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Research Article

Comparative *In-Vitro* Analysis and Secondary Metabolites Screening of *Uvaria afzelii* (Scott-Elliot) and *Tetrapleura tetraptera* (Schumach and Thonn) on Selected Multiple Antibiotics Resistant Isolates

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Abstract

The antibacterial effect of ethanol extract of *Uvaria afzelii* and ethyl acetate extract of *Tetrapleura tetraptera* was investigated against multidrug resistant organisms using the agar diffusion techniques. The agar diffusion was used to test the antibacterial potentials of the extract at different concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. The extracts were tested against three (3) Gram positive and seven (7) Gram negative bacteria. The ethanolic extract of *Uvaria afzelii* and ethyl acetate extract of *Tetrapleura tetraptera* displayed higher activities against gram positive multiple resistant bacteria isolates than the gram negative multiple resistant isolates. However, the ethyl acetate extracts have more inhibitory potential than the ethanolic extracts. The antibacterial screening shows that the diameter of zones of inhibition ranges from 10mm against *Salmonella gallinarum* to 22mm against *Staphylococcus aureus* on both extracts at 100mg/ml. The Minimum Inhibitory Concentration (MICs) and Minimum Bacteriicidal Concentration (MBCs) were determined and the MIC and MBC ranges from 12.5mg/ml to 100mg/ml for both plant extracts. The qualitative and quantitative secondary metabolites screening of *Uvaria afzelii* leaf extract and *Tetrapleura tetraptera* stem bark extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, steroids, saponins, tannins, anthraquin, pyrrolizidine alkaloid and reducing sugars as well as the value of each secondary metabolite in quantity while the presence of the volatile oil was not determined. These compounds are responsible for this broad antibacterial activity. The results suggest that the extracts possess some active components that may be used for the development of therapeutic agents for the treatment of infectious agents.

Keywords: *In-Vitro* Analysis; Secondary Metabolites Screening; Selected Multiple Antibiotics Resistant Isolates; *Tetrapleura tetraptera* (Schumach and Thonn); *Uvaria Afzelii* (Scott-Elliot)

Introduction

Tetrapleura tetraptera is a deciduous tree that sheds its leaf annually and grows approximately 20-25m in height. It is distinguished by a round smallish crown that tends to flatten when old. Younger trees of *Tetrapleura tetraptera* have slender bole however, the older ones have low and sharp buttress. The grey-brownish bark has a very smooth texture while the leaves

are glabrous and hairy in appearance. It bears up to 5-10 pairs of pinnae that measure approximately 5-10 cm long with 6-12mm leaves on both sides of the pinna stalk. The top of the tree can be marginally notched sometimes while the base is basically hairless with slender stems [1].

The flowers are pinkish-cream turning to orange and are densely crowded in spike racemes 5-12 cm long, usually in pairs in the upper leaf axes. The fruit is persistently hanging at the end of branches on stout stalks, 15-25 mm long by about 5 cm across the wing-like ribs; dark purple- brown, glabrous and glossy, usually slightly curved. Two of the wings are hard and woody and the other two filled with a soft sugary pulp. The seeds are hard, black, flat,

oval, about 8 mm long, embedded in the body of the pod which does not split. The wood is reddish to brown heart wood, fairly hard, sapwood white. The powdered fruit is used as fish poison and in ointment for the treatment of skin diseases [2]. The intensive odour produced when the fruit is roasted is claimed to repel insects and snakes. The methanol extract of the fruit which was linked to their saponin content has been reported to have molluscicidal property and its mechanism of action is by ultrastructural effects of the snail digestive system [3]. The leaves, bark, roots, fruits and kernels are used for medicinal purposes [4]. *Tetrapleura tetra-
raptera* is used for the treatment of skin diseases, stem bark extract for the treatment of gonorrhea [5]. The plant is used in West Africa to flavor soups and taken as general tonics and stimulant or as part of postpartum diet therapy [6]. The powdered fruit is used as fish poison and in ointment for the treatment of skin diseases [7]. The intensive odour produced when the fruit is roasted is claimed to repel insects and snakes [8]. The leaves, bark, roots, fruits and kernels are used for medicinal purposes [7]. *Tetrapleura tetra-
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raptera* is used for the treatment of skin diseases, stem bark extract for the treatment of gonorrhea [11]. The plant is used in West Africa to flavor soups and taken as general tonics and stimulant or as part of postpartum diet therapy [13]. *Tetrapleura tetra-
raptera* is a perennial tree which is commonly distributed along the Tropical regions of Africa. The common names of *Tetrapleura tetra-
raptera* are: Gum tree (English), Ìshíhí (Oshoho), Aridan (Yoruba), Ighimiaka (Bini) and Edeminnangi (Efik). In Nigeria, it is used for numerous purposes [14]. According to the international plant index, the plant *Tetrapleura tetra-
raptera* was classified as Kingdom: Plantae, Phylum: Angiosperms, (Unranked): Eudicots, (Unranked): Rosids Order: Fabales, Family: Fabaceae, Genus: *Tetrapleura*, Species: *tetra-
raptera* and its binomial name: *Tetrapleura tetra-
raptera*



Figure 1a: *Tetrapleura tetra-
raptera* (Source:11).

The stem and bark of this plant has an inhibitory effect on the leutenizing hormone released by the pituitary gland. This suggests why this plant has a contraceptive property. Both the stem, leaves and fruits are used as concoction for managing convulsion, hence, its anticonvulsant properties. The extract of this plant is known for its anti-inflammatory properties and this suggest its inhibitory impacts against certain human pathogens [11] As a result, it can be used for reducing inflammation of the body, arthritic pains and rheumatoid pains.

The dried tub fruit is also known for its distinguished aromatic and flavorful fragrance and as such used as a spice for flavouring assorted dishes such as meat pepper soup, palm karnel soup. The bark also supports the cardiovascular system due to its constituents of essential phytochemical and as such can be used for preventing and treating heart diseases [15]. The pods contain essential chemical compounds such as flavonoids, triterpenoid glycoside (aridanin) and phenols, which have been reported effective for healing wounds. The taub is an excellent source of antioxidants such as polyphenols, alkaloids, tannins and flavonoids.

