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PRELIMINARY ANTIBACTERIAL AND PHYTOCHEMICAL SCREENING OF THREE MEDICINAL PLANTS USED IN THE FOLKLORIC TREATMENT OF SKIN INFECTION

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ABSTRACT
The *in vitro* effects of *Schwenkia americana*, *Mormodica charantia* and *Lippia multiflora* extract in water, ethanol and ethyl acetate were evaluated on some pathogenic bacteria namely *Staphylococcus aureus*, *Pseudomonas aeruginosa* and β-heamolytic *Streptococcus pyogenes*. The work was carried out using the agar well diffusion method at concentrations ranging from 25mg/ml to 100mg/ml of extracts. The ethanol extract of *Schwenkia americana* and *Lippia multiflora* showed zones of inhibition of 24+1.19cfu against *Staphylococcus aureus*, at 100mg/ml stock concentration. The aqueous extract of *Lippia multiflora* showed a zone of inhibition of 22+0.60cfu at 100mg/ml against *Staphylococcus aureus* while the ethyl acetate extract of *Schwenkia americana* showed highest zone of inhibition of 24+1.19cfu at 100mg/ml of extract against *Staphylococcus aureus*, *Streptococcus pyogenes* was the least active of all the organisms to the test plants with *Lippia multiflora* showing a zone of inhibition of 10cm at 100mg/ml. Generally all the test plants are active against the organisms. Phytochemical screening revealed that the plants contain flavonoids, tannins, alkaloid, saponin, and steroids. In all the test plants, Positive antibiotic disk control and antiseptic test showed the microorganisms to be resistant to most of the antibiotic disk as well as the antiseptics used at concentrations ranging between 5%-20% of dettol, izal, ethanol and Lysol. The plants showing antimicrobial activities can be inculcated into the treatment of bacterial infections involving the test organisms to help fight the ever increasing antibiotic resistance.
Keywords: Pathogenic bacteria, *Staphylococcus species, Schwenkia americana, Lippia multiflora*, pharmacognosy, *Marmodica* species, *Pseudomonas* species.

INTRODUCTION

Pathogenic organisms have evolved numerous defense mechanisms against antimicrobial agents. As a result of this, newly produced drugs are on the rise. Despite this tremendous progress in human medicine, infectious diseases are still a major threat to public health. The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products[2]. Medicinal plants have been characterized for their possible bioactive compounds which have been separated and subjected to detailed structural analysis. Research in pharmacognosy and pharmacology of medicinal plants has evolved assay of bioactivity, identification of potential modes of action and target site for active phytomedicinal compounds. Animals live in equilibrium with the plants surrounding them, using these as sources of food and intuitively through the years of trials and error as medicine[13]. Based on current research and financial investments, medicinal plants will seemingly continue to play an important role as a health aid[7]. In spite of the millions of chemical compounds currently synthesized in the laboratory and available for screening for action of therapeutic value, natural products particularly of plant origin remain the most important sources of new drugs[14]. *Shwenkia americana, Mormodica charantia* and *Lippia multiflora* are used traditionally in the treatment of diabetes mellitus[6], malaria and hypertension. They are also shown to be active against gram positive and gram negative microorganisms[1]. Many traditionally proven drugs used in modern medicine were initially used in the crude form in traditional or folk healing practices or for other purposes that suggest potential useful biological activity. The primary benefit of using plant derived medicine is that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment[12]. This study is aimed at establishing the antimicrobial potentials of the medicinal values of these plants on bacterial pathogens.

MATERIALS AND METHODS

The plants samples were obtained from a traditional medicinal practitioner in Ibode, Ibadan, Oyo State of Nigeria. The botanical identities were determined and authenticated at the department of Botany, University of Ado-Ekiti, Ekiti State, Nigeria. Voucher specimens were deposited at the herbarium.

Plant processing and preparation

Fresh plant samples were collected, air dried and ground into powder using the laboratory blender. These were stored in air tight sterile bottles until use.

