Vol. 13(14), pp. 196-202, August, 2019
DOI: 10.5897/AJPP2019.5057
Article Number: 8D1C46161562
ISSN: 1996-0816

http://www.academicjournals.org/AJPP

ACADEMIC JOURNALS
expand your knowledge

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antibacterial activities of three medicinal plant extracts and their synergistic effect on *Staphylococcus aureus* isolated from burn wounds

Nwuna E. N.[1], Nwuzo A. C.[1], Afiukwa F. N.[1], Oke Boniface[1], Udu-ilbiam E. O.[1], Moses I. B.[1*], Ilang D. C.[2] and Aondaackaa A. D.[3]

[1]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, P. M. B. 053, Abakaliki, Nigeria.
[2]Department of Biological Science, Faculty of Science, Federal University Ndufu Alike, Ikwo, Ebonyi State, Nigeria.
[3]Department of Microbiology, Faculty of Science, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

Received 20 May, 2019: Accepted 17 June, 2019

This study was undertaken to investigate the antibacterial activities of three medicinal plant extracts and their synergistic effect on *Staphylococcus aureus* isolated from burn wounds. A total of 50 swab samples of burn wounds were collected from burn wound patients attending Federal Teaching Hospital Abakaliki and screened for *S. aureus* using standard microbiological techniques. Three plant materials (*Cucurbita pepo* leaf, *Alchornea cordifolia* leaf, and *Terminalia ivorensis* bark root) were dried under room temperature and ground into powdered form. Twenty grams of each plant materials was soaked in 100 ml of solvents (cold, warm water, ethanol, and methanol) for 24 h and filtered with muslin cloth. The crude extracts were mixed with dimethyl sulphoxide and subjected to 2 folds serial dilution. The results show that out of the 50 burn wounds swab samples collected, 32(64%) were positive for *S. aureus*. The susceptibility test results revealed that *Terminalia ivorensis* was the most active against the *S. aureus* isolates with an inhibition zone diameter (IZD) of 20 mm in warm water solvent at 100 mg/ml and 10 mm at 50 mg/ml. Results also revealed that a combination of ethanol extracts of *A. cordifolia* and root bark of *T. ivorensis* yielded 29 mm IZD. The combination of these two extracts exhibited a higher IZD against the *S. aureus* isolates. *C. pepo*, *A. cordifolia*, and *T. ivorensis* extracts could serve as putative agents for the development of novel drugs for the treatment of wound infections caused by *S. aureus*.

Key words: Medicinal plant, sensitivity, synergistic, bacteriological, bacteria and *Staphylococcus aureus*.

INTRODUCTION

Plants are very important for the health of humans and may serve as food source, medicinal, in environmental

*Corresponding author. E-mail: ben_iyke70@yahoo.com.

protection and beautification (Joshi et al., 2011). Plants are by far the richest source of drugs of traditional Systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Joshi et al., 2011). The concept of finding healing powers in plants is an ancient practice that is as old as humanity (Cowan, 1999). Therefore, medicinal plants are used as antimicrobials that addresses the problems of drug resistance of microbial pathogens. According to Merrian Webster, an antimicrobial agent is defined as an agent that destroys or kills microorganism or reduce their metabolic activity leading to retardation of their growth. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotic, use of antibacterial agents, host characteristics and environmental factors. This condition has forced scientists to carry out research for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents.