Antibacterial ability of the plant has been revealed by researchers that water extract and alcoholic mixture of Aridan fruit can inhibit the growth of *Staphylococcus aureus*. The presence of glycosides and tannins in ethanolic and water extract have been proven effective for inhibiting the growth of bacteria [16]. It is also used for dermatological care as the fruit can be dried and blended into powdered form for producing dermatological products such as soap. The great attention drawn to the use of this

plant for manufacturing soap is due to its high antimicrobial and antibacterial properties. It is worthy of note that the Aridan plant helps to promote soap forming as well as its hardness. To make soap with Aridan, the dried powdered herbs can be combined with shear butter, palm kernel oil or any other bases of choice. Studies reveals that the aqueous extracts from the stalk, leaves, bark and root of the Aridan plant contains molluscidal properties. This suggests why this plant acts as a pesticide for fighting against molluscs and pest. Aridan is normally used in gardening, planting and agriculture for offering protections and control against gastropod pests especially snails and slugs that feed on/damage crops and other valuable plants in the farmland [16].

Uvaria afzelii (UV) is a small tree or spreading shrub growing up to 5m tall. *Uvaria* is a genus of flowering plants in the soursop family, *Annonaceae*. The generic name is derived from the Latin “uva” meaning grape, likely because the edible fruit of some species in genus resembles grape. The tree is used locally, being harvested from wild for food and medicines. It is widely distributed and grown in the South and Eastern part of Nigeria, where it is known by various names such as “gbogbonishe” (Yoruba), “Umimiofia” (Igbo) and “osu-umimi” (Ukwani) (Odugbemi, 2015). Locally it is used in the treatment of cough, vaginal tumor, breast aches, swollen hands feet’s, diabetes as well as leucorrhoea and gonorrhea [17]. *Uvaria afzelii* is a scrambling shrub or small tree to 5m high, majorly found in the tropical part of West-Africa especially from Guinea to southern Nigeria [18]. According to the international plant name index, the plant *Uvaria afzelii* Scott Eliot was classified under the following: Kingdom: Plantae, Phylum: Angiosperms, Class: Magnoliids, Order: Magnoliales, Family: Annonaceae, Genus: *Uvaria*, Species: *afzelii* and its binomial name *Uvaria afzeli*

Ethno medicine of *Uvaria afzelii* (Scott-Elliot) A number of investigations carried out to ascertain the claimed uses of the plant includes its reported bactericidal activity against Gram-positive and acid-fast bacteria [19], antihelminthic and ant parasitic activities [18]. Other ethno medicinal uses of the plant includes its benefit as a remedy for jaundice, infections of the liver, kidney, and bladder. Silymarin is a standardized extract of the milk thistle plant (*Silybum marianum*) which majorly contains flavonoids: *silybin*, *silybinin*, *silydianin* and *silychristin* [20]. Seeds of this plant have been used for years to treat liver and gall bladder disorders, including hepatitis, cirrhosis and jaundice and to protect the liver against poisoning from chemicals, environment toxins, snake bites, insect stings, mushroom poisoning and alcohol [21]. The fruit is edible [17] the leaves are used for treating fever locally [22] and boiled with pepper are taken in draught, or rubbed on the skin for yellow fever in Nigeria. The plant is held to be good for bronchial troubles and for stomach ache in Ivory Coast and in the Gagnoa area pulped leaves are eaten with oil-palm.

The root is used in Nigeria for treating gonorrhea, and the root-bark is taken internally for catarrh, inflammation of the mucous membranes, bronchitis and also for gonorrhea. Some of its benefits also includes its contraceptive properties in that the leaf of this plant has inhibitory effect on the leutinizing hormones released by the pituitary gland. In the management of convulsion both the stem and the leaves are used as study reveals that aqueous extract of *Uvaria afzelii* exhibit anticonvulsant activities and this conforms its inhibitory effect on the Central nervous system. The extract of this plant is also known for its anti-inflammatory properties and this suggest it inhibitory impact on certain human pathogen. As a result, it can be used for reducing inflammation of the body, arthritic pains and rheumatoid pains. *Uvaria afzelii* also support the cardiovascular system due to its constituents of essential phytochemical and as such can be used for preventing and treating heart diseases. In folk medicine, the stem and leaves extracts of *Uvaria afzelii* can be used in the preventing and treatment of hypertension [23] Researchers agree that *Uvaria afzelii* is effective in preventing high blood pressure and for improving the oxidative position in salt model of hypertension patients.

The stem and bark of *Uvaria afzelii* can also be used for preparing herbal medicines for treating diabetes. Being an excellent source of key vitamins such as potassium, iron, calcium, magnesium, and zinc, *Uvaria afzelii*, helps to strengthen our immune system. Iron helps to regenerate lost blood, zinc offers protection against viruses especially those that can cause respiratory tract infections while calcium and potassium helps to manage, prevent and control bones and muscles disorder. *Uvaria afzelii* leaves is traditionally used for preparing special soup for new born mothers immediately they put to bed to avoid post-partum contraction [23].

Materials and Methods

Source and collection of plant samples

The leaves and stem-bark of the plant *Tetrapleura tetraaptera* and *Uvaria afzelii* were collected early in the morning into a polythene bag from Adekunle Ajasin University Akungba Ondo State with latitude of 7.4792 and longitude of 5.7484. The leaves and stem bark were dried in the laboratory (room temperature) for about two weeks and pulverized [24].

Authentication of plant samples

The plants were authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

Preparation of plant samples

The stem bark and leaves of *Tetrapleura tetraaptera* and *Uvaria afzelii* after collection were first washed thoroughly with sterile distilled water and appropriately air dried at room temperature [24].

Extraction solvents

The extraction solvents used were ethyl acetate and absolute ethanol

Extraction of plant material

The parts of various plants were dusted and air dried at room temperature and then soaked for seven days (10) and filtered with a muslin cloth and filter paper. Extracts were collected and concentrated under reduced pressure using rotary evaporator at 40°C, then reconstituted with 20% Dimethyl Sulphoxide (DMSO). The stock extracts were kept in the refrigerator at 4°C until use [25].

