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In vitro Action of *Vernonia perrottetii* Plant Extracts on *Staphylococcus* species Associated with Vulvovaginitis of Selected Pregnant Women in Lokoja, Nigeria

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ABSTRACT: The study was carried out to test for the *in vitro* action of *Vernonia perrottetii* extracts against *Staphylococcus* species isolated from women with Vulvovaginitis using medically certified standard procedures. A total of 50 samples of intravaginal swab (ICS) samples were collected from consented pregnant women in a State Specialist Hospital in Nigeria and investigated for the presence of *Staphylococcus* species and effect of *V. perrottetii* extracts on the isolates. The phytochemical screening of the methanolic and aqueous extract shows that the plant contain secondary metabolites such as flavonoids, alkaloids, saponins with absence of phenol and anthraquinone. Isolate *S. aureus* (c) and *Staphylococcus* species(c) were resistant to amoxillin which served as control while other isolates were susceptible to the plant extracts. At a concentration of 1000mg/ml, aqueous extract of the plant exhibited appreciable sensitivity with a zone of inhibition of 26.7mm and 20.7mm at 500mg/ml and 1000mg/ml for *S. xylosus.* The results of the study imply that extracts of *Vernonia perrottetii* is a potential biocontrol agents of Staphyloccoci infection.

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Herbal medicine is the oldest form of health care known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play an important roles in drug development in the pharmaceutical industry (Preethi *et al*., 2010). The global demand of herbal medicines is increasing rapidly because of their higher safety margin and low cost (Musyimi *et al*., 2008). In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Flood-Riccio *et al*., 2020).

There is therefore the need to search for plants of medicinal value. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Mathew *et al*., 2021). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. *Vernonia perrottetii* belongs to the family Asteraceae. The plant is called 'Burzu' in Hausa, 'Doko' chintara in Nupe, is a herb, with annual branched stem attaining a height of 60cm, Its leaves are linear, long and slender about 3cm long. The plant is being used locally by the Northern people of Nigeria as antimicrobial but little is known for its scientific bases as to its antimicrobial effects, hence, the study set out to investigate the *in vitro* action of *Vernonia perrottetii* extracts against *Staphylococcus* species isolated from consented women with vulvovaginitis using medically certified standard procedures at a State Specialist Hospital in Nigeria.

MATERIALS AND METHODS

Study area and population: The study was conducted in the Biological Sciences Laboratory, Federal University Lokoja. A total of (50) vaginal swabs were collected from pregnant women at the State Specialist Hospital Lokoja, Nigeria. Ethical approval for the study was granted by the authorities of the Hospital.

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Clinical Samples Collection: Intra Vaginal Swabs were collected from women attending antenatal with the assistance of medical personnel in the hospital and transported immediately in a cool box to the Department of Biological Sciences laboratory, Federal University Lokoja for culture and isolation. The swabs were inoculated on to the surface of Mannitol salt agar for *Staphylococcus* species at 37^0C for 24hours.

Presumptive *Staphylococcus* colonies were further purified and characterized using battery of morphological and biochemical tests including Gram's reaction, coagulase test, catalase, haemolysis on blood agar and hydrolysis of DNase test. The isolates were confirmed using Microgen ID Kit for *Staphylococcus* species.

Plant Collection and preparation: The plant materials were obtained from the botanical garden of Federal University Lokoja. The plants were brought to the Department of Biological Sciences for identification. The whole plant of *Vernonia perrottetii* were air dried for 2-3 weeks and powdered. The powdered materials were stored in an air tight container for future use.

Extraction of plant material: The extraction of the plant material of *Vernonia perrottetii* were carried out using known standard procedures (Pandey and Tripathi, 2014). The powdered materials were exhaustively extracted using distilled water and methanol. A total of 100g powdered sample of the whole plant part of *V.perrottetii* were separately macerated in distilled water for 24hrs and 70% methanol for 3 days respectively to obtain aqueous and methanol extracts of each plant for use in the analysis. Each extract were filtered and solvent evaporated under reduced pressure in a rotary evaporator and weighed.

Phytochemical screening of the Extracts: Phytochemical screening of aqueous and methanol extract of *Vernonia perrottetii* was carried out using standard phytochemical procedure of (Mikail *et al;* 2010). The following test were conducted to identify the chemical constituents.

Test for carbohydrate: Approximately 2ml of Molish's reagent and 2ml of concentrated sulphuric acid (H_2SO4) were added to 2ml boiling methanolic extract of *V. perrottetii*. A reddish ring indicates the presence of carbohydrate.

Test for reducing sugar: Approximately 2ml of methanolic extract of *V. perrottetii* were be added to boiling Fehling's solution for 5minutes. A brick red precipitate indicates the presence of reducing sugar.