The invention of antibiotics has completely transformed the face of medicine in the 21st century coupled with the introduction of vaccination which lead to eradication of disease such as polio and small pox in the developed world (Nathisuwan et al., 2001). Due to the fact that these drugs are easily accessible and equally effective it leads one to over use them in raising livestock prompting bacteria to develop resistance. The problems of bacterial resistance is happening every day in our different environments. That is why scientists are seriously making research or other alternative means of controlling pathogenic bacteria with herbal plants (Cowan, 1999). Herbal medicine is a practice that makes use of natural plant substances to treat and prevent disease (Tyler and Foster, 1999). Herbal medicine is the use of plants or plant parts, their water or solvent extracts, essential oil, gums whole resins, therapeutically to provide prevention and cure of disease (Blumenthal, 2008). Most herbal plants (leaf, bark, oil and seeds) which have indicated antimicrobial potentials are yet to be validated of their claimed effects and possibly drug production. Example of plants with antimicrobial potential are *Fluted pumpkin* (*Cucurbita pepo*) *Terminalia ivorensis* (Black afara) and *Alchonia cordifolia* (Dove wood). *Terminalia ivorensis, Fluted pumpkin* and *Alchonia cordifolia* plants were used to treat burns and skin infections. Most wounds infections are contaminated by the individual's own endogenous flora which is present on the skin, mucous membrane, or hollow viscera. Usual pathogens on the skin and mucous surface are gram-positive cocci, mainly *Staphylococcus aureus* (Rosenbluth et al., 2004). In other words, Gram-negative aerobic and anaerobic bacteria can contaminate skin wounds of the groin and perinea areas. Therefore, the aim for this study was to investigate the antibacterial activities of three medicinal plant extracts and their synergistic effect on *S. aureus* isolated from burn wounds.

MATERIALS AND METHODS

Study area

The sample collection of this study was carried out at Federal Teaching Hospital (FETHA II) Abakaliki, Ebonyi State Nigeria. The sample was analyzed at Ebonyi State university Microbiology Laboratory complex. Ebonyi state has a population of 2,173,501 people (NPC, 2006). It is situated between latitude 6° 03^{1}11.38N and longitude 8°09^{1}46.22"E. The occupation of the people within the area of study is majorly farming and the season is rainy (April - September) and dry (October - March).

Ethical clearance

Ethical clearance was obtained at Federal Teaching Hospital Abakaliki (FETHA 11) Ebonyi State, Nigeria.

Collection, Identification and preparation of plant materials

The plant materials used in this study include fresh leaves of *C. pepo* (fluted pumpkin), *A. cordifolia* (Ubu plant) and bark root of *T. ivorensis* (Black afara). The plant materials were collected from Obeagu Item forest in Ikwo Local Government Area of Ebonyi State, Nigeria and were identified by Prof. J. C. Okafor and Prof. S. C. Onyekwelu (Taxonomists) in the Department of Biological Sciences of Ebonyi State University, Abakaliki. The leaves of the plant materials were carefully washed with clean tap water and rinsed with distilled water. The plant leaves were air dried at room temperature of 25-28°C and then grinded into powdered form with manual grinding machine and stored in air tight containers (Kudi et al., 1999).

The extraction of active constituents of plants was carried out according to the method of Parekh and Chanda (2006). Briefly, the leaves and bark root of the plant materials were dried under room temperature and grinded into powdered form using manual grinder. Each of the grinded herbal sample was respectively weighed and 20 g of each of the sample was soaked in 100 ml of the solvents (cold water, warm water, methanol and ethanol) used for extraction. The cold water preparation was allowed to stand for 24 h only with interval of 30 min shaking. Warm water, methanol and ethanol preparations were allowed to stand for 48 h. After this, the preparations, were filtered using Muslin filter cloth. The filtrate was poured into flat plate and air dried at room temperature to recover the extracts. The crude extracts recovered was weighed accordingly and recorded.

Collection of wound swab samples

A total of fifty wound samples were collected by a nurse using sterile swab sticks from wound burn patients at Federal Teaching Hospital II Abakaliki, Ebonyi State (FETHA II). After collection, they were transported to Applied Microbiology Laboratory Complex, Ebonyi State University, Abakaliki for microbiological analysis.

Bacteriological analysis clinical samples

The following media, Nutrient agar, mannitol salt agar and (Oxoid, USA) Nutrient broth were prepared according to manufacturer's instructions. Each swabbed sample was inoculated on nutrient

Table 1. Antibacterial Activities of *C. pepo* extract against *S. aureus* at different solvent concentrations.

