



IN-VITRO STUDIES ON THE SENSITIVITY OF STAPHYLOCOCCUS AUREUS TO SOME ETHNO-MEDICINAL PREPARATIONS SOLD AROUND KANO, NIGERIA

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ABSTRACT

Five traditional herbal preparations were sampled between May-June, 2009 in Kano. The samples were investigated for invitro antibacterial activities against clinical isolates of Staphylococcus aureus. Likewise, phytochemical screening tests were conducted to determine some of the phytochemicals present in the ethanolic and water extracts of the samples. Various concentrations of the extracts were prepared using serial doubling dilutions (5000µl/ml, 2500µg/ml, 1250µg/ml, 625µg/ml and 312.5µg/ml). All the test extracts showed slight antibacterial activity against the test organism, with ethanolic extract of sample E having the highest zone diameter of inhibition, while sample H had the lowest diameter of inhibition. The standard antibiotic disc (Gentamicin) had demonstrated the highest activity on the test organisms. The results of the Phytochemical screening revealed the presence of steroid in all the samples, tannin in samples A, C, D and E, reducing sugars in sample A, D and E respectively. The result of the minimum inhibitory concentration (MIC) was found to be above 312.5µg/ml for samples C, D and E.

Keywords: *Staphylococcus aureus, Herbal preparations, antibacterial activity, Phytochemical screening and minimum inhibitory concentration.*

INTRODUCTION

Ethno-medicine is a sub-field of ethno-botany or medical anthropology that deals with the study of traditional medicines not only those that have relevant written sources (e.g traditional Chinese medicine, Ayurveda) but especially those whose knowledge and practices have been orally transmitted over the centuries (Acharya and Anshu, 2008). In the scientific arena, ethno-medical studies are generally characterized by a strong anthropological approach, or by a strong biomedical approach, particularly in drug discovery programs. The focus of anthropological studies is the perception and context of use of traditional medicines, while biomedical approaches often focus on discovering therapeutic molecules such as the anti-HIV/AIDS molecule prostratin (Acharya and Anshu, 2008.). According to World Health Organization (WHO) (2008), traditional medicine is the sum total of knowledge, skills and practices based on theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness. Practices known as traditional medicines include Herbal, Ayurvedic, Unani, Ancient, Iranian, Siddha, Islamic and traditional Chinese medicine, Acupuncture, Muti, Ifa and other medical knowledge and practices all over the globe (WHO, 2008). The scientific evidence from tests done to evaluate the safety and effectiveness of traditional medicinal products and practices is limited. While evidence shows that acupuncture, some herbal medicines and some manual therapies are effective for specific conditions; further study of products and practices is needed. Requirement and methods for research and evaluation are complex. The safety

effectiveness and quality of finished herbal medicinal products depend on the quality of their source materials (which can include hundreds of natural constituents), and how they are handled through production process (WHO, 2009).

The genus *S. aureus* encompasses gram-positive, catalase positive, non motile cocci that can cause a variety of human infections in all age groups (Boyce, 1981). It is a major causative agent of surgical wound infections and epidemic skin diseases in new born infants (Baldwin *et al.*, 1990). *S. aureus* infections may also be superimposed on superficial dermatologic diseases such as Eczema, Pediculosis and Mycosis (Kloos and Bannerman, 1995). Human Staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defences. The portal may be hair follicle but usually it is a break in the skin which may be a minute needle stick or a surgical wound. Foreign bodies, including sutures, are readily colonized by *Staphylococci*, which may make infections difficult to control. Another portal of entry is respiratory tract. *Staphylococcus pneumoniae* is a frequent complication of influenza. The localized host response to staphylococcal infection is inflammation, characterized by elevated temperature at the site, swelling, the accumulation of pus, and necrosis of tissue. Around the inflamed area, a fibrin clot may form, walling off the bacteria and leucocytes as a characteristic pus-filled boil or abscess. Several investigations have been conducted to study the antimicrobial resistance pattern of *S. aureus* and it has been shown that the organism is resistant to β -lactam antibiotics, aminoglycosides and macrolides (Maple *et al.*, 1989; Atkinson and Lorian, 1984).