Test for Tannins: Approximately 2ml of methanolic extract of *V. perrottetii*, 1ml of ferric chloride ($FeCl_3$) were added and blue-black or greenish –black precipitate indicates presence of tannins.

Test for saponins: Approximately 2ml of methanolic extract of *V. perrottetii*, 5ml of distilled water were added and the solution shaken vigorously for 30s, stable persistent frothing indicates saponin.

Test for flavonoids: Magnesium ribbon and few drops of concentrated HCl were added to 2ml of methanolic extract of *V. perrottetii*, pink or red colour indicates the presence of flavonoids.

Test for alkaloids: Approximately 10ml of ammoniacal chloroform solution were added to 2ml of methanolic extract of *V. perrottetii*. The extract was then treated with 10 drops of 10% sulphuric acid and tested with Meyer's reagent. Formation of white precipitate indicates the presence of alkaloid.

Test for steroids: Approximately 2ml of Methanolic extract of *V. perrottetii* 2ml of chloroform, acetic acid and 1ml of concentrated H_2SO4 were added. A blue-green indicates the presence of steroids.

Antimicrobial susceptibility testing: This was done using agar well diffusion method of Clinical Laboratory Standards Institute (CLSI) described by (Capenter *et al*., 2018). The washed overnight broth cultures was diluted appropriately using sterile distilled water to 0.5×10^6cfu/ml MacFarland Standard. Nutrient agar was poured into sterile petri dishes and allowed to set. The sterile Nutrient Agar plates were flooded with 0.1ml of the standardized isolates and these were spread uniformly using spread plate method. Wells of 6mm diameter were bored on the agar medium using a sterile cork-borer. Aqueous solution of amoxillin were prepared according to Kaur *et al*. (2011) which served as positive control. Exactly 0.1ml of the different concentrations of the extracts (1000mg/ml, 500mg/ml, 250mg/ml and 125mg/ml) as well as the antibiotics was placed in each well in the agar medium containing the culture using a sterile Pasteur pipette.

The plates were allowed to stand for one hour at room temperature to allow diffusion of the substrates to proceed before the growth of the organisms commenced. The plate was finally incubated at 37^0C. The presence of zone of inhibition around the hole containing the extracts as well as the antimicrobial drugs indicates the antimicrobial activity against the test organisms and this was measured and expressed in

terms of diameter zones of inhibition (mm) for susceptibility test.

Minimum inhibitory concentration (M.I.C.) of the plant extract: The least concentration that shows zones of inhibition from susceptibility testing was used to determine the minimum inhibitory concentration of the extracts. The least concentration that showed zone of inhibition for sensitivity was at 500mg/ml. These concentrations in sterile Muller- Hinton Broth were prepared in a test tube using double dilution method. 1ml each of the standardized organisms was taken and inoculated into a prepared Muller-Hinton Broth in a test tube and the inoculums was allowed to diffuse in the agar broth test tubes for 30mins after which it was incubated at 37^0C for 24hrs. The lowest concentration of the extract in the test tube that showed clear zone was considered as the M.I.C. of the extract against the *Staphylococcus* species.

Minimum bactericidal Concentration of the extract: A loopful of the broth culture from the MIC tube was inoculated onto Nutrient Agar plate. The plates were incubated at 37^0C for 24hr, after which it was examined for colony growth or lack of it. Absence of growth indicates that the plant extract is bactericidal and presence of growth indicates that it is bacteriostatic.

RESULTS AND DISCUSSION

The result from this study revealed the presence of *Staphylococcus* species associated with vulvovaginitis. The result agrees with the findings of Tang *et al.* (2020.) who revealed that different species of Staphylococci are found in patience with vaginitis. The results of this findings shows that *Staphylococcus xylosus*(a) isolated and subjected to the aqueous extracts of *V. perrottetii* has an inhibitory potential with an inhibition zone of 26.67±0.33 which even superseded the standard drug used (Amoxillin) which had a zone of inhibition of 20mm at 1000mg/ml. This could be due to the production of enzymes called Extended Spectrum Beta-lactamases (ESBL) (Nwinyi *et al*., 2009). The findings from this study also shows that *Staphylococcus xylosus* is the most susceptible to the extracts of the plant at 1000mg/ml compared to amoxillin. This is in agreement with Odunbaku *et al*. (2008) who reported that *V. amgydalina* had inhibitory activity against the gram positive bacteria (*S. albus* and *B. substilis*) and gram negative bacteria (*K. pneumonia*, *P. aeruginosa* and *P. mirabilis*) in a study of antimicrobial effect of ethanol leaf extract of selected medicinal plants on some human pathogenic microbes.

