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Phytochemical studies and Antimicrobial screening against Bacteria causing spleen abscess

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Abstract : *Leaves of Sesamum heudilotii, Annona senegalensis and Crossopteryx febrifuga used in traditional medicine for the treatment of spleen abscess were extracted individually using a sohxlet extractor. Their combined aqueous extract was also obtained. Preliminary phytochemical screening of the individual plant extracts showed the presence of tannins, saponins, glycosides, flavonoids and alkaloids. The presence of these components shows the pharmacological property of the plants. Their combined and individual extracts were found to inhibit Staphylococcus aureus. The minimum inhibitory concentrations of the extracts for Staphylococcus aureus were determined. The results are significant in health care delivery system and apparently justify the use of the plants in the treatment of Staphylococcus -related spleen abscess.*

Keywords: *Phytochemical screening, spleen abscess, bacteria, antimicrobial, Sesamum heudilotii, Annona senegalensis and Crossopteryx febrifuga*

I. Introduction

The use of plants as medicine predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds [2]. The use of herbs and spices in cuisine developed in part as a response to the threat of food-borne pathogens. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected. In all cultures vegetables are spiced less than meat, presumably because they are more resistant to spoilage. Angiosperms (flowering plants) were the original source of most plant medicines. Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, have medicinal properties [2, 3]. A large amount of archaeological evidence exists which indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, animals such as non-human primates, monarch butterflies and sheep are also known to ingest medicinal plants to treat illness [4]. Plant samples gathered from prehistoric burial sites are an example of the evidence supporting the claim that Paleolithic peoples had knowledge of herbal medicine. In this work we have attempted to analyse three plant species purportedly used for the treatment of spleen abscess.

II. Materials and Methods

Materials

Methanol, Sulphuric acid (concentrated and dilute form), Dragendorff's reagent, Meyers reagent, Wagners reagent Ammonia solution (3%), Acetic acid, Distilled water, Ferric Chloride (5%), Hydrochloric acid (1% and concentrated), Fehling's solution I and II, Chloroform, Nutrient Agar, Blood Agar. Soxhlet extractor, Mortar and Pestle, Measuring cylinder, Sample bottles, Heating mantle, Weighing balance, Bunsen burner, Oven, Retort stand, Pipette (graduated), Micro syringes, Funnels, Forceps, Autoclave, Filter papers, Refrigerator, Inoculation loops.

Methods

The leaves of Annona senegalensis, Sesamum heudelotii and Crossopteryx febrifuga were obtained near Lessel in Ushongo Local Government Area of Benue State, Nigeria. They were washed with distilled water, dried under shed for four weeks and powdered using mortar and pestle [2].

20 g of each of Annona senegalensis, Sesamum heudilotii and Crossopteryx febrifuga were mixed and put in a 500 mL beaker and 250 mL of distilled water was added. The mixture was heated using a Bunsen burner to boiling and the heating continued for 15 minutes and was allowed to cool for 20 minutes and filtered. This gave the concoction. The same process was repeated using 30 g of each plant and the concoctions were used for phytochemcial screening.

30 g of powdered Sesamum heudilotii leaf sample was poured into the soxhlet extractor and 250 mL was added. This was refluxed for about 7 hours. The extract was first distilled to recover part of the solvent and finally evaporated to dryness using a water bath. Sesamum heudilotii leaf extract was then stored at ambient temperature. The same procedure was repeated for the other two plant samples.

The aqueous extracts of each plant was evaluated for the presence of flavonoids, tannins, saponins alkaloids, phenols and glycosides [3-8].

Test for Flavonoids

3 drops of ammonia solution were added to 1 mL of each extract, then 0.5 M concentrated HC1 was added. A pale brown colour was observed indicating the presence of flavonoids.

Test for Tannins

2 drops of 5 % $FeCl_3$ were added to l mL of each extract. A dirty green precipitate indicated the presence of tannins for each extract.

Test for Saponins (Frothing Test)

2 mL of each leaf extract in a test tube was shaken vigorously for 2 minutes. Frothing was observed for each extract indicating the presence of saponins.

Test for Alkaloids

1 mL of 1 % HC1 was added to 3 mL of each extract in a test tube. Each extract was treated with few drops of Mayer's, Wagner's and Dragendorffs reagents separately. A creamy white (Mayer), a reddish brown (Wagner) and an orange brown (Dragendorff) precipitate were observed for each extract, indicating the presence of alkaloids.

