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ANTIMICROBIAL EFFECT OF ROOT EXTRACTS OF AFRICAN PEACH *(SARCOCEPHALUS ESCULENTUS AFZEL)* ON SOME HUMAN PATHOGENS

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ABSTRACT

The roots extract of Sarcocephalus esculentus (African peach) was tested on some human pathogens; *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Kleibsiella pneumonia,* and *Pseudomonas aeruginosa* were the bacteria isolates used. The organic solvents used for the extraction of active mycolides of the medicinal plant root were, ethanol, chloroform, methanol and petroleum ether. *S. typhi, K. pneumonia* and *S. aureus,* showed a high sensitivity to root extracts of S. esculentus, while *E. coli* and *P. aeruginosa* were resistant to all the extracts. The minimum inhibitory concentration value of the extracts on the organisms ranged between 0.0625-0.125mg/ml for *S. typhi* and 0.125-0.5mg/ml for K. pneumonia, and 0.125mg/ml for *S. aureus*. The solvent type of extract, concentrations of the root extracts and the organisms were factors that affected the antimicrobial activity.
Keywords. *Antmicrobial, human pathogens, mycolide, medicinal plant.*

INTRODUCTION

Sarcocephalus esculentus (African peach in English, "Uburu ilu" in Igbo, and "doundake" in Sierra Leone) is a multi stemmed tree or shrub up to 12m. It has an open canopy flowers with terminal spherical head –like cymes of smell whitish flowers. The flowers are joined by the calyces. The fruit is a syncarp (Dalziel, 1959). *S .esculentus alfzel* was formerly used as the sole constituent of arrow poisoins in the Rio Nunez area of West Africa Guinea (Charles, 1885). Nowadays, the very bitter stem or the roots are ingredients of *strophathes hispidous* – based arrow poisoins in Northern Nigerian and in the Northern part of Ivory Coast (Abbiw, 1990). In Nigeria, the fruit of the plant is eaten as a cough remedy (Asuzu, 1996). In Kinshasha, the plant is used by traditional healer to treat diabeties. In Nigeria, the roots, leaves and stem of the plant in powdered form is used as a cure for malaria fevers (Iwalewa, *et al.*, 2007). This is because it contains an alkaloid *strictosamine*. The human pathogens are the causes of various diseases in man. E. coli is associated with gastroenteritis and food poisoning (Etani,et al., 1998). S. typhi is the microorganism that causes malaria and typhoid (Assuzu, and Njoku., 1996) S. aureus is reponsible for the cause of wound diseases, while K. pneumonia and P. aeruginosa have been implicated in urinary tract infection. Plants have a great potential for producing new antimicrobials of great importance to mankind. The development of side effects and prevalence microbial resistance to existing antimicrobial drugs underscores the need for the continuous search for new antimicrobials (Olorundare, et al., 1998). The key for such a search is to screen natural products for active agents. Considering the afore said, the medicinal plant (based on its folk medicinal importance) Sarcocephalus esculentus was screened for its antimicrobial activities.

MATERIALS AND METHODS

Collection of Plant Material

In January 2008, roots of African peach (S. esculentus) were taken from the compound of Elder Ogbonnaya Okorafor in Arochukwu town, Abia State, Nigeria.The taxonomic identification of the plants was established in the haberium of Micheal Okpara University of Agriculture Umudike.

Test for Potency of Bacteria

The following organisms*, E. coli, S. typhi, K. pneumonia, P. aeruginosa, and S.aureus* were used. These bacteria were stock culture collection of the Federal Medical Centre Umuahia. They were identified according to standard method. Viability tests of each isolate were carried out by resuscitating the organisms in buffered Tryptone Soy Broth (Biotic, U.K) and thereafter sub-culturing onto appropriate solid media followed by overnight incubation at 37^0C. Enteropathogenic *E. coli* and *S. typhi* were sub-cultured onto MacConkey Agar (BL9 6AU-Lab-2, England); *S. aureus* onto Nutrient Agar (BL9-6AU Lab-8, England); *K. pneumonia* and *P.aeruginosa* onto Blood Agar (Biotec, U.K) and sabouraud Dextrose Agar (Biotec, U.K) and incubated for 24h at 37^0C, followed by refrigerator storage at 4^0C until required for use.

Organic Extract Preparation

The organic solvents used were ethanol (BDH Chemicals Ltd. England), chloroform (M&B Ltd., England), petroleum x63sether (James Burougy Ltd.,England), and methanol (H.E.Chemicals Ltd., U. K).

Ethanol Extract. The root of S. esculentus following complete dryness were powdered and sieved. Certain portion of *S. esculentus* was percolated with 10litres of ethanol for five days. Sterile filter paper was used to filter the extract. A rotary evaporator at 35^0c concentrated the filtrate. The solvent was recovered in the recovery flask while the extract remained in the sample holder, which was collected and stored in the refrigerator at 4^0C. A process called partitioning was employed to obtain the chloroform, petroleum and methanol extract.

