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RESEARCH ARTICLE

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Antimicrobial effects of *Gomphrena Celosioides* extracts on *Staphylococcus* species isolated from women with vulvovaginitis in Zaria, Nigeria

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ABSTRACT

This study carried out to assay for the activity of the phytochemical properties as well as Gomphrena celosioides (GC) against Staphylococcus species a prominent bacterial agent associated with vulvovaginitis. A total of 100 samples of high vaginal (HVS) were collected from women of reproductive age 15-50 years for suspected vaginitis at Ahmadu Bello University, Health Care Center and Samaru Clinic, Zaria, Kaduna State, Nigeria and investigated for the presence of Staphylococcus species and the effect of GC extracts on the isolates. A total number of 74(74%) were found to be infected with Staphylococcal vaginal infection. The result of the ten randomly selected Staphylococcus spp using Microgen kit also showed that S. xylosus and S. aureus and Staphylococcus spp were present in 3 samples, 3(1%), 3(1%) respectively. S. warneri 1(0.3%). The methanolic and aqueous extracts of the plant contain secondary metabolites such as alkaloids, flavonoids, saponins with the absence of phenols, anthraquinones and steroids. All the clinical isolates of the organism were sensitivie to ciprofloxacin, amoxicillin and Erythromycin with the exception of isolate S. aureus (c) and Staph sp(c) which were resistant to amoxicillin. At a concentration of 1000mg/ml, the methanolic extract exhibited appreciable inhibitory activity on most of the isolates with a zone of 20mm and 19.33mm for S. aureus and Staph. sp respectively. The aqueous extract with the same concentration was less effective showing activity on S. xylosus (a) and (c) with zones of 10.00mm and 10.33mm respectively.

Keywords: Vaginitis, HVS, *Staphylococcus* species, GC (*Gomphrena celosioides*)

INTRODUCTION

Africa, with a range of regions has a long and impressive list of medicinal plants spread through the forest and woodland regions of the continent [1] and many African plants are used in traditional medicine as antimicrobial agents though only a few have been documented. In developing countries, medicinal plants have been the most accessible source of medicaments and in rural areas, traditional medicine is part of the first line of treatment for common pathologies[2]. This is also occasioned by the fact that synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects [3]. Many medicinal plants provide valuable natural resources that are potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity, and some have been effectively deployed in the modern medicine [4]. Many species of Gomphreneae tribe have shown antimicrobial activity [5];[6]. *G. celosioides*, an annual documbent ascending herbs up to 30cm tall, with branches cloth with shaggy white hairs, is reportedly used as antimalarial against *Plasmodium falciparum* in traditional medicine system of Ghana [7]. Alcohol extracts of *G. celosioides* is

reported to be diuretic [8] and to possess antimicrobial properties against a range of organisms [9]. In South America, some species are employed in the treatment of bronchial infections, diarrhoea and malaria fever, while others had found application as analgesics, tonic/carminatives, diuretics [10]; [11] and abortifacient [12]. The need to search for plants of medicinal value especially in the vast forestry region of Nigeria will probably lead to discoveries of more plants with medicinal properties. One such plant under study is the amaranthaceae family which comprises of many species with biological activities some of which have used in nutrition and alternative medicine [13]. The aim of this study therefore is to assay for the activity of *Gomphrena celosioides* against *Staphylococcus* species a prominent bacterial agent associated with vulvovaginitis.

MATERIALS AND METHODS

Study Area and Population:
The Study was conducted in Selected Hospitals in Samaru, a suburb of Zaria in Kaduna State, Nigeria. The community is home to the largest University in Nigeria with a large number of Staff and Students patronizing these health facilities. A total of one hundred (100) female Staff, students and other members of the community who consented were recruited for the study. Similarly approval for the study was granted by the authorities of the University Health Services.