Test for Phenols

3 mL of each plant extract was added to 3 mL of $FeCl_3$. A deep green solution was observed instead of a deep bluish green solution, indicating the absence of phenols.

Test for Glycosides (General Test)

About 2.5 mL of dilute H_2SO_4 was added to 5 mL of each extract in a test tube and boiled on a heating mantle for 15 minutes. This was cooled and neutralized by adding 2.5 mL of 20 % KOH solutions. 5 mL of a mixture of Fehling's solution A and B was added and boiled. A brick red precipitate shows reducing sugars released as a result of the hydrolysis.

Antibacterial Screening

This was carried out at B.W.A Medix Medical Laboratory, Abu King Shuluwa Road, Shop 5, Kasevento Plaza, Wurukum, Makurdi Benue State.

Preparation of the Medium

The concoction and methanol extracts of each leaf were screened for antimicrobial activity using the disc diffusion method adopted by Alinnor [5, 9]. The nutrient agar medium was used as the growth medium for Staphylococcus aureus while blood agar medium was used as the growth medium for Streptococcus pyogenes.

The nutrient agar medium was prepared by dissolving 7.0 g of nutrient agar in 250 mL of distilled water in a flask, heated to dissolve and autoclave at 121 °C for 15 minutes. The same procedure was repeated for the blood agar medium. They were cooled and poured into sterile petri dishes to solidify. These petri dishes were sterilized by washing them with distilled water and drying them in the oven at a temperature of 121 °C. They were then left in the oven and collected when required for use.

Preparation of Cultures and Inoculation

Cultures of Staphylococcus aureus and Streptococcus pyogenes were obtained from Federal Medical Centre, Makurdi, Nigeria and were purified by sub-culturing into fresh nutrient agar slant and blood agar slant respectively. The bacteria were separately used to inoculate the petri dishes in a zigzag streaking manner. The inoculated plates were incubated at 37 °C for 24 hours.

Preparation of dilutions of Crude Extract Used for Antibacterial Activity

1 g of each extract was dissolved in 9 mL of distilled water in sterile test-tubes to obtain 100 mg/g concentration according to the equation.

$$Concentration = \frac{mass\ of\ solute}{mass\ of\ solution}$$

Using a micro syringe, 80 mg/g, 60 mg/g, 40 mg/g and 20 mg/g concentrations were obtained by taking 0.8 mL, 0.6 mL, 0.4mL and 0.2 mL respectively from the 100 mg/g solution and making the volume to l mL with distilled water.

Sterilized 6 mm filter paper discs were dipped in the extracts and placed on the plates using a sterilized forceps and incubated for 24 hours at 37 °C. At the end of the incubation, the plates were collected and the zones of inhibition that developed were measured. The minimum inhibitory concentration (MIC) was calculated by plotting the zones of inhibition against the natural logarithm of the concentration of extracts. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values.

III. Result and Discussion

Table 1: Percentage Yield of Concoction and Methanol Extracts of the Plants

Plants	Weight of Powdered Sample (g)	Weight of Sample Extracts (g)	Percentage Yield (%)
Concoction	60	10	16.67
S. heudilotii	30	5.4	18.00
A. senegalensis	30	3.5	11.67
C.febrifuga	30	3.0	10.00

Table 2: Preliminary Phytochemical Screening of aqueous extracts of the plants

Plants	Flavonoids	Tannins	saponins	Alkaloids	Phenols	glycosides
S. heudilotii	+	+	+	+	-	+
A. segalensis	+	+	+	+	-	+
C. febrifuga	+	+	+	+	-	+

Key + = active, - = absent

Table 3: Antibacterial Activity of Concoction and Methanol Extracts of Sesamum heudilotii, Annona senegalensis and Crossopteryx febrifuga.

Test organism	Conc. (mg/ml)	Zones of Inhibition (mm)			
		Concoction	S. heudilotii	A. sengalensis	C. febrifuga
Staphylococcus Aureus	100	10.0	09.5	09.0	09.0
	080	08.0	08.5	08.0	08.0
	060	07.5	08.0	07.5	06.0
	040	07.0	07.0	06.0	-
	020	06.5	06.0	-	-
	Control (G)	20.0	19.0	15.0	15.0
Streptococcus pyogenes	100	-	-	-	-
	80	-	-	-	-
	60	-	-	-	-
	40	-	-	-	-
	20	-	-	-	-
	Control (G)	20.0	18.0	18.0	21.0

= No Inhibition, G = Gentomycin.