Extraction procedure

The methods of Junaid *et al* (2006)[8] and Okwori *et al* (2007) were adopted for this study. Twenty grams each of every plant powder was used for extraction using 100ml of ethanol, ethyl acetate and cold sterile water. The solutions were filtered using sterile Whatman no 1 filter paper and the extracts were evaporated to dryness. The resulting crude extracts were reconstituted using 50% dimethlysulphoxide. Bacterial isolates: Stock clinical bacterial isolates of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus pyogenes* were obtained from the microorganism bank of the Obafemi Awolowo

University Teaching Hospital Complex, Ilesa, Osun State and were stored on nutrient agar slant at +4°C until use.

Standardization of Organisms

Standard inoculate were prepared by suspending a loop-full of bacterial culture in nutrient broth and incubated for 2-4h. The turbidity of the actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5Mcfarland units prepared by mixing 0.5ml of 1.75 (w/v) Barium chloride dehydrated with 99.5ml of 1% tetraoxosulphate (VI) acid. This turbidity was equivalent to approximately 1.2 x 10^8 colony forming units per millimeter (cfu/ml).

Antimicrobial bioassay

The preliminary antimicrobial study was conducted using the method of Inclu *et al* (2006). Stock concentrations ranging from 25mg/ml to 100mg/ml of each extract were used. This was done by dissolving 19g of each extract in 100ml of dimethyl sulphoxide and serial dilutions carried out to obtain the lower concentrations. One ml of the bacterial culture was inoculated into 19ml of molten nutrient agar and poured into sterile petri-dishes. The microbes were allowed to set. Thereafter, wells were bored on the gelled nutrient agar using a 6mm cork borer. All wells were filled with 0.5ml of extracts. A well was bored at the center of the plate and was filled with 0.5ml of 59% dimethyl sulphoxide to act as the control. The extracts were allowed to diffuse into the agar for about 1h before the plates were incubated in the incubator at 37^0C for 24h after which zones of inhibition were observed and recorded in millimeters. This experiment was carried out in duplicates and the mean values were recorded. The result of the antimicrobial zones of inhibition was analyzed using the one way ANOVA and the differences were regarded as significant when $P< 0.05$.

Phytochemical screening

The methods of Sofowora *et al* (2005) and Obdoni and Ochuko (2001) were used to screen for saponin, tannin, flavonoids, steroids, glycosides, tanins and alkaloids.

Positive control

Comparative analysis was carried out using conventional antibiotics such as gentamycin, tetracycline, penicillin, nitrofurantonin, contranizole, ciprofloxacin, augmentin, oflaxacin, perfloxacin on the test organisms using the disk diffusion methods (Cheeseborough, 2004) and conventional disinfectants such as izal, dettol, Lysol and ethanol using the agar diffusion methods of Inclu *et al* (2006).

Thin layer chromatography

The thin layer chromatography of plants extracts was carried out using different solvent fronts such as chloroform: water: methanol: petroleum ether: acetone in the ratio 1: 2: 2: 2:1 for *Schwenkia americana*, chloroform: water: ethyl acetate: methanol: acetone in the ratio 1:1:1:1:1:1 for *Mormodica charantia* and chloroform: water: ethyl acetate: petroleum ether: methanol in the ratio 1:2:1:1:2 for *Lippia multiflora.* Several bands were observed and these bands were tested for antimicrobial activities.

RESULTS

Ethanol extract of the plants showed appreciable antibacterial activities at stock concentration of 100mg/ml against *Staphylococcus aureus*. Zones of inhibition of 24±1.19mm were recorded at 100mg/ml concentration for the extracts of *Schwenkia americana* and *Mormodica charantia* against *Staphylococcus aureus*. Concentrations as low as 50mg/ml were active against this organism. Other solvent extract (aqueous and ethyl aetate) also showed high antibacterial activity. The aqueous and ethyl acetate extracts of *Schwenkia americana* and *Lippia multiflor*a showed minimal activity against *Streptococcus pyogenes* while the ethyl acetate extract of *Schwenkia americana* showed minimal activity against *Pseudomonas aeruginosa* (Table 1.) The control of the experiment (50% dimethyl sulphoxide) did not show any antimicrobial activity against the organism. Positive control using conventional antibiotics and disinfectant revealed that this test organism was resistant to most of the antibiotics except gentamycin and ciprofloxacin. However, *Pseudomonas aeruginosa* was susceptible to augmentin, ceftrizone, pefloxacin, penicillin and erythromycin (Table 2-3). *Staphylococcus aureus* and *Streptococcus pyogenes* were sensitive to 20% dettol, 15% v/v and 20% v/v of izal, 15% v/v and 20% v/v of lysol while they were resistant to ethanol at 5% v/v and 20% v/v. *Pseudomonas aeruginosa* was sensitive to 10% v/v, 15 v/v and 20% v/v of lysol (Table 4). The thin layer chromatography showed that ethanol extracts of plants had better chromatogram than other solvent extractions. However, *Schwenkia americana* did not show any visible chromatogram. Phytochemical screening revealed the presence of tannin, alkaloids and saponin in *Mormodica charantia* and flavonoids, tannin, glycosides, alkaloids, saponin and steroids in *Lippia multiflora* (Table 5).

