Antimicrobial potency of *Sphenocentrum jollyanum* on some human pathogenic bacteria

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Abstract: Water, ethanol, petroleum ether and chloroform were used individually to obtain extracts of the root of *Sphenocentrum jollyanum*. Each of the extracts was screened against some human pathogenic bacteria for antimicrobial activity. The human pathogenic bacteria were *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Streptococcus faecalis*, *Staphylococcus aureus*. The aqueous extract of the plant root showed the least antimicrobial activity. It was effective on gram positive *Staphylococcus aureus* at a diluted concentration of 83mg/ml and *Streptococcus faecalis* at undiluted concentration of 100mg/ml. Solvent extracts of the sample were effectively better than the aqueous, with petroleum ether having the highest potency than ethanol and chloroform. Comparison of inhibitory effect of the root extract against some broad spectrum antibiotics revealed that ciprofloxacin had the highest efficacy against the susceptible gram negative bacteria used. Antimicrobial activity of these extracts on gram positive and gram negative bacteria cannot be overemphasized as potency of these bacteria was as high as the most potent antibiotic tested.
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Introduction

Nature has been a source of medicinal agent for thousands of years and an impressive number of modem drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants are of great importance to the health of individuals and communities. In fact, herbal medicines are known to serve the health needs of about 80% of the world's population; especially for millions of people in the vast rural areas of developing countries (WHO, 2001). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found to have *in vitro*, antimicrobial properties (Edeoga *et al*., 2005). The medicinal value of these plants lies in some chemical substances that provide definite physiological action on the human body. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost consequently encouraged both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair *et al*., 2005).

Undoubtedly, medicinal plants are relevant in both developing countries and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes (Alanis *et al*., 2005). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy *et. al*., 2001; Adejuwon *et al*., 2011).

Sphenocentrum jallyanum was first discovered by Dieism England. It is called "obalabi" or" obanabe" in the south western part of Nigeria. Yorubas call it "Ajo" or "Akerejupon". It grows mainly in the rain forest areas usually in damp places under forest cover. The fruit is like mango and it is edible. *Sphenocentrum jollyanum* belongs to Menispennaceae family. In indigenous Yoruba language, It is called "Akerejupon". The roots are commonly used for healing purposes (Dalziel, 1955). This plant is known to be effective in treating chronic wounds, cough, tumours and other inflammatory conditions. lt is also known to have antioxidant and anti-angiogenic property and it contains flavonoids, 150 guinaine alkaloids such as palmatine, columbamine. deterpenes and some other alkaloids (Nair *et al*., 2005).

The purpose of study was to determine the antimicrobial effect of the root extract of *Sphenocentrum jollyanum* on different pathogenic microorganisms.

Materials And Methods

Collection of Plant Materials

The extract used for this study was the root of *Sphenocentrum jollyanum*. The already sun-dried roots were purchased from Bode market at Ibadan, Nigeria and identified at the Department of Botany, University of Ibadan, Ibadan, Nigeria. The roots were washed

thoroughly with distilled water, dried before milling into powder for experimental use.

Collection of Test Organisms

The following bacteria were used for this research work: *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Proteus rnirabilis*. They were all obtained from the Laboratory Unit, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria.

Glass Sterilization

The glasswares used were thoroughly washed with liquid detergent, rinsed with distilled water, drained and dried. They were wrapped with aluminium foil and sterilized at l60°C in a hot air oven (Fawole and Osho, 2002).

Validity of Test Organisms

The organisms were maintained in nutrient agar slants and kept in the refrigerator at about 4^0C. They were further sub-cultured into petri dishes using streak plate technique. Gram staining procedure, catalase, starch hydrolysis and oxidase tests were carried out on the samples to establish the validity of the test bacteria (Harrigan and McCane, 2001).