Sample Collection:
Clinical Samples: High Vaginal Swabs were collected from women attending Sick Bay and Samaru Clinic with the assistance of medical personnel in the hospital and transported immediately in a cool box to the Department of Microbiology laboratory, Ahmadu Bello University, Zaria, Kaduna, State Nigeria for culture and isolation. The swabs were inoculated unto the surface of Mannitol Salt Agar for *Staphylococcus* species at 37^{o}C for 24hrs. Presumptive *Staphylococcus* colonies were further purified and characterized using a battery of morphological and biochemical tests including Gram's reaction, coagulase, catalase, haemolysis on blood agar and hydrolysis of DNase. The isolates were confirmed using the Microgen ID Kit for *Staphylococcus* species.

Plant Collection and Preparation:
The plant materials were obtained from the botanical garden and environment of the main campus of Ahmadu Bello University, Zaria Kaduna State, Nigeria. The plants were brought to the Department of Biological Sciences for authentication with a voucher number of (864). The whole plant of *Gomphrena celosioides* was air dried for 2-3 weeks and powdered. The powdered materials were stored in an air tight container for future use.

Extraction of Plant Materials:
The extraction of the plant material of *Gomphrena celosioides* was carried out using a procedures described by [14]. The powdered materials were exhaustively extracted using distilled water and methanol. A total of 100g powdered sample of the whole plant part of *G. celosioides* 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 *Gomphrena celosioides* was carried carried out using standard phytochemical procedure of [15][16][17]. The presences of various phytochemicals were assayed for. These include carbohydrates, reducing sugar, tannins, saponins, flavonoids, alkaloids, phenols, anthraquinones and steroids.

Antimicrobial susceptibility testing of the isolates to Selected Antibiotics
This was done using agar well diffusion method of the National Committee for Clinical Laboratory Standards [18]. The washed overnight broth cultures were diluted appropriately using sterile distilled water to 0.5×10^{6}cfu/ml MacFarland Standard. Nutrient agar plates in the case of *Staphylococcus* sp were used. The sterile Nutrient Agar plates were each flooded with 0.1ml of the standardized isolates of *Staphylococcus* isolates. These were spread uniformly using spread plate method. Wells of 6mm diameter were bored on of the agar media using a sterile cork borer (No .1). Aqueous solution of three broad spectrum antibacterial drugs namely; ciprofloxacin, erythromycin and amoxicillin [19] were prepared, which served as positive controls. Exactly 0.1ml of the different concentrations of the standard antimicrobial drugs was placed in each well. 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 was measured using a transparent ruler and expressed in terms of zones of inhibition (mm) [20] and susceptibility determined.

Determination of Antimicrobial Activity of the Extracts:
This was done using agar well diffusion method of National Committee for Clinical Laboratory Standards [18]. An 18h broth culture of the organism was washed and diluted appropriately using sterile distilled water to 0.5×10^8cfu/ml. A total of 0.1ml of different concentrations (1000mg/ml, 500mg/ml, 250mg/ml and 125mg/ml) of the extract were added to each well bored on the agar medium and allowed to stand at room temperature for effective extract diffusion. 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).

RESULTS

The result showed that *Staphylococcus* species were isolated from 74 out of the 100 samples (74%). Further characterization revealed that *S. aureus* and *S. xylosus* were the most common species as presented in fig. 1. The methanolic and aqueous extracts of the plant contain secondary metabolites such as alkaloids, flavonoids, saponins with the absence of phenols, anthraquinones, steroids (table 1). All the clinical isolates of the organism were sensitivie to ciprofloxacin, amoxicillin and Erythromycin with the exception of isolate *S.aureus* (c) and Staph sp(c) which were resistant to amoxicillin (table 2). At a concentration of 1000mg/ml, the methanolic extract exhibited appreciable inhibitory activity on most of the isolates with a zone of 20mm and 19.33mm for *S. aureus* and Staph. sp respectively. The aqueous extract with the same concentration was less effective showing activity on *S. xylosus* (a) and (c) with zones of 10.00mm and 10.33mm respectively (table 3).