Solvent	Concentration (µg/ml)	Inhibition zone diameter (mm)	Control IPM (10 µg)
Cold water	100	10	10
	50	NI	
	25	NI	
Warm water	100	5	15
	50	NI	
	25	NI	
Methanol	100	12	20
	50	10	
	25	NI	
Ethanol	100	10	20
	50	NI	
	25	NI	

Key: IPM = imipenem, NI – No inhibition.

broth and was incubated for 18-24 h aerobrically and tubes which showed turbidity were re-inoculated onto nutrient agar and mannitol salt agar plates and incubated at 37°C for 18-24 h. After incubation, plates with growths were further characterized using standard microbiological techniques and biochemical tests including Gram staining, catalase, and coagulase tests.

Determination of antibacterial activities of plant extracts

This was determined by the method of Esimone et al. (2010) using agar well diffusion method. A 15-20 ml of molten Mueller-Hinton agar was aseptically poured into sterile petri dishes of equal sizes and was allowed to gel or solidify. The surface of the Mueller Hinton-agar was swabbed with the test organism (adjusted to 0.5 McFarland turbidity standards). Thereafter, a sterilized 6 mm cork borer was used to bore three holes on the Mueller-Hinton agar plates and the three holes were filled with 0.5 ml equal volume of the respective plant extracts at different concentrations of 100, 50 and 25 µg/ml. The aqueous extracts (cold water and warm water), methanol and ethanol extracts of *C. pepo, A. cordifolia* leaves and bark root of *T. ivorensis* were diluted with 4 ml of Dimethyl sulphoxide (DMSO) Imipenem 10 µg and ciprofloxacin 5 µg (Oxiod U.K.) paper disk which were used as positive control.

Determination of minimum inhibitory concentration of plant extracts on *S. aureus* isolated from burn wounds

The test organisms that were susceptible to the stock concentration of the herbal extract was further subjected to minimum inhibition concentration (MIC) using different concentration of 100, 50 and 25 µg/ml. The pure culture of the organisms were inoculated into nutrient broth and incubated at 37°C for 4-6 h. Then 0.5 ml of the broth culture of the bacteria were seeded on the surface of Mueller-Hinton agar plates and spread evenly. Three wells of 6 mm in diameter was cut on the seeded agar plates using a sterile cork borer, and the bored wells were each filled with the different herbal extracts concentration that is 100, 50 and 25 µg/ml in a separate agar plates. These were repeated for different plants *C. pepo*, *A. cordifolia* and *T. ivorensis* (cold and warm water, methanol and ethanol) and incubated at 37°C for 18-24 h. After which, the inhibition zone diameter (IZD) were measured to the nearest milliliter and the lowest concentration of that inhibited bacterial growth was taken as MIC.

Synergistic activities of herbal plant extracts against *S. aureus*

To determine the interaction of herbal extract combined, one gram each of the extracts was weighed and mixed together in the proportion of 1:1 ratio and was dissolved using 90% Dimethyl sulphoxide (DMSO) concentration. The mixture were used to fill the holes bored in the Mueller-Hinton agar seeded with 0.5 MacFarland turbidity standard of the isolates and were incubated at 37°C for 18-24 h. After which the zone of inhibition were measured and recorded.

RESULTS

Out if the 50 burn wounds samples collected from patients visiting FETHA II, 32 (64%) strains of *S. aureus* were isolated. The inhibition zone diameter of the plant extracts of *C. pepo* against *S. aureus* in different solvent and concentrations were shown in Table 1 The highest concentration of the *C. pepo* extracts was at 100 and 50 µg/ml in methanol (12 and 10 mm) and in cold water (10

Table 2: Antibacterial activities of *A. cordifolia* extract against *S. aureus* in different solvent concentrations.

Solvent	Concentration (µg/ml)	Inhibition zone diameter (mm)	Control IPM (10 µg)
Cold water	100	20	15
	50	15	
	25	5	
Warm water	100	10	15
	50	10	
	25	NI	
Methanol	100	10	14
	50	7	
	25	NI	
Ethanol	100	15	15
	50	15	
	25	5	

Key: IPM = imipenem, NI = No inhibition.

mm). No inhibitory zone diameter observed at 25 µg/ml as shown in Table 1.

