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ANTIBACTERIAL ACTIVITY OF ETHANOLIC ROOT EXTRACTS OF UVARIA CHARMAE ON STAPHYLOCOCCUS AUREUS

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ABSTRACT

Many different medicinal plants have been utilized in the treatment of infections caused by Staphylococcus aureus due to the increased antibiotic resistance exhibited by this organism. One of such medicinal plants is Uvaria charmae, a small wild shrub found in the south, east and northern parts of Nigeria. This plant has been incorporated into many herbal preparations sold under different proprietary names in Nigeria, with claims of antibacterial activity particularly against S. aureus. This study was carried out to determine the antibacterial activity of Uvaria charmae ethanolic root extract on S. aureus. The results of antibacterial susceptibility testing indicated that Uvaria charmae root extracts had no antibacterial effect on S. aureus at concentrations of 25mg/dl, 50mg/dl, 100mg/dl and 200mg/dl compared with standard commercial discs of Ciprofloxacin and Levofloxacin at similar concentrations which showed zones of inhibition >21mm depicting susceptibility. This study suggests that Uvaria charmae does not have antibacterial effect against S. aureus and is thus unsafe for continuous usage for this purpose as this could prove toxic. Public awareness on the effects of indiscriminate use of herbal medicines for treatment of bacterial infections should be encouraged. Government regulation should be intensified through its regulatory agencies in the verification of claims by alternative medicine practitioners before permitting sale of herbal products.

KEYWORDS: Antibacterial activity, Uvaria charmae, Staphylococcus aureus.

INTRODUCTION

Plants by nature have the inherent ability to synthesize aromatic substances such as phenols and their derivatives and these secondary metabolites possess therapeutic effects. Many plant extracts have been developed in recent times and proposed for use in food as natural antimicrobials.[1,2] Some of these metabolites have been used successfully in the treatment and prevention of infectious diseases and cancer or in stimulating the immune system. The medicinal value attributed to plants is a function of the bioactive phytochemical constituents that produce definite physiological action in the human body.[3] Phytochemicals are natural bioactive compounds of secondary metabolites found in plants that work with nutrients and fibers to act as a defense system against diseases. These phytochemicals are divided into two groups; primary and secondary constituents, according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids and phenolic compounds[1,4] and many more such as flavonoids, tannins and saponins. Uvaria charmae is one of such plants reported to have the above phytochemical constituents.[5,6]

Uvaria charmae is a small shrub growing up to 4mm tall, the plant is accessed locally and harvested from the wild as food and medicine in south, east and northern parts of Nigeria. It is known by various names such as “kaskaifi” or “atore” in Hausa, “eru ju” or “okooja” in Yoruba, “ufuri-aqu” in Igbo and “mboro inuen ikot” in Ibibio.[7] Uvaria charmae root has been reported to possess phytochemical components such as flavonoids, alkaloids, tannins, saponins and phenols in varied quantities.[7] These bioactive compounds may be responsible for the medicinal use of Uvaria charmae in treatment of bacterial infections, jaundice, yellow fever, sores, among other conditions.[8] It has also been reported to be good for the treatment of acute stomach ache, cough, dysentery and liver conditions.[9] The main objective of this study is to investigate the antibacterial activity of Uvaria charmae owing to its acclaimed efficacy by traditional herbalist in eradicating Staphylococcus aureus infection within 24 hours of administration.

MATERIALS AND METHODS

Collection and Preparation of plant root extracts (Uvaria charmae)

The fresh roots of Uvaria charmae for this study were obtained from Akpabuyo Local Government Area of

Cross River State, Nigeria. The study was carried out at the Human Anatomy research laboratory, University of Calabar. Identification of the plant specimen was done in the Herbarium Unit, University of Calabar and given a batch number BUC 397. Roots were thoroughly washed free of debris and cut into pieces, sun dried for few days and then grinded into fine powder. The roots were extracted exhaustively with 95% (v/v) ethanol for 48 hours. This was done by soaking the pulverized materials in 95% ethanol and shaking vigorously every 3 hours within the extraction period. Whatman number 3 filter paper was used to filter the extract after 48 hours. This was later condensed using a rotator evaporator (Rotavour, Biich R461, Switzerland) and given the code UC – Uvaria charmae.