Table 4: The Minimum Inhibitory Concentration of Concoction and Crude Methanol Extracts on Staphylococcus Aureus

Extract	Concoction	S. heudilotii	A. senegalensis	C. febrifuga
S. aureus	10.00	19.95	39.81	63.10

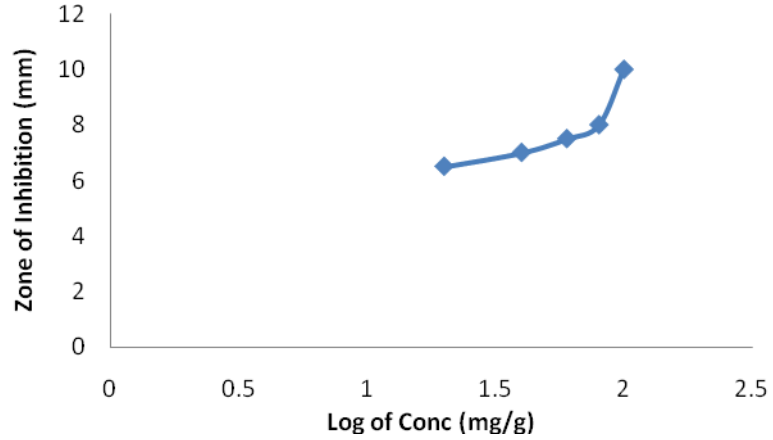

Fig 1 Graph of MIC of Concoction

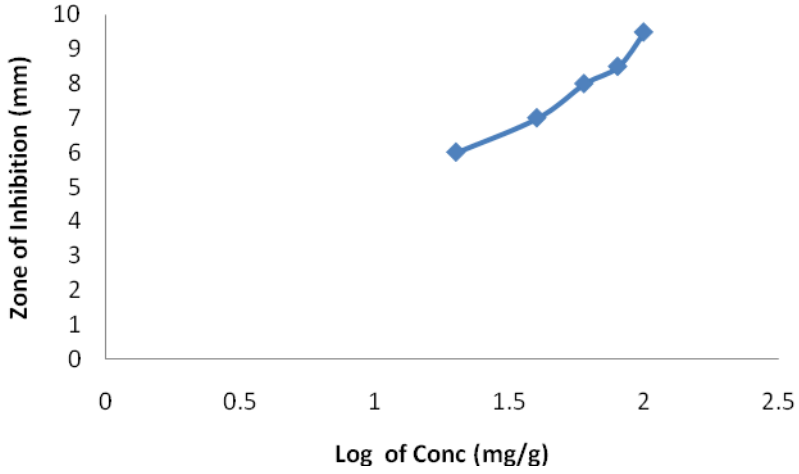

Fig 2 Graph of MIC of S. Heudilotii

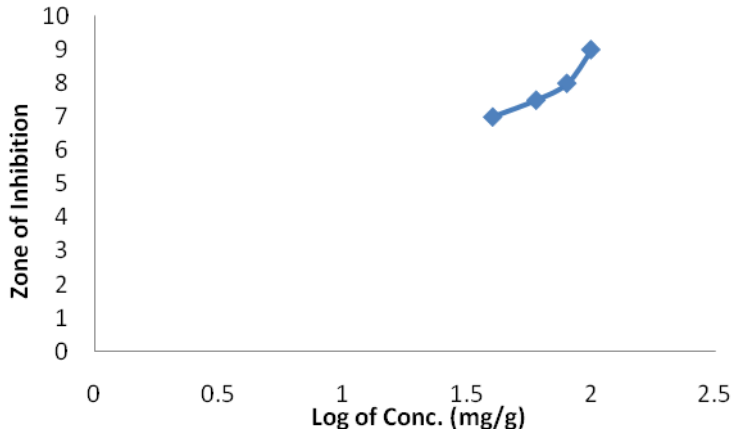

Fig 3. Graph of MIC of S. senegalensis

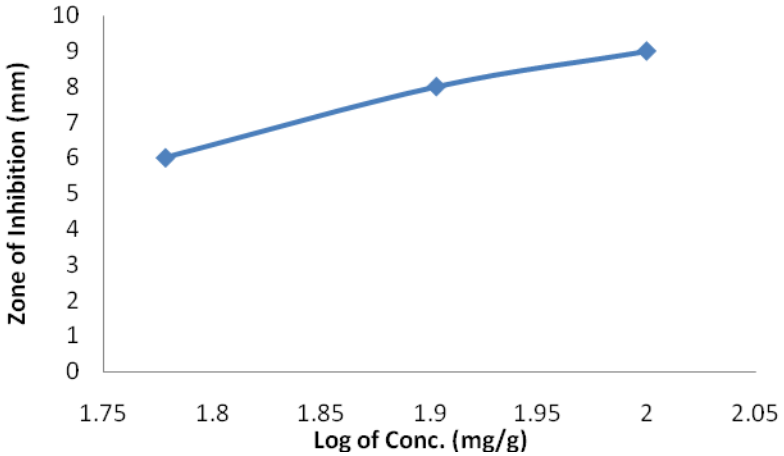

Fig 4 Graph of MIC of C. febrifuga

IV. Discussion

Table 1 shows the result of the weight of concoction obtained from a 60 g mixture containing 20 g of each plant sample and methanol extracts obtained from 30 g of each plant sample respectively. The concoction had a yield of 10 g representing 16.67 % while methanol extracts of Sesamum heudilotii, Annona senegalensis and Crossopteryx febrifuga had yields of 5.4 g, 3.5 g and 3.0 g representing 18.00 %, 11.67 % and 10.00 % respectively. The result obtained shows that methanol extraction of Sesamum heudilotii gave the highest yield while methanol extraction of Crossopteryx febrifuga gave the least yield.

The results of preliminary phytochemical screening are shown in table 2. The extracts of the three plants were found to contain flavonoids, tannins, saponins, alkaloids and glycosides. None gave a positive test for phenol.

Table 2 shows the result of antibacterial screening of the concoction and methanol extracts of the three plants at various concentrations. The result shows that increase in the concentration of the concoction and plants' extracts results in a corresponding increase in the size of the zone of growth inhibition on Staphylococcus aureus. None of the extracts had an effect on Streptococcus pyogenes. The highest zone of growth inhibition on Staphylococcus aureus was exhibited by the concoction giving a zone diameter of 10.0 mm when administered at 100 mg/g concentration. 