Chloroform Extract. A portion of ethanol extract was partitioned between Chloroform and water in the ratio of 1:1 (150ml of Chloroform: 150ml of water). The mixture was shaken for 1 hr and allowed to stand inside a separating funnel for 24hrs. After which Chloroform fraction was collected. Dried extract was obtained from this fraction on the exposure to the atmosphere.

Petroleum and Methanol extract. Petroleum and methanol extracts were obtained from *S. esculentus* by employing a second partitioning between petroleum ether and methanol solvents using a certain portion of the chloroform extract. The procedure is same as was described for the chloroform extract.

Antimicrobial Susceptibility Testing

Antimicrobial activity of the extracts was evaluated using the following organisms, *E. coli, S. typhi*, *K –pneumoniae, P. aeruginosa, S. aureus.* The ability of the various extracts to inhibit

growth of the clinically significant bacteria isolate was determined using the 8mm diameter hole in agar - diffusion technique. Sterile glass pippets of 8mm diameter were used to make holes on prepared agar medium. Aliquots of 0.2ml of the extracts were introduced into the holes made on pre-seeded appropriate gelled media containing each isolate of organism, at different concentrations of the extract, for a specific isolate. A control hole where the solvent used for the extraction was added to one of the plates. Plates were incubated at 37^{o}C for 24hrs in the incubator. Following the incubation, the diameter of zone of inhibition was recorded.

Determination of Minimum Inhibitory Concentration (MIC).

The minimum inhibitory concentration (MIC) of the extracts was determined by incorporating constant volumes (0.2ml) of each dilution of the extracts and antibiotics solutions into sensitivity dose prepared with white man filter paper No.1. This dilution was gotten by dissolving 0.2gml of the extract as well as the antibiotics in 100ml of sterile distilled water to obtain 2.0mg/ml. This 2.0mg/ml concentration was then doubly diluted in sterile distilled water to obtain concentrations of ½, ¼, 1/8, 1/16, and 1/32mg/ml. (100mg, 50mg, 25mg, 12.5mg, and 6.25mg). 0.2ml of diluted inoculums was introduced into the Petri dishes with agar, swab before introducing the sensitivity discs. The plates were incubated at 37^{o}C and examined after 24hrs and 48hrs growth. Zones of growth of inhibition were measured in millimeters using a plastic ruler. The positive control discs contain 2mg/ml of Ciprofloxacin sulphate produced by Pfizer (5mm diameter) tested concentration. The zone of inhibition is referred as the clearly visible zones of inhibition across a diameter disregarding the diameter of the disc.

RESULT

Certain Pathogens demonstrated susceptibility to the organic solvent extracts of *Sarcocephalus esculentus*. E.*coli* and *P.aeruginosa* showed resistance to the organic solvents , while S. *typhi*, K. *pneumonia*, and S. *aureus* were inhibited. The MIC in ethanol extract was 0.0625mg/ml for S. *typhi* , and 0.125mg/ml for both K.*pneumonia* and S. *aureus* which is notably the same result with the chloroform extract(Table1,& 2). Effects varied in all the four organic solvents extracts of S. esculentus root. In petroleum ether extract, S. *typhi* had an MIC of 0.125mg/ml and 0.5mg/ml for K. *pneumonia*, while S. *aureus* showed resistance in petroleum ether extract (Table 4). It is interesting to note that S. *typhi* with MIC of 0.0625mg/ml demonstrated the lowest MIC of all the micro-organisms that were sensitive to the S. *esculentus* root extract (Table 5). Also it is worthy to note that the ethanol extract exhibited a level of zone of inhibition closely related to that of the standard drug (Ciprofloxacin). In the Methanol extract, K. *pneumoniae* had a higher MIC of 0.5mg/ml (Table 3) whereas S. typhi and S. aureus were both 0.125mg/ml. In general zones of inhibition increased with concentration of the root extract of S. esculentus. S. *typhi* exhibited the highest zone of inhibition in all the extracts (Table 5). Ethanol extract demonstrated the highest zone of inhibition followed by the Chloroform extract, as expected, there was varied MIC and zones of inhibition of different extraction in the Pathogens (Table 5). It is interesting to know that S. typhi in chloroform extract, K. pneumonia in methanol extract, P. aeruginosa

and K. pneumonia in Petroleum ether extract all showed resistance to the standard drug Ciprofloxacin.

Table 1 Effects of Ethanol extract on the diameter of zone of Inhibition (mm) at varying concentration (mg/ml) of *S. esculentus*

Con (mg/ml) Diameter of zone of Inhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	MIC
E. coil	14	-	-	-	-	-	R
S. typhi	16	13	11	9	6	3	0.0625
K.pneumonia	13	4	2	1.5	1	-	0.125
P. aeruginosa	4	-	-	-	-	-	R
S.aureus	16	8	5	3	2	-	0.125

Note: - = No zone of inhibiton, R= Resistance, MIC = Minimum inhibitory Concentration

Table 2 Effects of chloroform extracts on the diameter of zones of Inhibition (mm) at varying Concentration(mg/ml) of *S. esculentus*.