Stock solution of Uvaria charmae extract was prepared by dissolving 55.9 mg/g body weight in 20ml of Dimethyl Sulphoxide (DMSO) diluted in 20ml of distilled water. Discs of different concentration strengths (25mg/dl, 50mg/dl, 100mg/dl, 200mg/dl) were prepared from the stock solution, dried and stored in the refrigerator at 4^0C until required.

Plate 1: Uvaria charmae plant.

Test Organisms

A standard strain, Staphylococcus aureus ATCC 25923 was obtained from the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria. This was first sub-cultured on blood agar and then used for antimicrobial susceptibility testing to determine the antibacterial activity of Uvaria charmae extract on Mueller-Hinton agar plates.

Plate 2: Luxuriant growth of Staphylococcus aureus ATCC 25923 on blood agar plate.

Preparation of turbidity standard (0.5 McFarland)

Half a gram of dehydrate barium chloride ($BaCl_2.2H_20$) was dissolved in 50ml of distilled water to prepare a one percent (w/v) solution of barium chlorine (Solution A). One millilitre of concentrated hydrogen tetra-oxo-sulphate (vi) acid (H_2S0_4) was added to 99ml of water and properly mixed to prepare a one percent (v/v) solution of H_2S0_4 (Solution B). Three-fifths of a millilitre (0.6ml) of Solution A was added to 99.4ml of solution B and properly mixed. A small volume of the turbid solution was transferred to a Bijou bottle and used as turbidity standard equivalent to 1.5 x 10^8 organisms/ml.[10]

Antimicrobial susceptibility testing of Staphylococcus aureus

A loopful of an overnight culture of the organism was inoculated into 5ml of sterile peptone water and incubated at 37^0C for 6 hours to achieve the same turbidity as the prepared 0.5 McFarland standard. Otherwise, sterile saline was added to adjust to turbidity standard equivalent to 1.5 x 10^8 organisms/ml.

A sterile cotton-tipped swab was dipped into the adjusted suspension and rotated 3-5 times in the medium. Excess inoculum was removed by pressing the swab firmly on the inside wall of tube above the fluid level. This was then used to streak over the surface of the Mueller-Hinton agar medium (Oxoid, UK), allowed to stand for 15minutes before the prepared discs of different concentrations (25mg/dl, 50mg/dl, 100mg/dl, 200mg/dl) were placed on the agar plates in accordance with CLSI guidelines.[11] The standard commercial discs of Ciprofloxacin and levofloxacin (Hardy Diagnostics, Santa Maria, USA) were also placed side by side for comparison. The plates were inoculated in duplicates, incubated at 37^0C for 18-24hours after which they were examined for zones of inhibition indicating susceptibility.

The results of the test for antibacterial activity of Uvaria charmae are as presented in plates 3 – 6 below.

Plate 3: 25mg/dl disc of Uvaria charmae extract in the middle as compared with 25mg/dl disc of ciprofloxacin and levofloxacin.

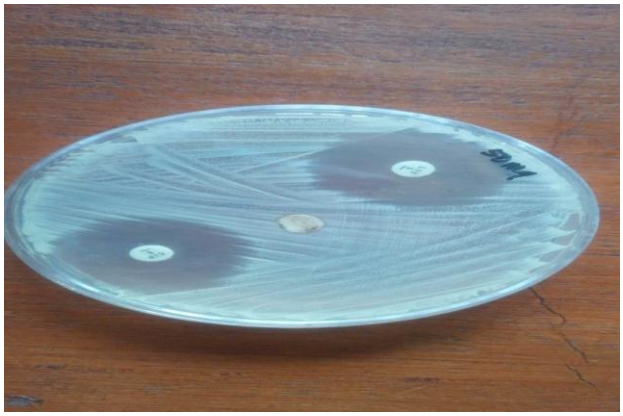

Plate 4: 50mg/dl disc of Uvaria charmae extract as compared with equal concentration of ciprofloxacin and levofloxacin.

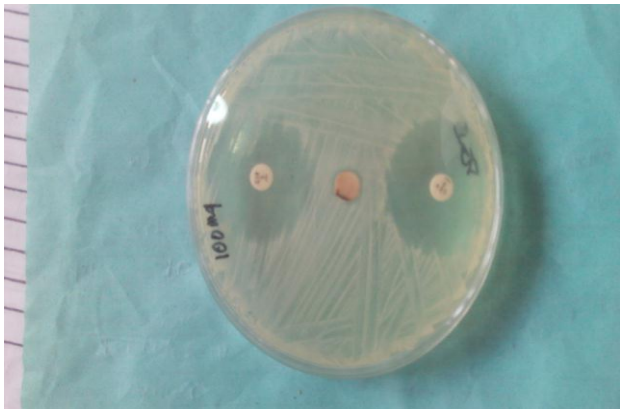

Plate 5: 100mg/dl disc of Uvaria charmae extract as compared with equal concentration of ciprofloxacin and levofloxacin.