DISCUSSION

The result obtained indicates that all the solvents had considerable antimicrobial activities with ethanol showing the best antibacterial activity. This may be as a result of phytochemical extraction capability of ethanol[8]. This is also in line with the report of Obi and Onuoha (2000)[15] which reported alcohol to be the best solvent for the extraction of most plant's active principles of medical importance. It can also be observed from this work that the higher the concentration, the more active are the plants extract and as concentration decreases, the activities also decrease. Hence an acceptable and effective dosage can be prepared by traditional healers for the control and eradication of bacterial pathogens. The result of the positive control showing a high level of resistance to the test organisms especially *Staphylococcus aureus* to antibiotics and antiseptics confirmed the emergence of resistant strains of pathogenic microorganisms (Fridkin *et al* 1999) and also reemphasize the need to search for new and more effective antibacterial agent so as not to return to the era before the development of antibiotics (Smith *et al* 1999). The phytochemical present in these plants are possibly responsible for the high antimicrobial activities.

Table 1: Antimicrobial activities of plant extract against test organisms

Plants	Microorganisms	Concentrations of extract in mg/ml and zones of inhibition in mm																				
		100			90			80			70			60			50			25		
		EE	EA	AQ	EE	EA	AQ	EE	EA	AQ	EE	EA	AQ	EE	EA	AQ	EE	EA	AQ	EE	EA	AQ
Schwenkia americana	*Staphylococcus aureus*	24± 1.19	24± 1.19	18± 1.46	22 ± 0.60	20± 0.00	15± 0.59	20± 1.19	14± 1.19	17± 0.84	17± 1.46	11± 0.84	14± 1.40	16± 0.84	10± 1.19	13± 0.84	--	--	--	--	--	--
	Streptococcus pyogenes	12± 1.19	10± 1.19	21± 0.84	10± 1,45	---	16± 0.84	---	---	15± 0.84	--	--	--	--	--	--	--	--	---	--	--	---
	Pseudomonas aeruginosa	21± 0.84	12± 0.84	16± 1.19	16± 0.84	14± 1.19	12± 1.19	15± 0.84	--	10± 0.00	14± 0.00	---	--	13± 1.46	---	---	---	---	---	---	---	---
Mormodica charantia	*Staphylococcus aureus*	21± 0.60	22± 1.19	20± 0.84	20± 1.19	19± 0.84	19± 0.84	17± 1.46	16± 0.84	14± 1.19	15± 0.84	12± 1.19	12± 1.19	12± 1.46	---	10± 1.19	10± 1.19	---	---	---	---	---
	Streptococcus pyogenes	21± 0.84	22± 1.19	20± 0.84	20± 1.19	19± 0.84	16± 0.84	14± 1.19	15± 1.46	14± 1.69	12± 1.19	13± 1.46	---	10± 1.19	---	---	---	---	---	---	---	---
	Pseudomonas aeruginosa	17± 0.84	16± 0.84	14± 1.19	14± 1.69	12± 1.19	12± 0.00	10± 0.84	10± 0.00	11± 0.00	---	---	13± 1.46	---	---	---	---	---	---	---	---	---
Lippia multiflora	*Staphylococcus aureuss*	24± 1.19	10± 0.84	---	21± 0.84	---	---	19± 0.84	---	---	15± 0.84	---	---	12± 1.19	---	---	10± 1.19-	---	---	---	---	---
	Streptococcus pyogenes	10± 1.19	10± 1.19	---	10± 1.19	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Pseudomonas aeruginosa	18± 0.84	18± 0.00	14± 1.19	17± 0.84	15± 0.84	13± 1.46	16± 0.84	10± 1.19	10± 0.00	14± 0.00	---	---	13± 1.19	---	---	10± 1.18	---	---	---	---	---