Table 2 revealed that the inhibition zone diameter of plant extracts of *A. cordifolia* against *S. aureus* in different solvents concentrations. The highest inhibition zone diameter of cold water extracts of *A. cordifolia* yielded 20 mm at 100 µg/ml while at 50 µg/ml, 15 mm was recorded. In ethanol extracts it yielded 15 mm at 100 µg/ml while at 50 µg/ml 15 mm also recorded. In warm water extracts, it yielded 10 mm at 100 µg/ml while at 50 µg/ml, 10 mm also recorded and in methanol extracts, it yielded 10 mm at 100 µg/ml while at 50 µg/ml, 7 mm was also recorded. The lowest inhibition zone diameter was observed in cold water and ethanol extract at 25 µg/ml, which yielded 5 mm.

The highest synergistic effect was observed in ethanolic extract of *A. cordifolia* and root bark of *T. ivorensis* which gave 29 mm while in warm water extract of root bark of *T. ivorensis* and ethanolic extract *A. cordifolia* yielded 26 and 25 mm respectively. The inhibition zone diameter of plant extracts *T. ivorensis* against *S. aureus* in different solvent concentrations. The highest inhibition zone diameter of warm water of *T. ivorensis* extracts yielded 20 mm at 100 µg/ml while at 50 µg/ml 10 mm was recorded. Ethanolic and cold water extracts gave 15 mm at 100 µg/ml while at 50 µg/ml 12 and 13 mm was recorded respectively. At 25 µg/ml, methanolic extracts yielded 11 mm while cold, warm water and ethanolic extracts gave 8 and 5 mm. It was observed that among the three plant extracts. *T. ivorensis* had highest inhibition zone diameter against *S. aureus.* It have inhibition zone diameter in all the solvents in comparing to the control drug (Table 3).

Table 4 showed the minimum inhibition concentration of all the three plant extracts (*C. pepo, A. cordifiolia* and *T. ivorensis*). The lowest minimum inhibition concentrations among the three plants were observed in *T. ivorensis* and *A. cordifiolia* extracts, in cold water and ethanol. Extracts gave 5 mm respectively. This showed that *T. ivorensis* has the highest healing component followed by *A. cordifiolia* in treatment of infection caused by *S. aureus*. Table 5 demonstrated the synergistic activities of herbal plantextracts against *S. aureus.* The highest inhibition zone diameter at 100 µg/ml was observed in ethanolic leaf extract of A*. cordifiolia* and ethanolic bark root extract of *T. ivorensis* (29 mm), followed by warm water extract of *T. ivorensis* and ethanolic leaf extract of *A. cordifiolia*, (26 mm) and warm water extract of *T. ivorensis* and *A. cordifiolia* (25 mm).

DISCUSSION

Antibiotic resistance is a great global concern. There have been an increasing incidence of multiple drug resistance in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial agents commonly used in the treatment of infectious diseases (Marjorie, 1999). This has forced scientists to search for new antimicrobial

Table 3. Antibacterial activities of *T. ivorensis* extracts against *S. aureus* at different solvent concentrations.

Solvent	Concentration (µg/ml)	Inhibition zone diameter (mm)	Control drug IPM (10 µg)
Cold water	100	15	15
	50	12	
	25	8	
Warm water	100	20	10
	50	10	
	25	5	
Methanol	100	12	15
	50	12	
	25	11	
Ethanol	100	15	25
	50	13	
	25	5	

Table 4. The minimum inhibition concentration of all the three herbal plant extracts against *S. aureus*.