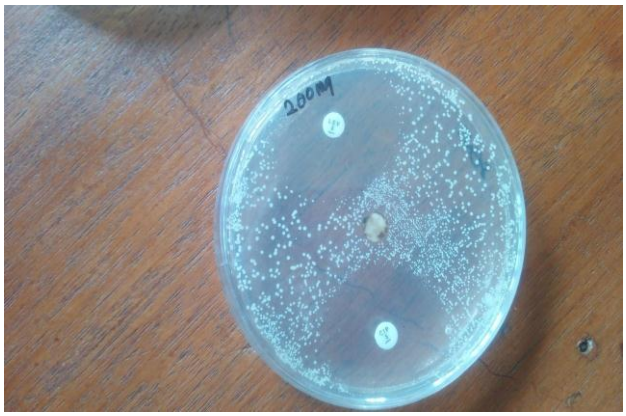

Plate 6: 200mg/dl disc of Uvaria charmae extract as compared with equal concentration of ciprofloxacin and levofloxacin.

At concentrations of 25mg/dl, 50mg/dl, 100mg/dl and 200mg/dl, Ciprofloxacin and levofloxacin showed zones of inhibition of S. aureus greater than 21mm which depicted sensitivity according to published interpretation chart, but the Uvaria charmae extracts under study did not show any zone of inhibition of the bacterial organism at similar concentrations and was thus regarded as non-sensitive.

The most predominant microorganism in Nigeria's clinical setting as reported by Onemu and Ophori[12] is Staphylococcus aureus with prevalence of infection up to 37.2%. The high prevalence rate of this bacterium and its attendant infective rate in Nigeria coupled with its resistance pattern to many established antibiotics have increased the tendency to seek alternative therapies. Uvaria charmae is one of the constituents of most herbal remedies such as Ruzu bitters sold commercially in many pharmaceutical and patent medicine stores in Nigeria. These herbal remedies have been widely used due to their low cost, availability and claims of antibacterial efficacy against S. aureus. However, the claim of antibacterial activity against S. aureus has raised many questions in the clinical setting. In this study, the antibacterial activity of Uvaria charmae extracts on S. aureus was assessed and it revealed that the extract had no activity compared with the standard antibiotic discs of ciprofloxacin and levofloxacin at similar concentrations as shown on Plates 3-6. Ciprofloxacin and levofloxacin are quinolones which have bactericidal activity on S. aureus. They act by inhibiting the bacterial DNA gyrase and topoisomerase II, which disrupts DNA replication, bacterial chromosome separation during division and other cell processes involving DNA.[13] On the other hand, the mechanism of action of Uvaria charmae according to Ogueke et al.[14] is by the action of the saponins which is less efficacious. The findings of this study disagrees with the findings of Ogueke et al.[14] which reported high antibacterial activity (16.5mm inhibition zone size at 200mg/dl) of Uvaria charmae extract against S. aureus. This disparity may have been due to the use of non-standard strain of the organism in the previous study as compared with the pure standard strain of S. aureus (ATCC 25923) used in the present study. However, a more recent study by Osuagwu and Ihenwosu[15] reported that the phytochemicals present in Uvaria charmae were alkaloids, phenols and saponins in small amounts and showed only a mild antibacterial sensitivity of less than 7.0mm which is in line with the results of this study. This study has shown that Uvaria charmae does not have sufficient antibacterial activity against S. aureus in order to result in its eradication. Therefore, the claim of antibacterial activity of herbal remedies such as Ruzu bitters on S. aureus should be done with caution.

CONCLUSION

Ethanolic root extracts of Uvaria charmae does not have sufficient antibacterial activity to inhibit the growth of standard strain of S. aureus (ATCC 25923) and so should not be used in the treatment of S. aureus infections. Ciprofloxacin and Levofloxacin are still effective agents in the treatment of infections from this bacterium. Although Uvaria charmae root contains bioactive constituents, its antibacterial remedy for Staphylococcus infections, as claimed by alternative medicine practitioners, is however discredited.

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Competing Interest
The authors declare no competing interest.

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