Percentage yield of the extracts

The 200g of the air dried bark of *Tetrapleura tetraptera* yielded 3g and 500g of *Uvaria afzelii* leaves yielded 5.5g of extract after the extraction.

Standardization of plant extracts

At aseptic condition, the extracts were reconstituted by adding 1g of each extracts to 2.5ml of DMSO and 7.5ml of sterile distilled water, making it 100mg/ml. For each extract, 5ml of distilled water is measured into three sterile bijou bottles. In bijou bottle, 3ml from the 100mg/ml bijou bottle was drawn and added into the sterile bijou bottle B, making it 50mg/ml. The serial concentration was prepared to get concentration of 50mg/ml, 25mg/ml and 12.5mg/ml respectively using the $C1V1=C2V2$ formula [26].

Test organism

The test organisms used were standard strains of bacteria and clinical fungal isolate. They include *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus typhii*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Salmonella gallinarum*

Source of test microorganisms

These organisms were obtained from the stock culture in the laboratory of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria

Standardization of test organisms

Slants of the various organisms were reconstituted at aseptic condition, using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, 0.1ml of the isolated colony was transferred into 9.9ml of sterile distilled water contained in each test tube using a sterile needle and syringe, and then mixed properly. The liquid now serves

as a source of inoculum containing approximately 10^6 cfu/ml of bacterial suspension [27].

Antibacterial screening of *Uvaria afzelii* and *Tetrapleura tetraptera* extract

All the test bacteria, were sub-cultured into sterile Mueller Hinton agar plates, and incubated at 37°C for 18-24 hours. Ten distinct colonies for each organism were inoculated into sterile Nutrient agar broth and incubated for 6-18 hours. All inocula were standardized accordingly to match the 0.5 McFarland standards, and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations; 100, 50, 25, 12.5mg/ml, using Dimethyl sulphoxide (DMSO). The susceptibility testing was investigated by the agar well diffusion method. A 0.1ml of 1: 10,000 dilutions (equivalent to 10^6 cfu/ml) of fresh overnight culture of the multiple resistant isolates grown in Nutrient agar broth was seeded into 40ml of Mueller Hinton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile Cork borer of 6mm diameter, equidistant wells were made in the agar. Drops of the re-suspended, (2ml per well) extracts with concentrations between 100 mg/ml to 12.5mg/ml were introduced into the wells till it was filled. Chloramphenicol 50 mg/ml was used as the control experiment for bacteria. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37°C for 24 hours for the bacterial isolates. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates [27].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the extract which resulted in maintenance or reduction of inoculums' viability was determined by macro broth tube dilution technique [28] for the bacterial isolates. Different concentrations ranging from 100mg/ml to 3.125mg/ml of the crude extract prepared by serial dilutions in double strength Mueller Hinton broth medium. A set of tubes was then inoculated with 1ml of the test organism. Two blank Mueller Hinton broth tubes, with and without bacterial inoculation, were used as the growth and sterility controls. The tubes were incubated at 37°C for 24 h. After the incubation period, the tubes were observed for the MICs by checking the concentration of the first tube in the series of tubes that showed no visible trace of growth. The lowest concentration in the series with no visible growth after the incubation period was taken as the MICs.

Determination of Minimum Bactericidal Concentration (MBC)

This was done using the National Committee for Clinical Laboratory Standard (1990) method. 1 ml sample from tubes used

in MIC determination which didn't show any visible growth after the period of incubation was streaked out on Nutrient Agar for 24 hours for bacteria to determine the minimum concentration of the extract required to kill the organisms [27] The lowest concentration of the extract that indicated a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC) [28].

Determination of Qualitative Secondary Metabolites Screening of *Uvaria afzelii* and *Tetrapleura tetraaptera* modified by [29].

Preliminary test / Preparation test

Plant filtrate were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate was used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

Test for Alkaloids

About 0.2 gram were warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicate the present of Alkaloids.

Test for Tannins

One milliliter of the filtrate was mixed with 2ml of $FeCl_3$, a dark green colour indicated a positive test for the tannins.

Test for Saponins

One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture was vigorously shaken and left to stand for 10min during which time, the development of foam on the surface of the mixture lasting for more than 10mm, indicates the presence of saponins.

Test for Anthraquinones

One milliliter of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test

Test for Anthocyanosides

One milliliter of the plant filtrate was mixed with 5 ml of dilute HCl; a pale pink colour indicates the positive test.

Test for Flavonoids

One milliliter of plant filtrate was mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the plant filtrate were mixed with 2ml of dilute NaOH; a golden yellow colour indicated the presence of flavonoids.

Test for Reducing Sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars.

Test for Cyanogenic glucosides

This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil.

Test for Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydride plus

Determination of Quantitative Secondary Metabolites Screening of *Uvaria afzelii* and *Tetrapleura tetraaptera* modified by Osuntokun et al. (2017)

Estimation of Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55°C, for 4 hours with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 rnl of diethyl ether were added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 rnl of n-butanol were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material

Estimation of Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weigh.

Estimation of Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydride plus H_2SO_4 .

Estimation of Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 M Hcl and 0.008 M potassium Ferro cyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract

Estimation of Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hours. This were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass.

Estimation of Phlobatannins

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrate was boiled in 2% HCl, red precipitate shows the present of phlobatannins.

Results

The results of the research work were demonstrated and recorded in Table 1 and Graphs 1-8.

Table 1 shows the initial weight of the different plant part used, the volume of the solvent used in mls and the percentage yield extracts of *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark used. The initial weight the *Uvaria afzelii* leaf and the *Tetrapleura tetraaptera* stem bark used weighed 400g and 200g respectively while 800mls of absolute ethanol was used to soak the *Uvaria afzelii* leaf and 400mls of ethyl acetate was used to soak the *Tetrapleura tetraaptera* stem bark the fiterate of the two plants were left to air-freeze the residue of the *Uvaria afzelii* ethanolic leaf extract was 5.5g while the residue of the *Tetrapleura tetraaptera* stem bark extract was 3g.