Solvents	Plant extracts Conc. (µg/ml)	Inhibitory zone diameter (mm)		
		C. pepo	***A. cordifolia***	***T. ivorensis* (5 mm)**
Cold water	100	Nil	25	25
Warm water	50	Nil	25	25
Methanol	25	Nil	Nil	25
Ethanol	100	Nil	25	25

Key: NI = No Inhibition, MIC = Minimum Inhibition Concentration.

substances from various sources like the medicinal plants. A total of 50 clinical samples were collected from burn wound patients, 32 were isolated and identified as *Staphylococcus* species based on microbiological and biochemical tests. The result showed Gram positive cocci and clustered in arrangement with purple colour. The result is in line with Sule et al. (2002) and Thanni et al. (2003) who reported that *S. aureus* is a normal flora of the skin and a major cause of both surgical and accidental wound infections. In this study, result obtained showed that the three plants used for the study possess bioactive compound against *S. aureus* isolated from wound burns. This is in agreement with Dweck (2001) who reported that medicinal plants possesses antimicrobial activity. In this work, *C. pepo* showed antibacterial activity against *S. aureus* in different solvents. *C. pepo* also revealed that different extracts in solvent have different compounds with antibacterial activity. This could be compounds with antibacterial activity. This could be due to the different classes of compounds or solvent used. This is in line with work of Marjorie (1999) who reported that different extracts in solvent have different compounds with antibacterial activity. Again, *C. pepo* extracts in methanol was observed to have highest inhibition zone diameter of 10 mm. This could mean that active ingredient of *C. pepo* extracts were not equally soluble in ethanol, methanol and water. This result is in agreement with the findings of who reported that alcohol/ ethanol as the best solvent for extraction of plant active substances of medical importance. Methanolic extract of *C. pepo* was also found to be active against *S. aureus. A. cordifolia* also revealed that almost the plant extracts in different solvents was equally able to inhibit the microbial growth of the isolate, *S. aureus*. It was observed that cold water and ethanolic extracts of *A. cordifolia* have the highest inhibition zone diameter (29 mm). This could be due to the infusion of dried and crushed leaves of the plant. *A. cordifolia* is soluble in cold water and ethanol moves faster in dried and crushed leaves of plant materials. This is in

Table 5. The synergistic activities of herbal plant extracts against the *S. aureus.*

Herbs	Inhibition zone diameter (mm) 100 (µg/ml)
A	14
B	9
C	11
D	14
E	19
F	18
G	19
H	19
I	29
J	19
K	26
L	25

Key: A = Cold water extract of *C. pepo* and *A. cordifolia, B* =Cold water extract of *C. Pepo and* root bark of *T. ivorensis,* C= warm water extract of *C . pepo and A .cordifolia,* D = warm water extract of *C .pepo* and root bark of *T .ivorensis,* E=Methanolic extract of *C. pepo* and root bark of *T. ivorensis,* F=Methanolic extract of *C .pepo* and *A. cordifolia,* G= Ethanolic extract of *C . pepo* and root bark of *T .ivorensis,* H= Cold water extract of *A.cordifolia* and root bark of *T .ivorensis,* I= Ethanolic extract of *A .cordifolia* and root bark of *T. ivorensis,* J = methanolic extract of *A. cordifolia* and root bark of *T. ivorensis,* K=warm water extract of root bark of *T. ivorensis* and ethanolic of A*. cordifolia,* L= warm water extract of root bark of *T. ivorensis* and *A .cordifolia.*

agreement with the work of Niemann et al. (2005) who reported that cold infusion of dried and crushed leaves of *A. cordifolia* acts as a durectic cicatrisant and antibacterial activities to wounds infections.

Among the three plant extracts used, *T. ivorensis* bark root have the highest inhibition zone diameter against *S. aureus*. This could be as a result of substance or constituent contained in the plant. This is in line with the work of Lawal et al. (2014) who reported that they contain substances like tannins, saponins, phenols, alkaloids and cyanogenic glucoside. Again alkaloids have been reported as the active ingredient in medicinal plants exhibiting potency as antibiotic, antidiabetic, and insecticidal agent (Abreu and Pereiru, 2001).