The antibiotic resistance of *S. aureus* had been debated in recent years; for example minimum inhibitory concentration (MIC) to penicillins encountered in more than one million strains tested in various hospitals in the USA, indicated that resistance has increased from 17% to more than 90% (Barret *et al.*, 1970). Due to medical importance of *Staphylococcus* species especially *S. aureus* in human and animals as it is responsible for many serious community and nosocomial-acquired infections and its popularity among traditional medicine vendors, there is need to ascertain the efficacies of various preparations (traditional) claimed to have higher activity on *S. aureus*. Therefore this study is aimed at determining the antibacterial activity of some traditional medicinal plants sold in Kano against *Staphylococcus aureus*.

MATERIALS AND METHODS

Sampling and sampling sites

Samples of five different herbal medicines were collected between May and June 2009 at different locations in Kano, Northern part of Nigeria. They were labeled sample A-E. Sample A and B were collected from Sabon Gari market Kano, Sample C from Ibrahim Taiwo Road, Sample D from Bayero University Old Campus and Sample E at Hotoro, Kwanar Sabo all from Herbal medicine sellers. Some of them disclosed the plant materials that were mixed while others failed to disclose it.

Bacterial isolates

The strain of test organism used in this work was *Staphylococcus aureus* obtained from Microbiology Department of Aminu Kano Teaching Hospital (AKTH), Kano. The isolate was subjected to biochemical tests according to standard procedures in order to confirm its identity (Cheesbrough, 2006).

Extraction of Samples

The samples were weighed and extracted by percolation using ethanol and water as solvent.

Fifteen grams of sample A, B and E were extracted in 150ml of ethanol for two weeks while

15g of sample C and D was extracted in 150ml of distilled water for two weeks. Sample A, B and E were extracted with ethanol because they are required to be taken with a fermentable substrate such as pap, while sample C and D were instructed to be taken with water.

After the percolation, the liquid phase of the extracts was filtered using Whatman No 1 filter paper. The crude extracts were concentrated using water bath at 40°C. The extracts were weighed and kept in the laboratory for further analysis.

Phytochemical Screening

The extracts were subjected to phytochemical tests to determine the groups of secondary metabolites present in the plant materials.

Test for Alkaloid

One half of a gramme (0.5g) of each extracts was stirred with 5ml of 1% aqueous hydrochloric acid (HBE) on a steam bath (GRIFFIN). One milliliter (1ml) of the filtrate was treated with a few drops of Mayer's reagent (HBE) and a second 1ml portion was treated

similarly with Dragendorff's reagent (BDH). Turbidity or precipitation with either of these reagents was considered as preliminary evidence for the presence of alkaloids in the extract Cuilci, (1994).

Test for Flavonoids

A piece of Magnesium ribbons were added to 2mls of 4mg/ml each of the fractions, followed by concentrated Hydrochloric acid (HBE) drop wise. A color change from orange to red indicated flavones and red to crimson indicates flavonoids (Sofowara, 1993).

Test for Reducing sugars.

One millilitre of each extract was taken in five separate test tubes. These were diluted with 2.0 ml of distilled water followed by addition of Fehling's solution (A+B) and the mixtures were warmed. Brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars in accordance with Brain and Turner (1975).

Test for Steroids

Two millilitres of the extract were taken into separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydrides and chloroform was then added. By means of a pipette concentrated sulphuric acid was added by the side of the test tubes. A brown ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer would indicate the presence of steroids as observed by Cuilci (1994).

Test for Tannins

Two milliliters of each extract were diluted with 2 ml of distilled water in separate test tubes, 2-3 drops of 5% Ferric Chloride solution was added. A green-black colour was observed which indicated the presence of tannins as reported by Cuilci, (1994).

Test for Glycosides

Two milliliters of each extract was placed in a sterile test tube. This was followed by adding 3ml of 3.5% iron III chloride (FeCl₃), then 3ml ethanoic acid. This gave a green precipitate and a dark coloured solution respectively. Finally, concentrated H₂SO₄ was carefully poured down the side of the test tube which resulted in the formation of brownish red layer, at the interface. This confirms the presence of glycoside (Sofowara, 1993).