Graphs 1-4 shows the zone of inhibition of bacterial growth at different concentration (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of absolute ethanol extract of *Uvaria afzelii* leaf against multiple resistant organisms. The antibacterial activities were expressed as the zone of inhibition in diameters (mm) produced by the plant extract. The ethanol extract of the leaf of *Uvaria afzelii* inhibited some of the bacteria tested with measurable zone of inhibition.

Effect of *Uvaria afzelii* (Scott-Elliot) and *Tetrapleura tetraaptera* (Schumach and Thonn) extracts on each organism

The ethanolic extract of *Uvaria afzelii* inhibited the growth of *Staphylococcus aureus* (Gram-positive bacteria). Four concentrations were used namely 100, 50, 25, 12.5 mg/ml and one control which was 50mg/ml chloramphenicol. *Staphylococcus aureus* shows higher zone of inhibition of 22mm at 100mg/ml and a lower zone of inhibition of 10mm at 12.5mg/ml while chloramphenicol which is the control shows 28mm at 50mg/ml. *Proteus vulgaris* (Gram negative bacteria) also shows higher zone of inhibition of 18mm at 100mg/ml and a lower zone of inhibition of 9mm at 12.5mg/ml while the control shows 21mm at 50mg/ml. *E. coli* (Gram negative bacteria) shows higher zone of inhibition of 17mm at 100mg/ml and a lower zone of inhibition of 5mm at 12.5mg/ml while the control shows 20mm at 50mg/ml. *Staphylococcus typhi* (Gram- positive bacteria) shows higher zone of inhibition of 16mm at 100mg/ml and a lower zone of inhibition of 9mm at 12.5mg/ml. *Bacillus cereus*, *klebsiella Pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella gallimarum* were resistant to the extract. The plant extract also inhibited the growth of *Salmonella typhii* and *S epidermydis* with zones of inhibition of 14mm and 15mm at 100mg/ml. The plant extract also inhibited the growth of *Staphylococcus aureus*, *Salmonella typhii* and *Proteus vulgaris* with zones of inhibition of 18mm, 14mm and 15mm at 50mg/ml while at this concentration *Bacillus cereus*, *klebsiella Pneumonia*, *Pseudomonas aeruginosa*, *Salmonella gallimarum*, *E coli* and *Staphylococcus typhii* had zones of inhibition of 11mm, 9mm, 9mm, 10mm, 9mm and 8mm at 50mg/ml.

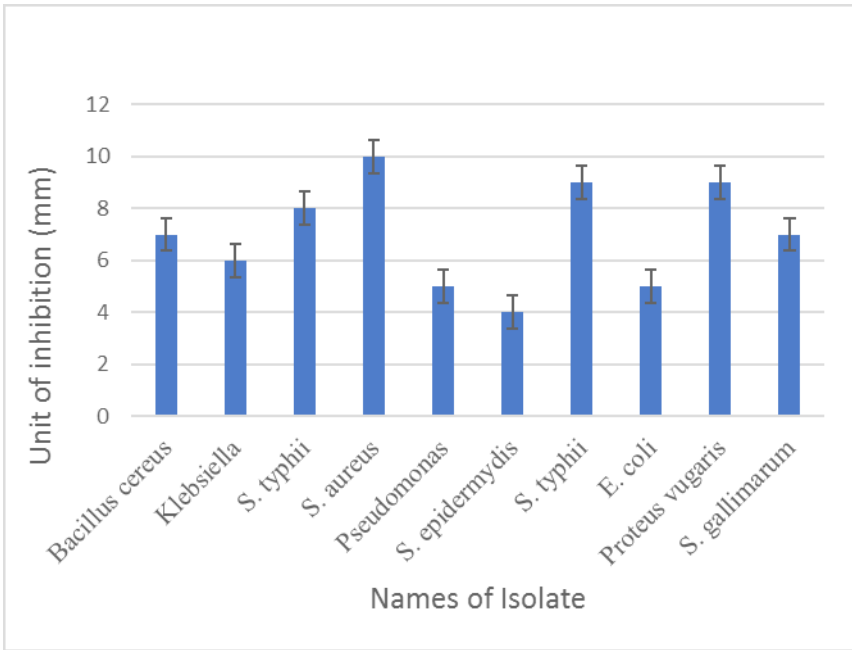
Figure 1 shows the MIC and MBC values of *Uvaria afzelii* leaf extract on the test isolates. The MIC values of *Uvaria afzelii* ranges from 25-50mg/ml and the MBC value ranging from 50-100mg/ml.



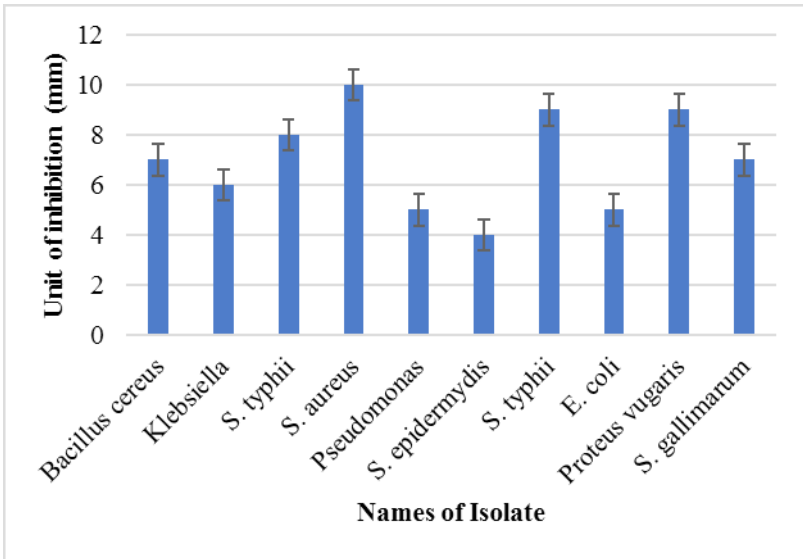
Figure 1b: *Uvaria afzelii* (Source:17).