The comparison of MIC of the ethanol, methanol and aqueous extracts of the plant leaves and bark root was observed that ethanol extract showed greater antibacterial activity of 5 mm compared to its corresponding extracts in methanol and aqueous extracts with 5 mm. The combination of the plant extracts were very significant especially with *C. pepo* which was resistant on single testing in different solvent concentrations but on combination was able to inhibit the isolate. This is in agreement with the work of Yang et al. (2009) who reported that effectiveness of the herbs when used in pairs than when used individually in treatment of various ailments like malaria, HIV, even cancer promotes the advantage of combination therapy in treatment of such ailments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abreu P, Pereiru B (2001). Indole alkaloids from *Sarcocephaluslatifolius*. Natural Product Letter in Microbiology 15(1):43-48.

Blumenthal M (2008) .Herbal Medicine; httpwww amsa.org/Icam/C7 doc. Retrieved (2009-2012).

Cowan MM (1999).Plant Products as Antimicrobial Agents*.* Clinical Microbiology Reviews 12(4):564-582.

Dweck AC (2001). Article for Cosmetics and Toiletries Magazine Enthnobotanical Plants from Africa. Black Medicare Ltd UK. pp. 809-811.

Esimone CO, Akah PA, Nwaru CS (2010). Efficacy and Safety Assesment of *T. angelica* Herbal Tonic, a Phytomedicinal Product Popularly Used in Nigeria. Evidence Based Complementary and Alternative Medicine 2:1-6.

Joshi B, Sah GP, Basnet BB (2011). Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). Journal of Microbiology and Antimicrobials 3(1):1-7.

Kudi AC, Umoh JU, Eduvie LO, Gefu J (1999). Screening of some Nigerian medicinal plants for antibacterial activity. Journal of Ethnopharmacology 67(2):225-228.

Lawal DI, Gulay KF, Vivek VK, Bala JA (2014). Phytochemical Screening and in Vitro Antibacterial Studies of Ethanolic Extract of Citrus Sinensis (Linn) peel against some Clinical Bacterial Isolates. International Journey of Advances in Pharmaceutical Biology and Chemistry 3(4):1-7.

Marjorie MC (1999). Plant products as antimicrobial agents. Clinical microbiology reviews 12(4):564-582.

Nathisuwan S, Burgesss DS, Lewiss JS (2001). Extended Spectrum Beta-Lactmase: Epidemiology, Detection and Treatment. Pharmacotherapy 21(8):920-928.

National Population Commission (NPC) (2006). Report of Nigeria National Population Commission on the 2006 census. Population Commission on the 2006 Census. Population and Development Review 33:206-210.

Niemann HB, Atreya SK, Bauer SJ (2005). The Abundances of Constituents of Titan's Atmosphere from the GCMS Instrument on the Huygens probe. Nature 438(7069):779.

Parekh J, Chanda S (2006). In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turkish Journal of Biology 31(1):53-58.

Rosen BM, Martines L, Silva J, Martinez-Romero E (2004). *Klebsiella varicola* a Novel Species with Clinical and Plant Associated Isolates Syst. Applied Microbiology 27:27-35.

Sule AM, Thanni LOA, Sule-odu OA, Olusanya O (2002). Bacterial Pathogens Associated with Infected Wounds in Ogun State University Teaching Hospital, Sagamu Nigeria. Africa Journals Clinical Experiment Microbial 3:13-16.

Thanni LOA, Osinupebi OA, Deji-Agboola M (2003). Prevalence of Bacterial Pathogens. In Infected Wounds in a Tertiary Hospital in Ogun State University, Sagamu Nigeria. African Journal of Clinical Experiment 3:13-16.

Tyler, Foster (1999). Tyler's Honest Herbal Physician Desk Reference for Herbal Medicine Annual Columbia Encyclopedia Columbia University Press pp. 1-2.

Yang HY, Wang JD, LOT C, Chen PC (2009). Increased Mortality Risk for Cancers of the Kidney and Urinary Organs among Chinese Herbalists. Journal of epidemiology 19:17-33.