Preparation Culture Media

Peptone water

The peptone water used for the culturing of the test organisms was buffered to pH 7.2. This was done by mixing 38ml of 3% monobasic sodium phosphate (Na_2PO_4) with 72ml of 7% dibasic I sodium phosphate ($Na_2HPO_4.12H_2O$). This solution was mixed and later homogenized. The mouth of the flask was then plugged with cotton wool and wrapped with aluminium foil paper. This was then sterilized in an autoclave at a temperature of 121°C for 15 minutes (151b/sq inch pressure). This peptone water was buffered to allow the medium to maintain its pH value, as microbial metabolite can alter the pH value of the medium with subsequent exhibition of variable gram characteristics (Valya *et al*., 2011; WHO, 2003).

Nutrient agar

According to the manufacturer's instruction, 8g of Nutrient Agar powder was dissolved into 250 mls of distilled water. The solution was properly mixed and placed in a homogenizer toensure proper solubility and even distribution. The mouth of the conical flask was then plugged firmly with cotton wool then wrapped with aluminium foil paper. This was then sterilized in an autoclave at a temperature of 121°C for 15minutes (Fawole and Osho, 2002).

Reactivation of Test organisms

The bacteria isolates were tested for viability by resuscitating the organisms in buffered peptone broth for 72 hours to allow heavy growth. It was then sub-cultured into the nutrient agar using streak plate techniques and incubated at 37^0C for 24hrs (Valya *et al*., 2010; Nwinyi *et al*., 2009).

Plant Extract Preparation

Forty grams of milled extract powder (*Sphenocentrum jollyanum*) were weighed and dissolved into 400ml of different solvent for 24 hours. The solvents were aqueous sterile distilled water, chloroform, ethanol and petroleum ether. The supernatant was filtered using muslin cloth first and then Whatman No 1 filter paper. The extract was used immediately and the remaining extract stored in the refrigerator for further study (Olayemi and Opaleye, 1990).

Antimicrobial Activity on Bacteria

Well diffusion technique

The extracts were tested for antimicrobial activity using the well diffusion method. This method depends on the diffusion of extracts from cavity through the solid medium in the petri dish such that growth of the cultured organism is restricted for a zone, thereby forming a circular area around the extract. The observed cleared zone and the diameter of such clearance is directly proportional to the efficacy of the extracts (Dairo and Adanlawo, 2007; Valya *et al*., 2010; Nwinyi *et al*., 2009).

Inhibitory tests

The test organism was streaked on the solid nutrient agar until it covered the petri dish using sterile inoculating loop. A sterile cork borer of about 8mm in diameter was used to cut deep uniform wells into the agar gel. Each well was then filled with the extracts prepared in different concentrations. The petri dishes were allowed to stand for 45 minutes at room temperature to allow proper diffusion. The control experiments were then set up using each of the solvents (without extracts). Sterilized distilled water was equally used as aqueous control sample.

Sensitivity Tests Disc (STD) was used side by side to compare the degree of clearance. The culture plates were transferred into the incubator for 24 and 48 hours at 37°C. Zones of clearance around each of the wells and their diameter were measured in millimeter (mm) value. The minimum inhibitory concentration (MIC) was determined by comparing the difference in concentration of the extracts with the control (Satish, 1998; Harrigan and McCane, 2001).

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using the agar streak technique (Nester *et al*., 2001). The concentration around the agar well that gave the least inhibition zone was regarded as the minimum inhibitory concentration.

The minimum inhibitory concentration of the extracts (*Sphenoccntrum jollyanum*) against the human pathogens used was determined by dissolving 40g of

the extracts sample with 400ml (0.4L) solvent. This was then filtered with muslin cloth and with Whatman No 1 filter paper into glass beaker before being concentrated in the water bath at 60°C for about 4 hours. The concentrated extract solution was then diluted serially four folds to give various diluted concentrations in mg/ml.

Determination of Minimum Potency Concentration (MPC)

The minimum potency concentrations for the extract were evaluated. According to Satish (1998), an inhibitory clear zone of 6mm diameter or less was considered non-potent on isolate.