Plant part used	Initial weight	Volume of solvent	Ethanol	Ethyl acetate
<i>Uvaria afzelii</i> leaf	400g	800ml	5.5g	2.9
<i>Tetrapleura tetra- raptera</i> stem bark	200g	400ml	4.5	3.0

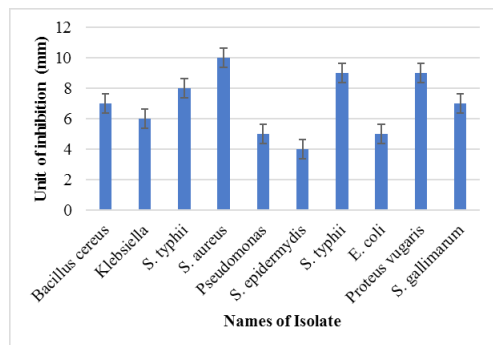
Table 1: Percentage yield extract of *Uvaria afzelii* and *Tetrapleura tetra-
raptera*.



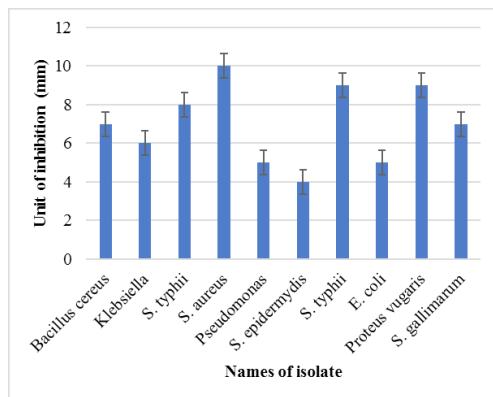
Graph 1: Measuring the zones of inhibition (Antimicrobial screening) of Ethanolic extract of *Uvaria afzelii* at Concentration of 100mg/
ml against selected multiple drug resistant isolates.



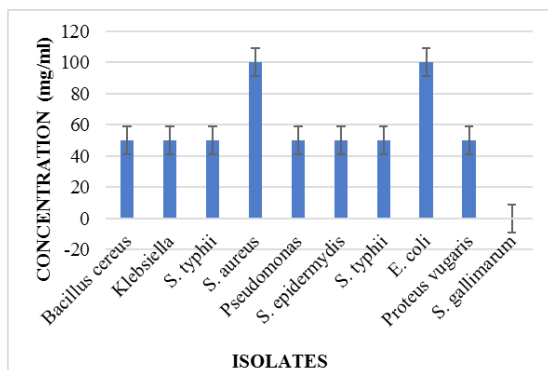
Graph 2: Measuring the zones of inhibition (Antimicrobial screening) of Ethanolic extract of *Uvaria afzelii* at 50mg/ml concentration
against selected multiple drug resistant isolates.



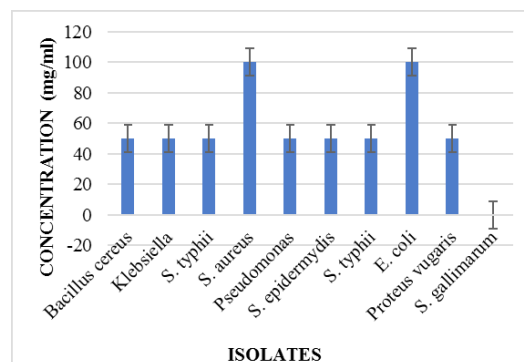
Graph 3: Measuring the zones of inhibition (Antimicrobial screening) of ethanolic extract of *Uvaria afzelii* at 25mg/ml concentration against selected multiple drug resistant isolates.



Graph 4: Measuring the zones of inhibition (Antimicrobial screening) of ethanolic extract of *Uvaria afzelii* at Concentration of 12.5mg/ml against selected multiple drug resistant isolates.



Graph 5: Measuring the Minimum Inhibitory Concentration of *Uvaria afzelii* leaf extract against selected multiple drug resistant isolates.

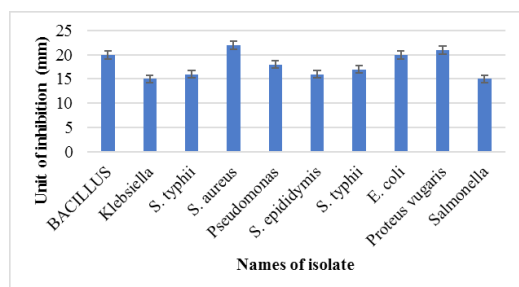


Graph 6: Measuring the Minimum Bactericidal Concentration of *Uvaria afzelii* leaf extract against selected multiple drug resistant isolates.

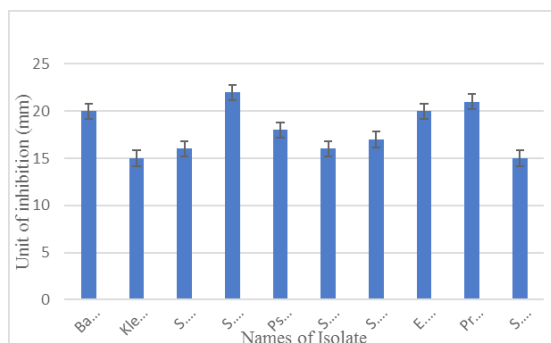
Graphs 7-10 shows that the ethyl acetate extract of *Tetrapleura tetrapectera* inhibited the growth of *Staphylococcus aureus* (Gram-positive bacteria) four concentrations were equally used namely 100, 50, 25 and 12.5mg/ml while chloramphenicol was used as control. *Staphylococcus aureus* showed higher zone of inhibition of 22mm at 100mg/ml and lower zone of inhibition of 8mm at 12.5mg/ml while control shows 27mm at 50mg/ml. *Bacillus cereus* shows higher zone of inhibition of 20mm at 100mg/ml and lower zone of inhibition of 8mm at 12.5mg/ml while 23mm at 50mg/ml. *E. coli* shows higher zone of inhibition of 20mm at 100mg/ml and a lower zone of inhibition of 10mm at 12.5mg/ml while the control showed 26mm at 50mg/ml. *Proteus vulgaris* shows higher zone 21mm at 100mg/ml and a lower zone of inhibition of 12mm at 12.5mg/ml and *Pseudomonas aeruginosa* with zones of inhibition of 12mm at 12.5mg/ml while the control showed 22mm at 50mg/ml. The *Tetrapleura tetrapectera* extract also showed higher inhibitory activity on *Klebsiella pneumonia* which measured 15mm at 100mg/ml and lower inhibition zone of 4mm at 12.5mg/ml. *Staphylococcus typhii* also showed higher zone of inhibition of 16mm at 100mg/ml and lower zone of inhibition of 7mm at 12.5mg/ml. *Staphylococcus epidermidis* showed higher zone of inhibition of 16mm at 100mg/ml and lower zone of inhibition 8mm at 12.5mg/ml while the control measured 20mm at 50mg/ml. *Salmonella typhii* also showed higher zone of inhibition of 17mm at 100mg/ml and lower zone of inhibition of 10mm at 12.5mg/ml while the control measured 20mm at 50mg/ml. *Salmonella gallinarum* also showed higher zone of inhibition of 15mm at 100mg/ml and lower zone of inhibition of 5mm at 12.5mg/ml while the control measured 20mm at 50mg/ml. The *Tetrapleura tetrapectera* extract also showed measurable zone of inhibition of *Staphylococcus aureus* with 15mm at 50mg/ml and lower zone of inhibition of 7mm at 12.5mg/ml.