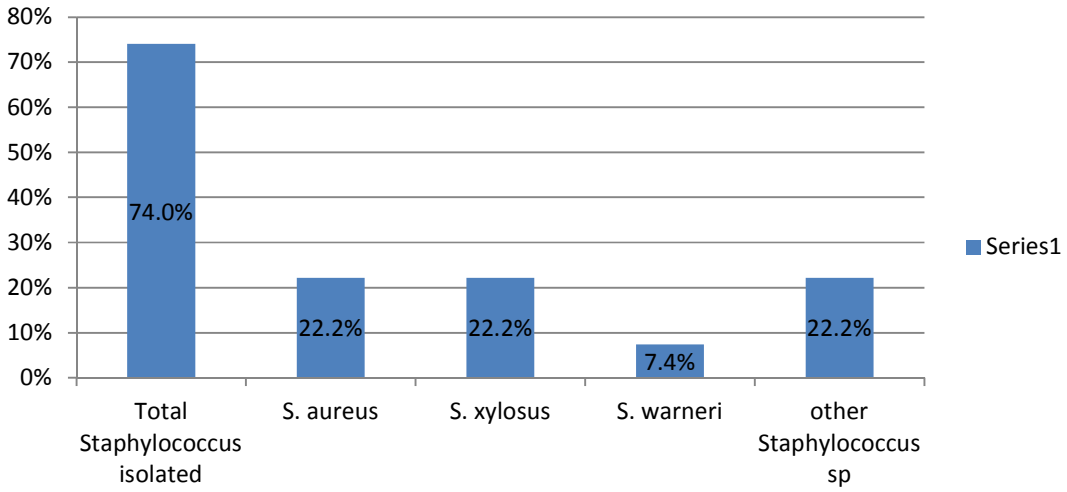

Figure 1: Prevalence of *Staphylococcus aureus* among the study population

Table 1: Phytochemical Properties of the Methanolic and Aqueous *Gomphrena celosioides* extracts

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

Table 2: Susceptibility Pattern of *Staphylococcus* species to selected antibiotics

Bacteria Isolates	Ciprofloxacin (500mg)		Amoxycillin(500mg)		Erythromycin(500mg)	
	S	R	S	R	S	R
S. aureus(a)	100	0	100	0	100	0
S .xylosus(a)	100	0	100	0	100	0
S .aureus(b)	100	0	100	0	100	0
S .warneri	100	0	100	0	100	0
S .aureus(c)	100	0	0	100	100	0
S .xylosus(b)	100	0	100	0	100	0
S.xylosus(c)	100	0	100	0	100	0
Staph sp(a)	100	0	100	0	100	0
Staph sp(b)	100	0	100	0	100	0
*Staph*sp(c)	100	0	0	100	100	0

Key: *n - number of samples, S - Sensitive, R - Resistant, Staph sp - Unidentified Staphylococcus species*

Table3: Determination of sensitivity of selected *Staphylococcus* species to Methanolic and aqueous extracts of *Gomphrena celosioides* (GC)

Clinical Isolates	Zones of Inhibition in mm				
	500mg/ml		1000mg/ml		
	GcMeth	GcAq	GcMeth	GcAq	Amoxycillin
S. aureus(a)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	35
S. xylosus(a)	8.67±0.33	0.0±0.0	11.33±0.33	10.0±0.0	20
S. aureus(b)	5.0±0.0	0.0±0.0	10.0 ±0.0	0.0 ±0.0	40
S. warneri	12.00±0.0	0.0±0.0	13.33±0.67	0.0 ±0.0	45
S. aureus(c)	0.0 ±0.0	0.0±0.0	20.0 ±0.0	0.0 ±0.0	0
S. xylosus(b)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	45
S. xylosus(c)	12.33±0.33	0.0±0.0	14.00±0.0	10.33±0.0	50
Staph sp(a)	10.00±0.0	0.0±0.0	13.00±0.0	0.0 ±0.0	46
Staph sp(b)	12.00±0.0	0.0±0.0	13.33±0.0	0.0 ±0.0	45
Staph sp(c)	15.33±0.33	3.33±3.33	19.33±0.33	5.00±5.00	0

GcMeth - Methanolic extract of Gomphrena celosioides;
GcAq – Aqueous extract of Gomphrena celosioides