Preparation of disc potencies

A No. 1 Whatman filter paper was punched to obtain 6mm discs which were placed in sterile bijour bottles and sterilized in an autoclave and allowed to cool. Five different concentrations of each extract from each of the samples investigated were prepared using serial dilution for the sensitivity testing i.e. 5000µg/ml, 2500µg/ml, 1250µg/ml, 625µg/ml and 312.5µg/ml respectively and placed in sterile bijour bottles. Subsequently 10 sterile discs each were aseptically placed in labeled bottles and 0.1ml solution of the various plant extracts in dimethyl sulphoxide (DMSO) were taken out using syringes and transferred into appropriate bottles containing the filter paper discs. For 5000µg/ml concentration 1g of the extracts was suspended in 2ml of DMSO.

For the 2500µg/ml concentration, 0.5ml of the 5000µg/ml solution was added to 0.5ml of DMSO. For the 1250µg/ml concentration 0.5ml of the 2500µg/ml concentration was added to 0.5ml of DMSO. For the 625µg/ml concentration 0.5ml of the 1250µg/ml concentration was added to 0.5ml of DMSO. For the 312.5µg/ml concentration 0.5ml of the 625µg/ml concentration was added to 0.5ml of DMSO.

Preparation of the Bacterial Culture using McFarland standard

The turbidity of the bacterial culture was compared with the standard McFarland 0.5 which is a barium sulphate standard against which the turbidity of the test and control inoculation was compared (Cheesbrough, 2006).

A sterile loop was used to pick the colonies of *S aureus* and emulsified in 4ml of sterile physiological saline which was compared with the turbidity of 4ml of the McFarland standard against a sheet of paper behind the test tubes for easier view.

Sensitivity assay of the test organism

The disc diffusion technique was used as described by Kirby and Bauer (1996). The standardized inoculum of the isolate was swabbed on the surface of Mueller Hinton agar using a sterile swab stick, then followed by placing the prepared discs of the extracts and the standardized antibiotic disc (Cephalexin and Gentamicin) on to the surface of the media at regular intervals. The plates were incubated at 37°C for 18 hours.

Determination of minimum inhibitory concentration for the extracts

Different concentrations of the plant extracts (3125, 1500 and 700µg/ml) were prepared by serial doubling dilution and incorporated into test tubes of Mueller Hinton broth (MHB) (Scharlau Microbiology, Belgium). Standardized inocula of the test organism were introduced into the test tubes containing the extract concentrations. Then a positive control test tubes containing the test extracts and MHB and a negative control test tube containing the

standardized inoculums of the organism and the MHB were also prepared. All the setups were incubated at 37°C for 24 hours. The tubes were observed after incubation to determine the minimum inhibitory concentration that shows no evidence of growth (turbidity) (Cheesbrough, 2006).

RESULTS

Steroids were found in all the samples. Tannin was found in sample A, C, D and E. Alkaloids, Flavonoids, Glycosides and Reducing sugars were found together in samples A and E (Table 1). Samples B, C, D contain Alkaloid, Flavonoid and Glycosides respectively.

Sample A yielded extracts amounting to 20.7% of the initial powdered plant materials when subjected to ethanol extraction. The extract was slightly active against the clinical isolate of *S. aureus* at 5000µg/disc concentration and inactive against the isolate at the remaining concentrations tested (Table 3). Seven and half percent (7.5%) of sample B was yielded after subjecting it to ethanol extraction. It has slight activity at all concentrations tested (Table 4). Sample C yielded 9.6% of the initial powdered plant material after subjecting it to water extraction.. It has slight activity at all concentration tested (Table 4). Sample D yielded highest percentage amounting to 40% of the initial powdered plant materials after subjecting it to water extraction. The extracts have a little activity against *S. aureus* at all concentrations with the exception of 312.6µg/disc concentration (Table 4). Sample E gave a yield of 9.7% of the initial powdered plant materials when subjected to ethanol extraction. The extract has slight activity against *S. aureus* at all concentration (table 3). The texture and appearance of each extract obtained was shown on table 2. Minimum inhibitory concentrations (MIC) of sample C, D and E against *S. aureus* were found to be above 1500µg/ml. (Table 5).