Pseudomonas aeruginosa also showed higher zone of inhibition of 15mm at 50mg/ml and lower zone of inhibition of 8mm at 12.5mg/ml. *Salmonella typhii*, *Bacillus cereus*, *E. coli*, and *Proteus vulgaris* with higher zones of inhibition of 15mm, 18mm and 18mm at 50mg/ml and lower zones of inhibition of 7mm, 7mm and 10mm at 12.5mg/ml. The plant extract was resistant to the growth of *Klebsiella pneumonia*, *Staphylococcus typhii*, *S. epidermidis* and *Salmonella gallinarum* at 50mg/ml. The extract also inhibited the growth of *Salmonella typhii*, *E. coli*, and *Proteus vulgaris* of 14mm, 14mm and 16mm while the other bacteria isolates were resistant to the extract at 25mg/ml. At 12.5mg/ml all the bacteria isolate tested on the extract were resistant. It is therefore worthy of note that on the two extracts used *Tetrapleura tetraaptera* and *Uvaria afzelii* *Staphylococcus aureus* has the highest zone of inhibition at all the four concentration (100, 50, 25 and 12.5mg/ml).

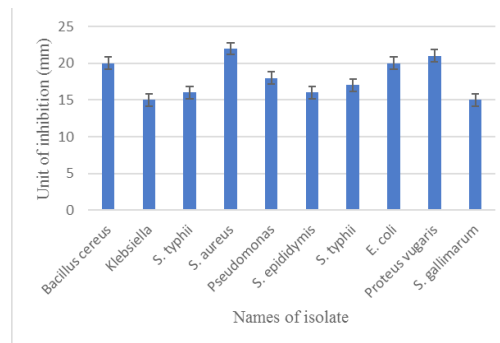
Graphs 11 and 12 shows the MIC and MBC values of the *Tetrapleura tetraaptera* stem bark extract the MIC values ranges from 12.5-25mg/ml and the MBC value ranging from 25-50mg/ml.



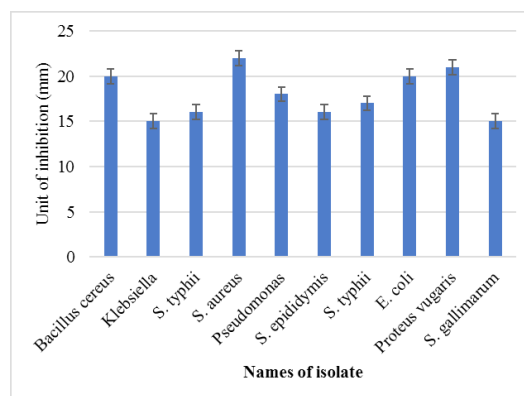
Graph 7: Measuring the zones of inhibition (Antimicrobial screening) of Ethyl acetate extract of *Tetrapleura tetraaptera* at 100mg/ml concentration against selected multiple drug resistant isolates.



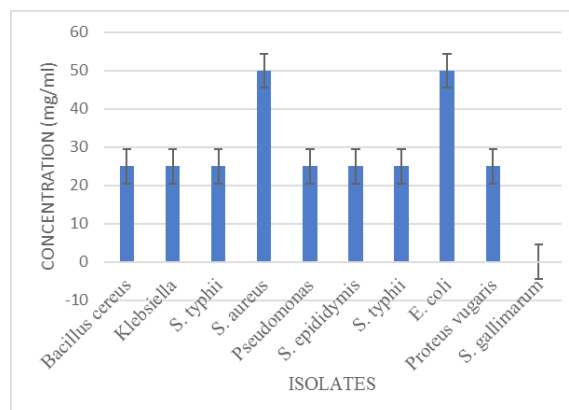
Graph 8: Measuring the zones of inhibition (Antimicrobial screening) of Ethyl acetate extract of *Tetrapleura tetraaptera* at 50mg/ml concentration against selected multiple drug resistant isolates.



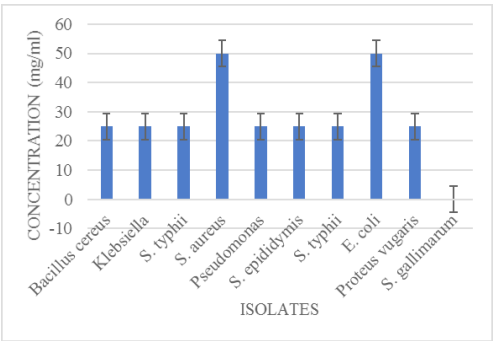
Graph 9: Measuring the zones of inhibition (Antimicrobial screening) of Ethyl acetate extract of *Tetrapleura tetraaptera* at 25mg/ml concentration against selected multiple drug resistant isolates.