Results

Aqueous, petroleum ether, ethanoic and chloroform extracts of *Sphenocentrum jollyanum* at concentrations of 72, 77, 83, 91 and 100 mg/ml were used in the present study. In Table 1, we report the clearance and zones of inhibition by extracts of *Sphenocentrum jollyanum* within 24hr incubation. In Table 2, we have zones of inhibition within 48hr. Isolates tested were *Streptococcus faecalis*, *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*.

In Table 3 we have sensitivity to commercially available standard antibiotics discs. *Escherichia coli* and *Proteus mirabilis* were sensitive to ciprofloxacin (ClP) and Gentamycin (GN). *Proteus mirabilis, Staphylococcus aureus and Streptococcus faecalis* were sensitive to Augmentin (AG). Other antibiotics tests disc used were non-reactive to the test organisms. These were Amoxillin (AX), Erythromycin (E), Norfloxacin (NB), Nitrofurantoin (N), Tetracycline (T), Chloramphemcol (C), Ampicillin (AM), Nalidixic Acid (NA), Cefuroxine (CF), Drovid (D), Cephalexin (CX), Clindamycm (CD), Septrin (SXT) and Ampiclox (AC). The isolates were resistant to these.

Table 1: Inhibition of isolates by extracts of *Sphenocentrum jollyanum* at 24hr incubation

	Microbial Isolate	Extract	Conc. 100mg/ml	Conc. 91mg/ml	Conc. 83mg/ml	Conc. 77mg/ml	Conc. 72mg/ml	Control
A	*Streptococcus faecalis*	Water	10	-	-	-	-	-
		Petroleum Ether	50	40	38	34	30	18
		Ethanol	24	20	-	-	-	-
		Chloroform	26	18	-	-	-	-
B	*Staphylococcus aureus*	Water	30	30	18	-	-	-
		Petroleum Ether	48	32	29	24	24	20
		Ethanol	33	24	-	-	-	6
		Chloroform	20	15	13	-	-	8
C	*Klebsiella pneumonia*	Water	-	-	-	-	-	-
		Petroleum Ether	60	55	50	45	40	38
		Ethanol	36	21	18	-	-	5
		Chloroform	48	42	35	30	-	22
E	*Escherichia coli*	Water	-	-	-	-	-	-
		Petroleum Ether	34	28	25	16	-	12
		Ethanol	20	20	-	-	-	-
		Chloroform	14	-	-	-	-	-
	Proteus mirabilis	Water	-	-	-	-	-	-
		Petroleum Ether	23	18	12	12	-	5
		Ethanol	22	15	13	-	-	8
		Chloroform	13	5	-	-	-	-

Table 2: Inhibition of isolates by extracts of *Sphenocentrum jollyanum* at 48hr incubation

	Microbial Isolate	Extract	100mg/ml	91mg/ml	83mg/ml	77mg/ml	72mg/ml
A	*Streptococcus faecalis*	Water	10	-	-	-	-
		Petroleum Ether	32	22	20	16	12
		Ethanol	24	20	-	--	-
		Chloroform	26	18	-	-	-
B	*Staphylococcus aureus*	Water	30	30	18	-	-
		Petroleum Ether	28	12	9	4	4
		Ethanol	27	18	-	-	-
		Chloroform	12	7	5	-	-
C	*Klebsiella pneumonia*	Water	-	-	-	-	-
		Petroleum Ether	22	17	12	7	2
		Ethanol	31	16	13	-	-
		Chloroform	26	20	13	8	-
D	*Pseudomonas aeruginosa*	Water	-	-	-	-	-
		Petroleum Ether	21	12	9	-	-
		Ethanol	24	14	14	9	-
		Chloroform	21	14	6	-	-
E	*Escherichia Coli*	Water	-	-	-	-	-
		Petroleum Ether	22	16	13	4	-
		Ethanol	20	20	-	-	-
		Chloroform	14	-	-	-	-
F	*Proteus mirabilis*	Water	-	-	-	-	-
		Petroleum Ether	18	13	7	7	-
		Ethanol	14	7	5	-	-
		Chloroform	13	5	-	-	-

