diameter of zones of inhibition of different organisms by crude extracts and control drugs, for root extracts, crude N-hexane extract of *Uvaria chamae* root UNR_c (400 μ g) showed highest clearance among all plant extract parts and this clearance was for *Staphylococcus aureus* (25.00 \pm 0.47) significantly comparable with inhibition seen in the control Ciproxin (5 μ g) (29.00 \pm 1.25) $p < 0.05$. Zones of clearance for UML and UMR also compared significantly between both control drugs. Crude UMR extract also showed highest clearance for *Proteus vulgaris* (23.33 \pm 0.54), significantly comparable with inhibition seen with Gentamycin control drug (16.67 \pm 0.72) ($p < 0.05$). Compounding factors could explain the reasons for choice of resistance or susceptibility of an organism for a plant part dissolved in a particular solvent as factors may combine to determine the susceptibility pattern of a plant extracts.

Izah (2018) noted that environmental factors (pH of a medium, temperature, water activity, oxygen, nutrient availability, choice of solvent, source of the organism, biochemistry, physiology, metabolism, adaptation strategies of the microbes, plant species, biochemistry, age, concentration of plant extract and period of extraction are important contributory factors to consider.

The high activity seen in the leaf extracts could be because they are aerial plant part and have rich metabolites in their reproductive parts. In general, whether more active metabolites are found on root or leaf extract may also have depended on if the herbal plant is annual or perennial, as there is a general observation that annual plants have more secondary metabolites in their reproductive parts, while perennial plants contain theirs in their roots, rhizomes and bark (Wink, 2010).

Oluremi *et al.* (2010) compared the methanolic extracts of root, stem bark and leaves of *Uvaria chamae* against Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, and typed strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC27853 and found that the stem bark extract inhibited the growth of all the tested organisms with leaf extract showing the least antibacterial activity.

There was a variation in selectivity of their action using different solvents in the present research which could be as a result of their choice in ability of the solvent to diffuse into the plant cell, dissolve the secondary metabolite and move back out of the cell. This observation supports the fact stated by Caecer and Cech (2019) on the need to be aware that a single active compound obtained through bioassay – guided fractionation is not enough to capture the potential a plant may have as an antibacterial agent. Metrouth-Amir *et al.* (2015) noted that extract yield and the resultant plant material biological activities depend on nature of solvent used in extraction, which is a function of differences in chemical characteristics and polarity.

The excellent inhibitory activities of the crude extracts of the plant parts against *Pseudomonas aeruginosa*, seen especially in crude UML and UMR is an important finding owing to the involvement of this organisms in hospital acquired infections. The mean zones of inhibition *Pseudomonas aeruginosa* by crude UML and UMR were all comparable to those of the clinical antibiotics used. The antibacterial activities of this plant thus justifies its use in folk medicine for the treatment of infectious diseases. This is also supported by findings in various literature studies that have confirmed bioactive compounds present in *U. chamae* to contain different phytochemicals such as alkaloids, flavonoids, phenols, tannins, and terpenoids with antibacterial, antifungal, and antimalarial effects (Okwu and Iroabuchi, 2009; Oluremi *et al.*, 2010).

In conclusion, the study proved that the extracts of the leaves and roots of *Uvaria chamae* have antimicrobial metabolites such as flavonoids, alkaloids and tannins etc. and had activity against spore bearers' Gram - positive and Gram-negative bacteria. These substances even had activity when diluted, although the exact active components in the extracts metabolites that showed this effect were not identified. Standardizing methods of extraction and in-vitro testing for a more systemic interpretation of the test results could be facilitated as well harnessing the plant parts for antibiotic use. However, it is recommended that more work should be done to purify the active chemicals from the crude, via positioning with various

solvents and chromatographic analysis to obtain various fractions including using chemical structure analysis using bioautographic, radioimmune or other new molecular assay as well as testing it against other microbes.

CONSENT

It is not applicable.

REFERENCES

Abu T, Rex-Ogbuku EM, and Idibiye K. (2018). A review: secondary metabolites of *Uvaria chamae* p. Beauv. (Annonaceae) and their biological activities. *International Journal of Agriculture, Environment and Food Sciences* 2(4);177-185.

Abubakar AR and Haque M. Preparation of Medicinal Plants: Basic extraction and Fractionation procedures for experimental purposes. *Journal of Pharm Bio allied Science*.2020;**12**(10): 1-10.

Caecer LK, and Cech NB. Synergy and antagonism in natural product extracts: when 1+1 does not equal to 2. *Natural Products Report*. 2019; **36**: 869-888.

Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*.1999; 12 (4) P.564-382.

Eloff JN. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. *BMC Complement Alternative Medicine*. 2019;**19**, 106(109).

Eloff JN. Which extract should be used for the screening of antimicrobial components from plants? *Journal of Ethnopharmacology*. 1998; **160**:1-8.

Enin GN, Shaibu SE, Ujah GA, Richard O, Ibu RO and Inangha PG., Gbulie J.N, Ogueke C.C. and Nwanebu F. N. Antibacterial properties of *Uvaria chamae*, *Congronematifolium*, *Garcinia kola*, *Vernoniaamygdalina* and *Aframomiummelegueta*. *African Journal of Biotechnology* 2021;**6** (13): 1549-1553.

Inyang IJ, Fischer V, Eyo AAO, and Udongkang MI. Antibacterial activity of ethanolic root extracts of *Uvaria charmae* on *Staphylococcus aureus*. *European Journal of Pharmaceutical and Medical Research*.2017;**4** (9); 127-130.

Iwu EC. Ethnobotanical importance of some Nigerian plants. *Journal of Ethno medicine*. 1993;**55**: 893-906.

Izah SC. Some determinant factors of antimicrobial susceptibility pattern of plant extracts. *Research and Review Insights*. 2018; **2** (3): 1-4.