Graph 10: Measuring the zones of inhibition (Antimicrobial screening) of Ethyl acetate extract of *Tetrapleura tetraaptera* at 12.5mg/ml concentration against selected multiple drug resistant isolates.



Graph 11: Measuring the Minimum Inhibitory Concentration of *Tetrapleura tetraaptera* stem bark extract against selected multiple drug resistant isolates



Graph 12: Measuring the Minimum Bactericidal Concentration of *Tetrapleura tetraaptera* stem bark extract against selected multiple drug resistant isolates.

Tables 2,3,4,5 shows the qualitative secondary metabolite screening of *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark it was observed that Alkaloids, glycosides, steroids, Anthraquins, phenol, tannins, saponins, pyrrolizidine alkaloids, reducing sugar, terpenoid and cardiac glycoside were present in the leaf extract of *Uvaria afzelii* and stem bark of *Tetrapleura tetraaptera*. The volatile oil of both plants were not present while the flavonoid of both *Uvaria afzelii* leaf extract and *Tetrapleura tetraaptera* stem bark extract were not determined.

Table 2 shows the quantitative analysis of secondary metabolites screening of *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark using different solvents. These table shows the quantity in value of the secondary metabolites present in the plants.

Sample	Alkaloid	Glycoside	Steroid	Anthraquin	Phenol	Tannins	Saponin	Flavonoid	Pyrrolizidine alkaloid	Reducing sugar	Terpenoid	Volatile oil	Cardiac glycosides
<i>Tetrapleura tetraaptera</i>	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	ND	+ ve	+ ve	+ ve	- ve	+ ve
<i>Uvaria afzelii</i>	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	ND	+ ve	+ ve	+ ve	-ve	+ ve
Key: ND- NOT DETERMINED +ve – POSITIVE -ve - NEGATIVE													

Table 2: Shows the Qualitative Analysis of Secondary Metabolite Screening of *Uvaria afzelii* and *Tetrapleura tetraaptera*.

Table 3 which is for methanol, shows that saponin is the most abundant secondary metabolite in *Uvaria afzelii* leaf with 6.12 and others such as the alkaloid, glycoside, steroid, phenol, tannins, flavonoid, reducing sugar, terpenoid and cardiac glycoside had values ranging from 2.13-3.55 while flavonoid is the most abundant secondary metabolites in *Tetrapleura tetraaptera* stem bark with 5.21 and alkaloid, glycoside, steroid, tannins, phenol, anthraquin and cardiac glycoside have 2.20, 2.10, 2.32, 2.30, 2.25, 3.23, 2.10 and 2.37 respectively. Volatile oil was not determined in both plants.

Sample	Alkaloid	Glycoside	Steroid	Anthraquin	Phenol	Tannins	Saponin	Flavonoi	Pyrrolizidine alkaloid	Reducing sugar	Terpenoid	Volatile oil	Cardiac glycosides
<i>Tetrapleura tetraaptera</i>	2.20	2.10	2.32	2.37	2.30	2.25	3.23	0.1	2.10	2.20	2.10	1.0	2.37
<i>Uvaria afzelii</i>	3.50	3.55	2.50	2.47	3.42	3.47	6.12	0.1	3.41	3.50	3.55	1.0	2.47
Key: ND- NOT DETERMINED													

Table 3: Shows the Quantitative Analysis of Secondary Metabolite Screening of *Uvaria afzelii* and *Tetrapleura tetraaptera* (METHANOL).

Table 4 which is for ethanol, shows that saponin is the most abundant secondary metabolite in *Uvaria afzelii* with value of 4.55 and others such as alkaloid, glycoside, steroid and pyrrolidizine alkaloid had values of 2.50, 3.57, 2.56, 2.49, 3.49, 3.45, 2.80, 3.57, 2.56, 2.49 and volatile oil was not determined for *Uvaria afzelii* while saponin is also the most abundant secondary metabolite in *Tetrapleura tetraptera* stem bark with value of 5.87 while other secondary metabolites such as tannins, phenol, pyrrolidizine alkaloid, alkaloid and cardiac glycoside had values ranging between 2.10- 2.37 and the volatile oil had the least value of 0.23.s.

Sample	Alkaloid	Glycoside	Steroid	Anthraquin	Phenol	Tannins	Saponin	Flavonoid	Pyrrolizidine alkaloid	Reducing sugar	Terpenoid	Volatile oil	Cardiac glycosides
<i>Tetrapleura tetraptera</i>	2.20	2.10	2.32	2.37	2.30	2.25	4.55	0.0	2.10	2.32	2.37	0.2	2.25
<i>Uvaria afzelii</i>	2.50	3.57	2.56	2.49	3.49	3.45	5.87	0.0	3.57	2.56	2.49	0.0	3.45
Key: ND- NOT DETERMINED													

Table 4: Shows the Quantitative Analysis of Secondary Metabolite Screening of *Uvaria afzelii* and *Tetrapleura tetraptera* (ETHANOL).

Table 5 which is for ethyl acetate, shows that flavonoid and alkaloid are the most abundant secondary metabolites with highest value of 20.34 for the *Uvaria afzelii* and other such as phenol, tannins, and cardiac glycoside had high values of 9.82 and 5.95. Also, glycode, anthraquin, saponin, pyrrolidizine alkaloid and terpenoid had values of 4.34, 3.18, 2.35, 4.43 and 3.18. Reducing sugar has the least value of 0.72 while the volatile oil of this plant was not determined. For *Tetrapleura tetraptera*, the most abundant secondary metabolite is phenol with value of 8.09. anthraquins also had high values of 6.09, 6.70 and 6.42. Alkaloid, steroid, flavonoid and reducing sugar has values of 4.03 and 2.31 while glycoside and pyrrolidizine alkaloid has the least value of 0.14 and the volatile oil for both plant was not determined.