Table 1. Phytochemical properties of the methanolic and aqueous extracts *Vernonia perrottetii*

	Methanolic Extract	Aqueous Extract
Carbohydrate	+	+
Reducing sugar	+	+
Tannins	++	++
Saponins	++	++
Flavonoids	+	+
Alkaloids	+	+
Phenols	-	-
Anthraquinones	-	-
Steroid and Triterpene	+	+
Cardiac glycoside	+	+

Key: + = positive ++ = highly positive - = Negative

Table 2. *Staphylococcus* Spp Sensitivity Test of Aqueous Plant Extract at 500mg/Ml

Test organism	VP (AQ) 500mg/ml (mm)	VP (AQ) 1000mg/ml (mm)	VP(ME) 500mg/ml (mm)	VP(ME) 1000mg/ml (mm)	Amoxillin
S. aureus(a)	0.0±0.0	0.0±0.0	0.0±0.0	23.0±0.0	35
S .xylosus(a)	20.7±0.3	26.7±0.3	15.3±0.3	20.0±0.0	20
S .aureus(b)	6.0±0.0.0	12.3±0.3	16.7±0.3	19.3±0.3	40
S .warneri	12.0±1.0	16.3±0.3	19.0±0.0	20.0±0.0	45
S .aureus(c)	18.3±0.3	19.3±0.3	0.0±0.0	0.0±0.0	-
S .xylosus(b)	12.7±0.7	20.0±0.0	12.0±0.0	21.3±0.7	45
S.xylosus(c)	19.3±0.3	22.3±0.3	10.0±0.0	15.0±0.0	50
Staph spp(a)	10.0±0.0	14.7±0.3	18.0±0.0	20.0±0.0	46
Staph spp(b)	12.0±1.0	16.0±0.0	15.0±0.0	20.0±0.0	45
Staph spp(c)	20.0±0.0	22.3±0.0	20.3±0.2	25.3±0.3	-

Key: VP (AQ) = Vernonia perrottetii aqueous extract, VP (ME) = Vernonia perrottetii methanolic extract

Statistical findings from the study also show that aqueous extract of *V. perrottetii* (20.7±0.3, 26.7±0.3) on *S. xylosus*(a) has higher inhibitory potential than methanolic extract (15.3±0.3, 20.0±0.0) at concentrations of 500mg/ml and 1000mg/ml respectively. This is in agreement with the findings of Dar *et al*. (2016) who revealed that the aqueous extract of *Rheum spiciformis* had greater inhibitory potential than the methanolic extract against *Proteus vulgaris* and *Bacillus subtilis.* This can be as a result of the change in the potential of the bioactive components of botanical due to different solvent properties. *S. warneri* was the least susceptible organism to both aqueous and methanolic extracts of the plant. These findings are supported by Ochei and Kolhatkar, (2006) who reported that if drugs shows less activity or no

activity against microorganisms, it is an indications of development of resistance by the test organisms.

Table 3. Minimum Inhibitory Concentration of *V. Perrottetii* Aqueous Extracts at 125mg/ml/ 250mg/ml/ 500mg/ml

Organism	MIC(mg/ml	MBC(mg/ml)
S. aureus	125	250
S .xylosus	250	500
S .aureus	250	500
S. aureus	500	>1000
S .xylosus	125	250

Literature study done on *V. perrottetii*, showed that there was no previous research work on the antimicrobial activity of crude extracts of the whole plants part of *V. perrottetii*.

Table 4. Minimum Inhibitory Concentration of *Vernonia perrottetii* Methanolic extract at 125mg/ml, 250mg/ml/500mg/ml of the extracts

Organism	MIC(mg/ml)	MBC(mg/ml)
S .warneri	500	>1000
S .xylosus	500	>1000
Staph spp	500	>1000
Staph spp	500	>1000
Staph spp	500	>1000

As such, this could be the first report on such activity and could be a start point for novel antimicrobial drugs. MBC value was found higher than the MIC value of the extracts tested against the microorganism, indicating bacteriostatic effects of the extracts. Aqueous extracts of *V. perrottetii* were found to be bactericidal against *S. aureus* in two isolates, *S. xylosus* in two isolate.

The bactericidal activity may be possibly due to high content of essential oils such as monoterpenes and sesquiterpenes (Nwinyi *et al*., 2009). Only *S. aureus* were found to be bacteriostatic to this extracts. Methanol extraction of this plant were found to be bacteriostatic against *S. warneri*, S. *xylosus* and *Staph* spp in 3 samples isolated at MIC500/ and MBC>1000, respectively. The entire microorganism showed bacteriostatic activity to the methanol extracts of the plant.

Conclusion: The study justifies the use of aqueous extracts of *V. perrottetii* as its use in the traditional medicine among the Northern part of Nigeria and is found to contain secondary metabolites such as alkaloids, flavonoids, saponins with the absence of phenol and steroids and exhibited appreciable inhibitory activity on most of the isolates. Both the aqueous and methanolic extracts of the whole plant part show no activity on *S. aureus* (a) at both concentration. Further studies on inability of this extracts to control *Staphyloccocus aureus* (a) is therefore suggested.

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