EE-Ethanol extract; EA-Ethyl acetate extract; AQ- Aqueous extract

Table 2: Effects of antibiotic disc on test organisms (Gram negative)

Antibiotics	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Pseudomonas aeruginosa*
Amoxicillin	---	---	---
Ofloxacin	---	---	---
Augmentin	---	---	10mm
Ceftriazone	---	---	12mm
Nitrofurantonin	---	---	---
Gentamycin	10mm	13mm	12mm
Pefloxacin	---	---	10mm
Cotrimoxaxole	---	---	---
Ciprofloxacin	14mm	---	8mm
Tetracycline	---	---	---

Legend; --- No activity

Table 3: Effects of standard antibiotic disc on test organisms (Gram positive)

Antibiotics	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Pseudomonas aeruginosa*
Penicillin	---	---	8mm
Clindamycin	---	---	---
Gentamycin	---	---	14mm
Fusidi acid	---	---	---
Erythromycin	---	---	12mm
Trimethoprim	---	---	---
Sulphamethoxazole	---	---	---
Tetracycline	---	---	---
Pefloxacin	---	---	---
Chloramphenicol	---	---	---

Legend; --- No activity

Table 4: Comparative antimicrobial test of conventional disinfectant against test organisms

Organisms	Disinfectant (zones of inhibition in mm)															
	Dettol				Izal				Lysol				Ethanol			
Staphylococcus aureus	5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%
Streptococcus pyogenes	---	---	---	10	---	---	10	13	---	---	15	18	---	---	---	---
Pseudomonas aeruginosa	---	---	---	---	---	---	---	---	---	10	14	15	---	---	---	---

Legend ---- no activity

Table 5: Phytochemical screening results

Plants	Phytochemical constituents						
	Flavonoids	Glycosides	Tannins	Alkaloids	Saponins	Steroids	Tannins
Schwenkia americana	+	-	+	+	+	+	-
Mormodica charantia	+	-	+	+	+	+	-
Lippia multiflora	+	+	+	+	+	+	-

Key + Means presence - Means not observed

Constituents such as flavonoids, tannins, saponins and alkaloids which are generally known to be part of bioactive components in any ethno- medical plants has been a subject of discussion amongst intellectuals and traditional practitioners. These constituents have been shown to possess medicinal values as well as exhibiting physiological properties[8]. Flavonoid is used in medicine as antimicrobial, anti-inflammatory and antioxidant agent. The presence of steroids and flavonoids in medicinal plants has been variously reported by researchers (Okwu, 2001. Matu *et al* 2003). Tannins, one of the phytochemicals in these plants, has been reported to inhibit the growth of HIV and herpes simplex virus (Okuda *et al* 1999).

Alkaloids, apart from their uses in the treatment of bruises and superficial wounds, have also been found to interfere with cell division of micro organisms (Kirtikar and Basu 1999). It was reported that aqueous extract of the plants are most effective in the leakages of Na and K ions than the ethanol extracts on these test organism. This also explains the effectiveness of the aqueous extract of the plants under present investigation. The higher number of bands as observed with ethanol extract during the thin layer chromatography confirmed the claim of Obi and Onuoha (2000)[15] that ethanol is a better extraction solvent than other solvents. It also agrees with the views of Kirtikar and Basu (1999) who claimed that the solubility of each constituent in a herb is very specific to different solvents used in the extraction procedure. Hence chemical nature as well as the pharmacological activities of herbal extracts obtained using the same herb with different solvents will be different. Conclusively, the plants used in this study conform to their traditional use in the treatment of skin infections since they are effective against the organisms implicate in the infection.

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