Sample	Alkaloid	Glycoside	Steroid	Anthraquin	Phenol	Tannins	Saponin	Flavonoi	Pyrrolizidine alkaloid	Reducing sugar	Terpenoid	Volatile oil	Cardiac glycosides
<i>Tetrapleura tetraptera</i>	4.03	0.14	2.31	6.09	8.09	6.70	6.42	0.3	0.14	2.31	6.09	ND	6.70
<i>Uvaria afzelii</i>	20.34	4.34	0.72	3.18	9.82	5.95	2.35	0.0	4.34	0.72	3.18	ND	5.95
Key: ND- NOT DETERMINED													

Table 5: Shows the Quantitative Analysis of Secondary Metabolite Screening of *Uvaria afzelii* and *Tetrapleura tetraptera* (ETHYL ACETATE).

Discussion

The purpose of this research work is to determine the comparative *In-vitro* analysis and the secondary metabolites screening of *Uvaria afzelii* leaf extract and *Tetrapleura tetraptera* stem bark extract against selected multi-drug resistant organisms and to provide scientific validation for the use of this medicinal plants. The choice of plant used in this study *Uvaria afzelii* and *Tetrapleura tetraptera* was based on their reported local

uses in the treatment of various diseases and this study reveals their antimicrobial activities on selected multiple resistant organisms using absolute ethanol and ethyl acetate as the extracting solvents. In this study, leaves of *Uvaria afzelii* and the stem bark of *Tetrapleura tetraptera* were extracted and were tested for their antibacterial activity against: *Bacillus cererus*, *Klebsiella pneumonia*, *Staphylococcus typhii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermydis*, *Salmonella typhii*, *Escherichia coli*, *Proteus vulgaris* and

Salmonella gallinarum which are all multiple resistant isolates [30].

The crude plant extracts tested in this study showed antibacterial activity against all the test organisms and each of the organisms gave distinctive differences. This difference could be attributed to the differences in the concentration of the plant extracts as four different concentrations were used (100, 50, 25 and 12.5mg/ml) which all produced varied measurable zones of inhibition. The diameter of inhibition zone decreased with decrease in concentration of the ethanol and ethyl acetate extract this is in accordance with [31].

Results obtained from this study indicate that the ethanol and ethyl acetate extracts of *Uvaria afzelii* and *Tetrapleura tetraaptera* had inhibitory effects on the test organisms with zones of inhibition that ranged from 10-22mm. The antimicrobial activity was more pronounced on the multiple resistant gram positive bacteria (*Staphylococcus aureus*), *Salmonella gallinarum* was resistant to *Uvaria afzelii* at 100mg/ml while *Tetrapleura tetraaptera* inhibited this organism at this concentration. The reason for difference in sensitivity between Gram positive and Gram negative bacteria may be ascribed to the differences in the morphological constitutions between these microorganisms. Gram negative bacteria have an outer phospholipids membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances [30]. The Gram positive bacteria on the other hand have only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram negative microorganisms which are more complex than Gram positive ones act as a diffusion barrier and make them less susceptible to the antimicrobial agents than are Gram positive bacteria [32]. In fact, Gram negative bacteria are frequently reported to have developed multiple drug resistance to many antibiotics, of which *E. coli* is the most prominent [33,34].

Despite this permeability difference, however some of the extracts exerted some degree of inhibition against Gram negative organisms like *Salmonella typhi* and *Pseudomonas aeruginosa*, hence the extract can be referred to as having a broad spectrum activity, having the ability to inhibit or kill both Gram positive and Gram negative bacteria. This is in conformity with the work of [35]; in his review he observed that the plant had inhibitory activity against both Gram positive and Gram negative bacteria. Although *Bacillus cereus* is a Gram positive bacteria like *Staphylococcus aureus*, but it was not as inhibitory as *Staphylococcus aureus* to the plants extracts. The reason for this resistance is due to the presence of endospore which serves as an encystment against the extracts. This goes in line with the work of [36] he said in his review that *Bacillus spp* has an endospore that is centrally located in the cell which is capable of forming a cyst enclosed the extracts from getting into the cell. However, both plants can be combined together to fight infections caused by these multiple resistant organisms.

Furthermore. In this study, *Uvaria afzelii* and *Tetrapleura tetraaptera* was studied for its Minimum Inhibitory Concentration MIC and Minimum Bacteriostatic Concentration MBC. The MIC result on the test isolates for *Uvaria afzelii* ranges between 25 to 50mg/ml while the MBC values range between 50 to 100mg/ml while the MIC values for *Tetrapleura tetraaptera* range between 12.5 to 25mg/ml while its MBC values range from 50 to 100mg/ml. These differences could also be attributed to many pharmacologically bioactive compounds such as alkaloids, flavonoid, tannins and phenolic compounds which have been associated with their antibacterial activities of the plants [36]. The qualitative secondary metabolite screening of *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark revealed the presence of medicinally active constituent such as cardiac glycoside, steroids, phenol, tannins, saponin, flavonoids, pyrrolizidine alkaloid, alkaloid, anthraquinones and reducing sugar while volatile oil was not determined, some of which have been previously associated with antibacterial activity as observed by [37]; he observed in his work that these plants possess tannins, phlobatannins, alkaloids, saponins. The quantitative secondary metabolites screening of *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark using methanol, ethanol and ethyl acetate, showed the presence of different secondary metabolites in different quantities.

Conclusion

The degree of the antibacterial activities exhibited by the ethanolic leaf extract of *Uvaria afzelii* and the ethyl acetate extract of *Tetrapleura tetraaptera* at different concentrations has demonstrated that the two plants showed broad spectrum activity and that the use of herbs for the treatment of infections and diseases has proven to be effective as an alternative means of treatment therefore, *Uvaria afzelii* leaf and *Tetrapleura tetraaptera* stem bark extract should be further proven scientifically to establish its toxicity, safety and also its standard dosage which can serve as lead structures in the future for the production of purified, novel, effective and inexpensive drugs of great importance.

Recommendation

From the result obtained from the research carried out on this plant extracts, I recommend that *Uvaria afzelii* and *Tetrapleura tetraaptera* be used as antimicrobial agents against infections caused by multiple resistant organisms because of the great antimicrobial property exhibited in these research. As it can be used as antibiotics, preservatives or expectorants.

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Conflicts of interest

Author has declared that no competing interests

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