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* was studied to demonstrate its potential as a chemotherapeutic agent. Test organisms include: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi and Proteus vulgaris* were used. Fresh tender leaves and roots of *Uvaria charmae* were collected, air- dried, grounded and soarked in methanol, n-hexane and aqueous solvents. Phytochemistry was done using standard procedures and Antimicrobial activities were determined using agar well diffusion method(punch hole) then MIC was determined. Qualitative Phytochemical analysis confirmed the presence of alkaloids, tannins, terpernoids, glycosides, saponins and flavonoids. Antimicrobial analysis of the plant parts using disc-diffusion method showed Ten (10mg/ml) MIC concentration for aqueous extract, 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, *Pseudomonas aeruginosa* (23.67± 0.27mm), clearance higher 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 the 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 its 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. Comparing inhibitory property of (UML (20.33± 0.27mm) and UMR (23.33± 0.54mm) against *Proteus vulgaris* was significantly greater than that for 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 treating *Salmonella typhi* while UML_c and UMR could be harnessed for *Proteus vulgaris* and UML for *Pseudomonas aeruginosa* eradication. *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); "Kas Kaifi" in Hausa; "Eruju" or "Okoaja"in Yoruba; "Nkarika Ikot" 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 micro-organisms 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.

The aim of the work therefore are 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 7days, separately grounded to a fine powder with a mechanical grinder and pulverized. The resulting powder was weighed, 20 g collected and extracted using water, methanol and n-hexane as described by Eloff (1991) after soaking in 100 ml 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 $2\text{-}4^0$ 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, terpernoids,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 10 mg/ml (10,000µg/ml). One hundred (100 mg) of the concentrated aqueous extracts was dissolved in 10 ml of normal saline to obtain a stock of 10 mg/ml. One hundred (100 mg) of the metabolic extracts (leaves / roots) and 100 mg of n-hexane extracts (roots) were separately dissolved in 10 ml of dimethylsulphoxide (DMSO).
The n- hexane extracts of the leaves were dissolved in 10 mls of 3% tween 85 to obtain a stock of 10 mg/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 Preparation 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 (10 mg/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 organisms and all processes repeated in triplicate. On each plate, (10 µg) Gentamycin and (5 µg 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 consider 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, terpernoids, alkaloids, glycosides (anthracene derivatives), saponins). More of tannins, flavonoids and terpernoids 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 UMLc(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	+++	++
Terpernoids	+++	+++

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	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Proteus vulgaris*
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 Effect of methanolic and n-hexane extracts of leaves of *Uvaria chamae* on the growth of test organisms.

Extract	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Proteus vulgaris*
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:Effect of methanolic and N-Hexane extracts of roots of *Uvaria chamae* on the bacteria species

Extracts	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Proteus vulgaris*
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
Staphylococcus aureus	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)
Bacillus subtilis	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)
Pseudomonas aeruginosa	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
Proteus vulgaris			(x = 16.67 ± 0.72)	(x = 22.33 ± 0.72)
	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)
E.coli	UML	15.33 ± 0.27	P <0.05	P <0.05
	UMR	14.67 ± 0.27	P <0.05	P <0.05
Salmonella typhi			(x = 23.00 ± 0.82)	(x = 32.00 ± 0.47)
	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, terpernoids, Alkaloids, Glycosides (anthracene derivatives), saponins). Root extracts contained more of tannins, flavonoids and terpernoids and the leaves more of alkaloids and terpernoids. 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). The observed antimicrobial effects of the extracts may be attributed to a single or combined effect of these bioactive constituents.

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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 (400µg) 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 dissolves 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 is in agreement 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 Unaeze *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(400µg) (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 (5µg) (33.67 ± 0.27) and Gentamycin (10µg) (24.67 ± 0.72mm) in 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 (10µg) (x = 33.67 ± 0.27mm) in clearing *B. subtilis* ($p<0.05$).Also in 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 (400µg) 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} (40µg),it still had activity for *Bacillus subtilis*(13.33±0.54mm) *Pseudomonas aeruginosa* (13.00±0.47mm)and *Staphylococcus aureus* (11.33 ± 0.54mm) 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.