Table 1: Some physical parameters of the sample obtained

Physical parameter	Sample A	Sample B	Sample C	Sample D	Sample E
Weight extracted (g)	15	15	15	15	15
Weight of extract (g)	3.1	1.13	1.5	1.6	2.95
Percentage yield (%)	20.7	7.5	10	40	19.7
Colour	Brown	Green	Brown	Reddish brown	Light brown
Texture	Gummy	Gummy	Soft	Gummy	Soft

Table 2: Some secondary metabolites in the samples obtained

Phytochemical	Sample A	Sample B	Sample C	Sample D	Sample E
Alkaloids	+	+	-	-	+
Flavonoids	+	-	+	-	+
Glycosides	+	-	-	-	+
Reducing sugars	+	-	-	+	+
Steroids	+	+	+	+	+
Tannins	+	-	+	+	+

Key: +=Present, - = Absent

Table 3: Antimicrobial activity of ethanol extracts of the herbal preparations against *S. aureus* clinical isolate

Extracts	Disc potency µg/disc					Cep	Gen
	5000	2500	1250	625	312.6		
	Diameters of Zone of Inhibition (mm)						
A	7	6	6	6	6	6	30
B	9	8	7	7	7	6	30
E	10	10	9	9	8	6	30

Key: A=Sample A; B= Sample B; E= Sample E, Cep=Cephalexin, Gen=Gentamicin

Table 4: Antimicrobial activity of aqueous extracts of the Herbal preparation against *S. aureus* clinical isolate

Extracts	Disc potency µg/disc					Cep	Gen
	5000	2500	1250	625	312.6		
	Diameter of Zone of Inhibition (mm)						
C	8	8	8	7	7	6	30
D	7	7	7	7	7	6	30

Key: C=Sample C; D=Sample D; Cep=Cephalexin, Gen=Gentamicin

Table 5: Minimum Inhibitory Concentration of the Plant extracts on *S. aureus*

Extracts	Concentration of the extracts µg/ml		
	312.5	700	1500
C	+	+	+
D	+	+	+
E	+	+	+

Key: C, D and E =Sample C, D and E respectively, +=Growth observed

DISCUSSION

The herbal samples screened in this study exhibited varying degrees of antibacterial activity against the test organism. Sample E showed the highest antibacterial activity among all the samples even though with a slight antibacterial activity when compared with the standard Gentamicin with zone diameter of inhibition of 10mm at 5000 and 2500µg/disc concentrations. The antibacterial activity of sample E may be due to the presence of the plant *Commiphora kerstingii* which was highly effective in suppressing the growth of *S. aureus* invitro. The plant contains tannins as one of the classes of natural products detected during phytochemical screening which was reported by Scalbert (1991). Sample E also contains *Anogiessus latifolia* as one of the plants which is also used as medicine in India. Its leaves contain large amount of tannins (Shiva, 1995). Similarly the antimicrobial activity of sample E could also be attributed to the presence of other phytochemicals such as flavonoids and alkaloids which were found to possess some antibacterial activity according to Tschehe (1971), and Singh and Bhat (2003).

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Sample C had very little activity against the test organism at concentrations of 5000, 2500 and 1250µg/disc with a zone diameter of inhibition of 8mm respectively. The presence of *Piper nigrum* among the plants in sample C may be responsible for the little activity shown by the sample because it had been documented that *Piper nigrum* (black pepper) figure in remedies in Ayurveda, Siddha and Unani medicine in India (Sofowora, 1993). Sample C as well contains tannins and alkaloids which are well documented for antimicrobial activity. The slight antibacterial activity of sample D and A might be due to the presence of tannins in both samples and in addition flavonoids and alkaloids in sample A. Sample B contains alkaloids which might be responsible for the slight antibacterial activity exhibited by the sample

The general performance of the test samples was far lower than the standard gentamicin disc at 10µg/disc with average of inhibition of 30mm. From this study it can be concluded that standard Gentamicin has superior activity compared to the test samples even though all had demonstrated varying degree of activities.

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