Jalil J, Attiq A, Hui CC, Yao LJ, and Nurul Aimi Zakaria NA.(2020). Modulation of inflammatory pathways, medicinal uses and toxicities of *Uvaria* species: potential role in the prevention and treatment of inflammation. *Infammo pharmacology* 28:1195–1218.

Kigigha LT, Izah SC. and Ehizibue M. Activities of *Aframomiummelegueta* seed against *Escherichia coli*, *S. aureus* and *Bacillus* species. *Point Journal of Botany and Microbiology Research*. 2015; **1**: 23-29.

Koudokpon H, Armstrong N, Dougnon TV, Fah L, Hounsa E, Bankole HS, Loko F, Chabriere E, and Rolain JM. (2018). Antibacterial Activity of Chalcone and Dihydrochalcone Compounds from *Uvaria chamae* Roots against Multidrug-Resistant Bacteria. *Biomed Research International* 2018:1453173

Legba B, Dougnon V, ChabiY, Gbaguidi C, Aniambossou A, Deguenon E. Dougnon J, Kpodekon M, and Baba-Moussa L.(2020). Evaluation of in-vivo anti-*Salmonella* activity of *Uvaria chamae*, *Lantana camara* and *Phyllanthusamarus* used in Benin, West Africa. *BMC Veterinary Research* **16**, 49.

Lovian V. Antibiotics in Laboratory Medicine. 7th ed. Pg 20 Williams and Williams, Baltimore, England.1980.

Metrouh- Amir H, Duarte CMM, and Maiza F. Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of *Matricariapubescens*. *Industrial Crops and Products* .2015; 67: 249-256.

Obasi DC and Obasi JN. (2021). Comparative study on the levels of phytochemicals present in n-hexane, ethanol, aqueous, ethylacetate and methanol extracts of *Uvaria chamae* stem. *Journal of Medicinal Plants Studies* 2021; 9(3): 195-200.

Ogbu SI, Okafor EO, Obeagu EIC, Ogbu CC, Esimai BN, Okeke NJ, and Ukeekwe CO. (2020).. Effect of oral administration of the leaf extract of *Uvaria chamae* (Mmimiohia) in Albino Wistar Rats. *Journal of Pharmaceutical Research International* 32(24): 52-56.

Ogbuanu CC, Amujiogu SN, and Agboeze E. Secondary metabolites investigation and TLC analysis of leaves, stem bark and root extracts of *Uvaria chamae* (UDAGU). *Journal of Natural Science Research*. 2020;10(10): 2224-3186.

Okwu DE, and Iroabuchi F. (2009). Phytochemical composition and biological activities of *Uvaria chamae* and *Clerodendronsplendens*. *Journal of Chemistry*. 6(2):553-560.

Okwuosa OM, Chukwura EI, Chukwuma GO, Okwuosa CN, Enweani IB, Agbakoba NR, Chukwuma CM, Manafa PO, and Umedum CU. Phytochemical and antifungal activities of *Uvaria chamae* leaves and roots, *Spondiamombin* leaves and bark and *Combretumracemosum* leaves. *African Journal of Medicine and Medical Science*. 2012;41 suppl:99-103.

Olumese FE, Onoagbeb IO, Eze GI, and Omoruyi FO. Safety assessment of *Uvaria chamae* root extract: acute and sub-chronic toxicity studies. *Journal of African Association of Physiological Science*.2016;4 (1): 53-60.

Oluremi BB, Osungunna MO, and Omafuma OO. (2010). Comparative assessment of antibacterial activity of *Uvaria chamae* parts. *African Journal of Microbiology Research*4(13): 1391-1394.

Omale J, Ebiloma UG, and Idoko GO. *Uvaria charmae* (Annonaceae). Plant extract neutralizes some biological effects of *Najanigracolis* snake venom in rats. *British Journal of Pharmacology and Toxicology*. 2013; 4(2): 41-50.

Opoku F and Akoto O. Antimicrobial and phytochemical properties of *AlstoniaBoonei* Extract. *Organic Chemistry Current Research* 4. Phytochemical and Nutritive Composition of *Uvaria chamae* P. Beauv. Leaves, Stem Bark and Root Bark. *Chem Search Journal* .2015;12(1): 9 – 14.

Padma KR, Don KR and Josthna P. (2020). Herbal Plant *Uvaria* Species and Its Therapeutic Potentiality. *World Journal of Environmental Biosciences* 9 (1) :33-38.

Popoola TD, Awodele O, Babawale F, Oguns O, Onabanjo O, Ibanga I, Godwin H, Oyeniyi T, Fatokun AA and Akinloye O. (2020). Antioxidative , antimitotic, and DNA-damaging activities of *Garcinia kola* stem bark, *Uvaria chamae* root , and *Olaxsubscorpioidea* root used in the ethnotherapy of cancers. *Journal of Basic Clinical Physiology and Pharmacology*31(3): 2019007.

Rabe T and Von-Staden J. Antibacterial activity of African Plants used for Medicinal purposes. *Journal Ethnopharmacology*. 1997; 56 (1): 81-87.

Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria.1993: 289.

Trease GE, and Evans WC. Pharmacognosy 11th edition. BrailliarTiridel Can. Macmillian publishers. 1989.

Unaeze BC, Ochiabuto OMTB, Ejike EC, Obi MC, Nwankpa SN. Antimicrobial activities of *Irvingia gabonensis* leaf against diarrhea causing agents. *International Journal of Advanced Engineering Research and Science*. 2019;6 (3): 234-243.

Wink M. Introduction. *Annual plant Reviews, biochemistry of plant secondary metabolism*. (Hoboken, NJ, USA: Blackwell Publishing Ltd). 2010; 40, 1-20.