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Relative antimicrobial activities of ethanolic extracts of roots of *Hydnora africana* (Sub. Family-Hydnoraceae)

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Abstract
The root of *Hydnora africana* plant may show therapeutic activities against a specific condition in traditional medicine systems. In the current research crave to compare the antimicrobial activities of roots of *Hydnora africana* (Sub. Family-Hydnoraceae). From the research using agar well diffusion and the broth dilution methods the antimicrobial activities of ethanolic extracts of the roots of *Hydnora africana* (Sub. Family-Hydnoraceae) were investigated against some microbes. From the research the root extracts exhibited the greatest antimicrobial activity of average diameters of zones of inhibition in the range of 18 mm for 0.5 ml/l concentration and 16 mm for 0.25 ml/l. The research study has shown that the root extracts each of *Hydnora africana* (Sub. Family-Hydnoraceae) has antimicrobial activity.

Keywords: IS means this means *Hydnora africana*, antimicrobial, root and mic

Introduction
The *Hydnora africana* (Sub. Family-Hydnoraceae) is the plant root extract used for the treatment of dysentery, diarrhoea and has antimicrobial activity. This *Hydnora africana* is found from the Western Coastal areas of South Africa of Namibia, Botswana as far as Ethiopia. This *Hydnora africana* with no leaves and chlorophyll. It is a dwelling underground parasitic plant it get all of sugars, minerals and water by attaching to the roots of the host plant and takes some nutrients that makes from photosynthesis. But the *Hydnora africana* has a unpleasant and very strong smell. In this research I watch the antimicrobial effects for the microorganisms of Streptococcus Faecalis, Edwardsiella Tarda and Candida Albicans.

Materials and Methods
1. **Plant Material:** When First time this Plant is came from Africa and after it is collected by Lab from Malbazar. My first research it used after some plant samples were left so, this also used second time for this research. The root of *Hydnora africana* (Sub. Family-Hydnoraceae) collected from Malbazar by the Lab. The sample is identified by me. The samples were washed, air dried and chopped into pieces and ground into coarse powder.
2. **Extraction of Plant Materials:** The samples was extracted with 96% ethanol, 100gm of each of the coarsely powderd root extract of plant material was soaked in 400ml of ethanol for 4days with occasional shaking to facilitate extraction of constituents. Each mixture filtered by a plug of cotton wool (Followed by filtration by filter paper) after labelled appropriately and the filtrate is allowed to dry by exposing it to air in a fume chamber.
3. **Test Microorganisms:** The microorganisms used for the study of Gram positive bacterial (Streptococcus Faecalis), Gram negative rod shaped like structure Edwardsiella Tarda and yeast like fungus Candida Albicans. These organisms were obtained from lab.
4. **Antimicrobial Assay:** In this research the cupplate method was used, 0.1ml of overnight broth culture of each microorganism 106 cfu/mL unto the surface of nutrient agar plate was introduced and uniformly distributed using a sterile cotton swab. Five wells of 5mm depth and each of about 6mm diameter were made on the agar using a cork borer number 3 and labelled appropriately as representing roots, Tobramycin and water methanol respectively. The extracts then dissolved in a mixture of methanol and water (1:9) to prepare different concentrations, a 0.5 ml/l and 0.25 ml/l. Tobramycin at a concentration of 0.1 mg/ml and methanol water (1:9) were used as the controls. A volume of 0.1 ml of the extracts (roots) at a concentration of 0.5 ml/l was dispensed into their corresponding well. The controls of same volume (Tobramycin and methanol water mixture) was also

dispensed into the corresponding wells In the laboratory bench for 30 minutes the plates were left before incubating for 26 hours at 37 °C. Then repeated the procedure by using 0.25 ml/l concentration of the extracts. By triplicates the procedure was carried out for each of the concentrations used. By using a ruler sensitivity of the test organisms to the extracts were determined by measuring the diameters of the zones of inhibition surrounding the wells and the average of each recorded.

5. **Determination of Minimum Inhibitory Concentration (MIC):** Cultures of the organisms were incubated at 37 °C and were prepared in nutrient broth (sigma). The root extracts of the plant *Hydnora africana* (Sub. Family-Hydnoraceae) were each reconstituted in 1:9 ratio of methanol water. To make up the required concentration each was diluted with nutrient broth. By using the well dilution method the MIC's of the extracts and the controls were determined with triplicate wells and two independent experiments. By serially each extract diluted two fold with nutrient broth to present a dilution range of 8mg/ml to 0.008 mg/ml in sterile 96 well microtitre plates. One hundred microlitres (100 µL) of overnight broth culture of the organisms (105 cfu/mL) was added to each well and incubated at 37 °C for 20 hrs. Control wells didn't contain extracts and by contained scalar dilutions the vehicle control wells of the 10% methanol. Serial dilution of Tobramycin (from 2 mg/ml to 0.002 mg/ml) was used as the positive control. The plates were examined for growth after 20 hrs of incubation.