DISCUSSION

Staphylococci have been known to be associated with vulvovaginitis causing bacterial vaginitis. The prevalence in this study disagrees with the report of Campos *et al.*, [21] who reported a prevalence of 22.79% for *S. aureus* though their report on other staphylococci were lower than that in our finding. *S. aureus* is known to cause various human diseases and unlike the other species which are opportunistic pathogens *S. aureus* is considered a true pathogen. The incidence of *S. xylosus* and *S. warneri* were also reported by Campos as associated with bacterial vaginitis [21]. In their work on pubertal girls, Stricker *et al* [22] reported a 36% incidence of bacterial vulvovaginitis and *S. aureus* was among the organisms isolated alongside other bacteria. The incidence of bacterial vulvovaginitis in their report was lower than that in this finding. This could be because their work was also among pubertal girl who are not sexually active while the 74(74) % in our study was among women of reproductive age most of whom are married. *Staphylococcus* are not considered among the sexually transmitted diseases however [23] and many factors such as personal unhygienic practices, improper care during menstruation, multiple sexual partners and contraceptive use must have contributed to the high incidence of bacterial vaginitis in the study population [24]. *Staphylococcus* occurs as normal flora in various body folds and may gain entrance into the vaginal tract where a local colonization may occur. The vaginal flora is known to be dynamic and varying with pH and hormonal changes, multiple sex activities, use and abuse of antibiotic therapy for infections occurring in other locations [25]. The result of the antibiotic showed that *S. aureus*(c) was resistance to amoxycillin. Similarly, there was also a resistance to *Staph* spp (c). This result is in agreement with the study of Simeoni *et al*.,[26] who reported that there was a similar pattern of antibiotic resistance between CNS (coagulase negative *Staphylococcus*) and *S. aureus*. However, in another study by Marino *et al*., [27] showed that CNS were phenotypically less susceptible to antimicrobial agents than CPS (coagulase positive *Staphylococcus*), in a study of Biofilm, protease and lipase properties and antibiotic resistance profile of *Staphylococcus* isolated from various foods. Resistance in microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. The number of antibiotics with increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin.

The abuse of antibiotics has given rise to resistance by various organisms necessitating the search for more antibiotics and screen of more plants since the primary and secondary metabolites of many plants are known to possess antibacterial activities. The phytochemical analysis revealed the presence of compounds like saponins, steroids, amino acids, non-reducing sugars, phenols and flavonoids from the methanol extract of *G. celosioides* which agrees with the work of Dosumu *et al*. [28] and Abalaka *et al.,* [29]. These compounds were also demonstrated in the aqueous extract of the plant however the aqueous extract had little activity compared to the methanolic extract. This was also reported by Abalaka *et al*, [28]. These primary and secondary metabolites account for the inability of most microorganisms which are unable to colonize living plant tissue [30]. The extracts were found to possess inhibitory property of the test organism. The inhibitory property of *G. celosioides* on *S. aureus* was also reported by several workers [28][29][30]. The activity of the extract was more profound at 1000mg/ml. this concentration is actually high but considering that it was a crude extract, further purification and fractionation could help establish the real active fraction, leading to lower concentrations with high activity for use.

CONCLUSION

The study established the association of Staphylococcus species with vulvovaginitis in the study area with a prevalence of 74%, and *S. xylosu*s and *S. aureus* as the most prominent isolates. The study also justifies the use of methanolic extract of *Gomphrena celosioides* (GC) as the plant was found to contain secondary metabolites such as alkaloids, flavonoids, saponins with the absence of phenols, anthraquinones and steroids and at a concentration of 1000mg/ml, exhibited appreciable inhibitory activity on most of the isolates with a zone of 20mm and 19.33mm for *S. aureus* and *Staph*. sp respectively. The aqueous extract with the same concentration was less effective showing activity on *S. xylosus* (a) and (c) with zones of 10.00mm and 10.33mm respectively. Further study should be done by using different type of solvent different from the present study for the extraction processes. Toxicity study should also be carried out using animal model to know the precise dose of the plant extract

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