Table 3: Reaction of pathogenic bacteria to antibiotics Reactive antibiotics:

Trade code	Commercial name	Diameter of inhibition	Microorganism
CIP	Ciprofloxaxin	18mm	*Proteus mirabilis*
CIP	Ciprofloxacin	12mm	*Escherichia coli*
GN	Gentamyci	15mm	*Proteus mirabilis*
GN	Gentamycin	5mm	*Escherichia coli*
AG	Augumentin	9mm	*Staphylococus aureus*
AG	Augmentin	14mm	*Proteus mirabilis*
AG	Augmentin	8mm	*Streptococcus faecalis*

Non Reactive antibiotics:

Amoxillin (AX), Erythromycin (E), Norfloxacin (NB), Nitrofurantoin (N), Tetracycline (T), Chlorampheмcol (C), Ampicillin (AM), Nalidixic Acid (NA), Cefuroxine (CF), Drovid (D), Cephalexin (CX), Clindamycin (CD), Septrin (SXT) and Ampiclox (AC).

Discussion

The antimicrobial activity of the solvent extracts of *Sphenocentrum jollyanum* root against the human pathogenic bacteria indicates the assessment of the potency of the root extracts as observed in the inhibition zones that occurred on the plates. The results obtained showed that two of the root extracts

(petroleum ether and ethanol) had significant antimicrobial activity against the pathogenic bacteria tested (*Pseudomonas aeruginosa*, *Proteus mirabilis* and *Streptococcus faecalis*).

Petroleum ether was found to be the most active against the tested bacterial strains followed by ethanol among all extracts used. This is similar to the findings of Girija *et al.* (2011), who reported alcohol as a good organic solvent for the extraction of most plant bioactive constituents of medicinal importance. Nester *et al.* (2001) reported that genetic properties of tested organisms play a major role in the resistance effects of microorganisms on extracts.

Klebsiella pneumonia was found to be the most susceptible to petroleum ether extract with an inhibition zone diameter ranging between 60mm and 55mm at concentration between 100 mg/ml and 91 mg/ml followed by *Streptococcus faecalis* with an inhibition zone diameter ranging between 40mm and 50mm at concentrations between 100 mg/ml and 91 mg/ml. *Staphylococcus aureus* had an inhibition zone diameter of 48mm at 100 mg/ml. The remaining three bacteria strains were resistant to the ethanol extract.

Petroleum ether extract showed maximum antimicrobial activity against *Streptococcus faecalis* at concentration between 91 mg/ml and 100 mg/ml with inhibition zone diameter ranging between 22mm and 32mm. *Escherichia coli* had an inhibition zone diameter between 16mm and 22mm at concentrations ranging between 91 mg/ml and 100 mg/ml. *Proteus mirabilis* showed least susceptibility with inhibition zone diameter ranging between 13mm and 18mm. From these results, it can be inferred that the activity of the extract is concentration dependent. This is in agreement to an earlier report that an increase in the concentration of an antimicrobial agent might result in an increase in its effectiveness (Aspen, 2000).

The alcohol extracts showed greater antimicrobial activity than the corresponding aqueous and petroleum ether extracts. This finding is interesting in that the African traditional method of treating a bacterial infection is by administering a decoction of the plant parts or whole plant in water whereas, from these results, preparing an extract with an organic solvent seem to provide a better antimicrobial activity which is in accordance with the results obtained by Nair *et al.* (2005). African traditional healers in history, used aqueous solutions to extract biologically active compounds because of ease of availability (Shale *et al*., 1999), however, the findings of these experiments show that alcoholic extracts seem to have greater antimicrobial activity.