Results and Discussion

Antimicrobial activities of root extract of *Hydnora africana* (Sub. Family-Hydnoraceae)- The antimicrobial activity of each of the *Hydnora africana* extracts was assessed against each test microorganism and the result obtained for the 0.5 ml/l and 0.25 ml/l concentrations used are shown in Table 1 and Table 2 respectively. The roots of *Hydnora africana* contain various phytochemicals which are responsible for therapeutic activities of the plants. The root *Hydnora africana* also known to have useful activities including antimicrobials. The current research shows the potential antimicrobial activities when the activities were compared using ethanol extract of each part and tested at a concentration of Streptococcus Faecalis, Edwardsiella Tarda and Candida Albicans. This morphological part of the plant has also been reported to exhibit antimicrobial activity against different strains of bacteria. The pharmacological activities of plants due to phytochemicals present in the roots of *Hydnora africana*. There is a relatively good activity of the extracts against Candida Albicans (a fungi) but if further research used more doses than it will be better to be a good result and Edwardsiella Tarda is the Gram Negative bacteria it causes both intestinal and extra-intestinal infections and it imapaired immune systems of human body and by this disease fishes, mammals and aquatic mammals also infect many times but in this present research Hynora Africana shows a good result against this infection. In this research a mixture of methanol water (1:9) used as negative control and one part of methanol used to dissolve each extract followed by nine parts of sterile water. But this mixture unable to show any antimicrobial activity indicating that the activity exhibited by the root extracts came from the extracts. Tobramycin used as positive control to compare the activities of the extracts is used to several types of bacterial infection. It showed higher zones of inhibition (28 mm) even though a relatively smaller concentration of 0.1mg/ml was used. It also gave an MIC in range of 0.016 and 0.032 mg/ml). Tobramycin is a refined product containing only one compound (which binds to 30s subunit of bacterial ribosome) interrupting with protein sysnthesis and inhibit the growth of microorganism.

Table1: Average of inhibition of *Hydnora africana* root extract against the test organisms using 0.5 ml/l concentration

	Average zones of inhibition (mm) (n=3)	
Microorganisms	**Root Extract(IS)**	**Tobramycin**
Streptococcus Faecalis	18±0.4	28±2.5
Edwardsiella Tarda	18±0.0	25±2.0
Candida Albicans	15±2.0	NT

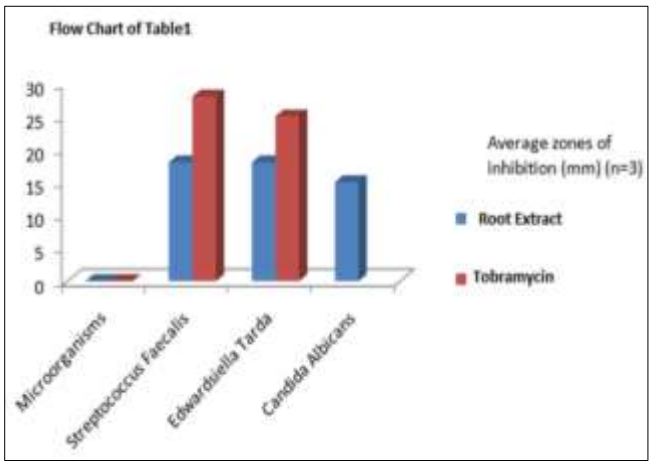

Fig 1: Average of inhibition of *Hydnora africana* root extract against the test organisms using 0.5 ml/l concentration

Table 2: Average of inhibition of *Hydnora africana* root extract against the test organisms using 0.25ml/l concentration

	Average zones of inhibition (mm) (n=3)	
Microorganisms	**Root Extract(IS)**	**Tobramycin**
Streptococcus Faecalis	16±1.7	28±2.5
Edwardsiella Tarda	16±0.6	25±2.0
Candida Albicans	13±2.0	NT

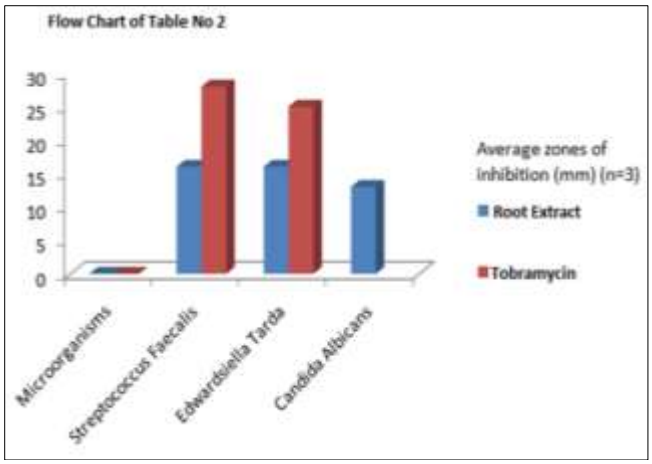

Fig 2: Average of inhibition of *Hydnora africana* root extract against the test organisms using 0.25ml/l concentration

Conclusion

The root extracts of *Hydnora africana* exhibit antimicrobial activity. Ina comparative study of the antimicrobial activities the root extract has the highest antimicrobial activity against both gram positive and gram negative bacteria and yeast like fungus Candida Albicans employed in this research. This root extract shown that ethanolic root extract of *Hydnora africana* has a more potent antimicrobial activity.

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