100 mg/g, 80 mg/g, 60 mg/g and 40 mg/g concentrations of Annona senegalensis and Crossopteryx febrifuga had effects on Staphylococcus aureus while their 20 mg/g concentrations showed no activity. All concentrations of the concoction and Sesamum heudilotii inhibited S. aureus growth. The lowest zone of growth inhibition was observed with 40 gm/g concentration of Crossopteryx febrifuga which gave a zone of inhibition measuring 5.5 mm while the highest zone of growth inhibition was exhibited by 100 mg/g concentration of the concoction measuring 10.0 mm.

The minimum inhibitory concentrations (MIC) of the concoction and methanol extracts on Staphylococcus aureus are shown in Table 3. The lowest minimum inhibitory concentration was produced on Staphylococcus aureus by the concoction with a concentration of 10.00 mg/g while the highest MIC was produced by Crossopteryx febrifuga with a 63.10 mg/g concentration. Sesamum heudilotii and Annona senegalensis had MICs of 19.95 mg/g and 39.81 mg/g respectively.

The results obtained in this study indicate that the concoction and methanol extracts of the individual plants inhibited the growth of Staphylococcus aureus but were ineffective against Streptococcus pyogenes. This shows that the extracts contain substance(s) that can inhibit Staphylococcus aureus growth. The observed antibacterial effects of the extracts are believed to be due to the presence of alkaloids, tannins and flavonoids which are found present in the plants and this is supported by the following evidence: highly aromatic alkaloids such as berberine and harmane have the ability to intercalate with microorganisms' DNA; tannins have the ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins; while flavonoids, which are synthesized by plants in response to microbial infection, have the ability to complex with extracellular and soluble proteins and bacterial cell walls [10].