The mechanism of action of the bioactive constituents of *Sphenocentrum jollyanum* may be difficult to speculate, though many antibacterial agents may exhibit their action through inhibition of nucleic acids, proteins and membrane phospholipids biosynthesis (Franklin *et al.*, 1987). The strong extracting capacity of the organic solvent has produced greater number of bioactive constituents responsible for antimicrobial activity (Okigbo and Omodamiro, 2005). The bioactive components contained in a plant are connected with its antimicrobial properties (Adegoke *et al*., 2009).

Plants are rich reservoir of antimicrobials. It is observed that a single plant is known to contain several bioactive principles of biological significance (Cowan, 1999). The antimicrobial properties of *Sphenocentrum jollyanum* probably connote its traditional use for treating bacterial disease. Akinpelu and Obuotor (2000) indicated that different solvent extracts of some plants may exhibit different pharmacological properties. The observed variation in susceptibility pattern of the test bacteria used in this research may be related to the genetic properties of test organisms which plays major role in their resistance to the effects of the extract. Also, the susceptibility of the pathogenic bacteria to the alcohol extracts or their resistance to the extract at varying concentrations might be ascribed to the differences in the morphology of the cell structure and chemical composition within these organisms as well as variation in permeability and osmotic potential (Hailu *et al.,* 2005).

From the results of this experiment, gram negative bacteria used are more susceptible to the plant extract than gram positive bacteria which contradicts previous reports that plant extracts are more active against gram positive bacteria than gram negative bacteria (Tulukeu *et al.*, 2009; Delmare *et al*., 2007; Tepe and Daferera, 2005).

It is a general belief that gram positive bacteria are more susceptible than gram negative bacteria to antimicrobials due to differences in the cell wall structure. Gram negative organisms are considered to be more resistant to antimicrobials because of their outer membrane acting as a barrier to many environmental conditions including antibiotics (Tortora *et. al.,* 2001). Of all the microorganisms used, *Streptococcus faecalis* had the highest susceptibility with Petroleum ether extracts. This is an indication that the extract could be a good first line basis for drug production with high potency against infection caused by this bacterium.

Proteus mirabilis is frequently associated with urinary tract infection, bacteraemia, pneumonia and focal lesions in debilitated patients or those receiving intravenous infusions (Brooks *et al.,* 2007). According to Nester *et al.* (2001), *Proteus mirabilis* seem to be more resistant to many of the commonly used antibiotics and may be liable to cause super-infection during antibiotic therapy. We observed that Norfloxacin and Ciproflaxin had significant

antimicrobial activity against our strain of *Klebsiella pneumonia* when compared with the organic solvent extract and aqueous extract of the herb. The aqueous extract of the herb had no observable antimicrobial activity on this isolate. We observed no noticeable antimicrobial activity of both the aqueous and organic solvent extracts on our strain of *Escherichia coli.* A similar result was for the broad spectrum antibiotics. Our observations on *Staphylococus aureus* and *Escherichia coli* were similar.

Conclusion

Antibiotics provide the main basis for the therapy of bacterial infections. However, the genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. In recent years, development of multidrug resistance in pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases. Multidrug resistance and other problems such as toxicity of certain antimicrobial drugs in the host tissue have triggered interest in search of new antimicrobial substances/drugs of plant origin, considering, perhaps the rich diversity of *Sphenocentrum jollyanum* in bioactive constituents. Screening of various natural organic compounds and their identification to reveal the active principle by isolation and characterization of their antimicrobial constituents must be considered as a fruitful approach in the search of new herbal drugs for folkloric usage.

Antimicrobial activity can be advanced if active components of plants are purified with the determination of adequate dosage for proper administration. This may go a long way in preventing the administration of inappropriate concentrations which is a common practice among many folklore medicine practitioners. Finally, the results obtained from *in vitro* antimicrobial assay should confirm the therapeutic potency of *Sphenocentrum jollyanum* used in folklore medicine. The antibacterial properties of this herb makes it of interest in the development of new drugs in pharmaceutics.

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