Con (mg/ml) Diameter of zone Of inhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	MIC
E. coil	15	-	-	-	-	-	R
S. typhi	-	8	7	5	2	-	0.0625
K. pneumonia	1	3	2	0.5	0.5	-	0.125
P. aeruginosa	11	-	-	-	-	-	R
S.aureus	16	6	4	2	1	-	0.125

Note: - =No zone of inhibition, R = resistance, MIC =Minimum Inhibitory Concentration

Table 3: Antimicrobial effects of methanol extract on the diameter of zones of Inhibition (mm) at varying Concentration (mg/ml) of S, *esculentus*.

Con (mg/ml) Diameter of zone OfIinhibition (mm)	Drug	1.0	0.5	0.25	0.125	0.0625	MIC
E. coil	16	-	-	-	-	-	-
S. typhi	15	7	5	3	2	-	0.125
K. pneumonia	-	1	0.5	-	-	-	0.5

P. aeruginosa	1	-	-	-	-	-	-
S.aureus	11	5	3	2	1	-	0.125

Note: - = No zone of Inhibition R = resistance, MIC =Minimum Inhibitory Concentration

Table 4: Antimicrobial Effects of pet. ether extracts on the diameter of zones inhibition (mm) at different Concentration (mg/ml) of *S. esculentus* on the listed pathogens .

Con (mg/ml) Diameter of zone of inhibition (mm)	**Drug**	**1.0**	**0.5**	**0.25**	**0.125**	**0.0625**	**MIC**
E. coil	18	-	-	-	-	-	R
S. typhi	16	6	4	2	1	-	0.125
K.pneumonia	-	3	1	-	-	-	0.5
P.aeruginosa	-	-	-	-	-	-	R
S.aureus	11	-	-	-	-	-	R

Note: - = No zone of Inhibition R = resistance, MIC = Minimum Inhibitory Concentration

Table 5 Minimum Inhibitory Concentrations (MIC) of all the pathogens

Pathogens	**Ethanol Extract**	**Chloroform extract**	**Methanol extract**	**Pet. extract**
E .C*oli*	R	R	R	R
S. typhi	0.0625	0.0625	0.125	0.125
K. pneumonia	0.125	0.125	0.5	0.5
P. aeruginosa	R	R	R	R
S. aurues	0.125	0.125	0.125	R

Note R = resistance

DISCUSSION

The results of this investigation revealed that all the extracts of S. *esculentus* possess appreciable antimicrobial activity against commonly encountered micro- organisms. S. *typhi*, K. *pneumonia* and S. *aureus* were all susceptible to all extracts except petroleum extract which showed no effect on S. *aureus*. This finding could indicate a possible treatment for *Samonellosis* diseases (Assuzu, and Njoku, 1996). The inhibition of the extract on K. *pneumonia* shows that there is an active component in the S. *esculentus* root extract that is able to cure *pneumonia* infection as the cause of *pneumonia* infection is attributed to K. *pneumonia* (Latta et al., 1998). This study tried to show that the inhibition of the growth of the S. *aureus* indicates that S. *esculentus* root extract can be used in the treatment of serious illness like *meningitis*, *septicamia*, diarrhea, billary tract infection. The results obtained from all the organic solvents extract showed a maximum susceptibility of the extracts on S. *aureus*.

The inability of the extract to inhibit the growth of E.*coli* indicates that the bioactive component of the extract can not be used in the treatment of gastro enteritis that has been associated with E. *coli* (Gill, 2004). Also the non -inhibition of growth of P. *aeruginosa* which is an aetiological agents of tract infection, osteochondritis and dermatitis (Latta,et al., 1998) however shows that the active components of the root extract cannot kill or cure any diseases caused by these organisms. S. *typhi* registered the highest diameter of zones of inhibition of all organisms used and therefore was very sensitive to the active components of the plant root extract of S. *esculentus*. This is in line with the local use of the plant in the treatment of typhoid fever and high fever (Assuzu, 1996). The ethanol root extract demonstrated a maximum antibacterial activity and synergistic effect on S. *typhi*. This fact indicates that the combination of the ethanol extract and the standard drug Ciprofloxacin can be used for the treatment of diseases associated with *salmonella typhi*. Also the ethanol extract was the most competent of the extraction solvents because it produced the highest zone of inhibition on the respective organisms at different concentration. There are many factors that influence the active principles present in plant which include the age of plant, extracting solvent method of extraction and time of harvesting plant materials (Amadioha, 1999, Quasem., 1996, Okigbo, 2003, Okigbo, 2004, Okigbo, 2005). This work has highlighted the antimicrobial effects of the root of S. *esculentus afzelius Holl* on known human Pathogens. The consequence of drug resistance implies that new drug's both synthetic and natural must be sought to treat diseases for which known drugs are no longer useful.Hence the uncovering of antimicrobials from plants will generate many more active recipes for diseases.

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