The primary curative agents are believed to be alkaloids and flavonoids as studies have shown that alkaloids isolated from the water extracts of Sensiviera zeylanica inhibited the growth of Staphylococcus aureus [10]. Also, the flavonoids, galangin (3,5,7-trihydroxyflavone) which is derived from the herb, Helichrysum aureonitens, show activity against a wide range of gram-positive bacteria [10].

The sizes of the zones of inhibition around the discs containing various concentrations of the concoction and plants' extracts reflect the degree of susceptibility of Staphylococcus aureus to the concoction

and the plants' extracts. From this, it is observed that that the concoction is more potent against Staphylococcus aureus than the individual plant extracts.

The values of the MIC exhibited by the concoction and the individual plant extracts also give more credence to the above observation. These values also show that Sesamum heudilotii extract is the most potent of the three plants extracts and therefore can be said to be the most important plant used in preparing the concoction. This observation corroborates the claim of the traditional healer.

In preparing the concoction, fresh leaves of the plants that are free from insect attack should be collected as the flowers are beginning to open to ensure maximum yield of the active product [11]. Sesamum heudilotii leaves should constitute a higher percentage since it is the most important of the three plants used in preparing the concoction. They should be washed with clean water to free them from dust and other particles, placed is cooled water and heated for about 15 minutes or longer (up to one hour) and then allowed to stand for another 15 minutes. The aqueous extract can then be decanted or filtered and taken when required [13, 14]. The extract, if it is to last for days or weeks, should be warmed up only and not heated to boiling to prevent decomposition of active components. Administration of the concoction should take into consideration the age and weight of the patient [11].

V. Conclusion

This investigation shows that the concoction prepared from the leaves of the plants have bacteria activities at concentrations ranging from 20 mg/mL to100 mg/mL on Staphylococcus aureus. The result also indicates that the traditional medicine use of the concoction has scientific backing and hence the bioactive constituents responsible for antibacterial activities should be elucidated.

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References

[1]. Lewington, A. (1990): Plants for the People; National History Museum, Crownwell Road, London. Pp.vii.
[2]. Tapsell L.C., Hemphill, I., Cobiac L et al. (2006). Health benefits of herbs and spices: the past, the present, the future. Med. J. Aust. **185** (4 Suppl): S4–24. PMID 1702243
[3]. Oyewole, A.O.; Audu, O.T. (2007): The Medicinal Potential of Aqueous Extracts of Six Flora of West Africa; Journal of Chemical Society of Nigeria, Vol. 32, No 1. Pp.150.
[4]. Kubmarawa, D.; Wase, G.A.; Ayinla, O.G. (2007): Preliminary Studies on Phytochemical Analysis & Antimicrobial Evaluation of Extracts of Commiphora kerstingii, Journal of Chemical Society of Nigeria, Vol. 32, No. l.Pp. 39.
[5]. Alinnor, I. J. (2007): Preliminary Phytochemical and Antibacterial Screening of seeds of Garcinia Cola; Journal of Chemical Society of Nigeria, Vol. 32, No. Pp. 42, 43.
[6]. Http://www.dx.doi.org/10.3738/wjg. 14308. Retrieved 06/09/2008
Totora, G. J., Bardell, R. F., Christine, L. C. (2007) Microbiology- An Introduction Seventh Edition. Pearson Education Publishers. Pp 582-585. Http://www.aluka.org/action/dosearch?searchtext=annona+senegalensis. Retrieved 26/07/2008.
[8]. Keay, R.W.J. (1989): Trees of Nigeria. Oxford University Press, New York. Pp.30, 425, 426, 427.
[9]. Keay, R.W.J. Onochie, C.F.A.; Stanfield, D.P. (1960): Nigerian Trees, Vol.1, Federal Government Printer, Lagos. Pp. 58-59.
[10]. 10] Http://www.pubmedcentral.nih.gov/articlerender.fcgi?artd⁻⁻88929.retiev-ed 20/09/208.
[11]. Sofowora, A. (1982) Medicinal plants and Traditional medicine in Africa. Chiechester, John Willey. Pp 54-59, 142
[12]. Pandey, B.P. (2001): Economic Botany; S. Chand and Company LTD, Ram Nagar, New Delhi. Pp.5.
[13]. Eugene, W. N. Denise, G. A. Evans, C. R. J. Nancy, N. P. Martha, T. N. (2007): Microbiology, A Human Perspective. 4th Edition Mc-Graw-Hill, New York, America.