There was no activity at all observed for UML_C 10^{-1} (4µg) and in *Uvaria* n-hexane crude leaf extract, $10^{-1}(UNL_c)$ (40µg) and its corresponding 10^{-2} (4µg) diluted concentrations respectively (7mm). This could be attributed to the dose-response relationship (Eloff, 2019). UNL_c (400 µg) 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 10µg (21.33± 0.72) $p<0.05$.

In table 4, UNR crude root extracts of the non-polar solvent were also potentand at 10^{-1} (40µg) 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 microbial classes.

Crude methanolic extracts of the roots of *Uvaria chamae* (400µg) had greater activity than other root concentrations, clearing all bacteria tested and highest for *Proteus vulgaris* (23.33 ± 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 µg) concentration against *Staphylococcus aureus* (9.67 ± 0.27) among all tested. 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} (40µg) extract on *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*. UMR and UNR 10^{-2} (4µg) dilutions of root concentrations extracts were both inactive against all bacteria tested (7mm), maybe caused by lowered potency of the extracts.

While comparing between the diameter of zones of inhibition of different organisms by crude extracts and control drugs, in root extracts, crude N-hexane extract of Uvaria *chamae* root UNR_c (400µg) showed highest clearance among all plant extract parts and this clearance was for *Staphylococcus aureus* (25.00 ± 0.47) significantly comparable with inhibition seen in the control Ciproxin (5µg) (29.00±1.25)$p<0.05$. Zones of clearance for UML and UMR also compared significantly with both control drugs. Crude UMR extract also showed highest clearance for *Proteus vulgaris* (23.33 ± 0.54), significantly comparable with inhibition seen with Gentamycin control drug (16.67 ± 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, and parts, 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).

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 P*seudomonsa 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 *Pseudomonsa 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.

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.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable

REFERENCES

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

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

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

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

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

Enin G.N, Shaibu S.E, Ujah G.A, Richard O, Ibu R.O and Inangha P.G.Gbulie J.N, Ogueke C.C. and Nwanebu F. N. Anti-bacterial properties of *Uvaria chamae, Congronemalatifolium, Garcina kola, Vemoniaamygdalina and Aframomiummelegueta*. *African Journal of Biotechnology* 2021;**6** (13): 1549-1553.

Inyang I.J, Fischer V, Eyo A.AO, and Udongkang M.I. 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 E.C. Ethnobotanical importance of some Nigerian plants. *Journal of Ethno medicine*. 1993**;55**: 893-906.

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

Kigigha L.T, Izah S.C. and Ehizibue M. Activities of *Aframomum melegueta* seed against *Escherichia coli, S. aureus* and Bacillus species. *Point Journal of Botany and Microbiology Research* . 2015;**1**: 23-29.

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

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

Ogbuanu C.C, Amujiogu S.N, 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.

Okwuosa O.M, Chukwura E.I, Chukwuma G.O, Okwuosa C.N, Enweani I.B, Agbakoba N.R, Chukwuma C.M, Manafa P.O, and Umedum C.U. Phytochemical and antifungal activities of *Uvaria chamae* leaves and roots, *Spondia smombin* leaves and bark and *Combretum racemosum* leaves. *African Journal of Medicine and Medical Science*. 2012;41 suppl:99-103.

Olumese F.E, Onoagbeb I.O, Eze G.I, and Omoruyi F.O. 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.

Omale J, Ebiloma U.G, and Idoko G.O. Uvaria charmae (Annonaceae). Plant extract neutralizes some biological effects of *Najanigricolis* 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.*

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

Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria.1993; p. 289.
Trease G.E, and Evans W.C. Pharmacognsy 11th edition. BrailliarTiridel Can. Macmillian publishers. 1989.

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

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