MUHIMBILI UNIVERSITY COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMARCY



PROJECT REPORT

TITLE : **ANTIMICROBIAL AND BRINESHRIMP LETHALITY OF THE EXTRACTS OF *TERMINALIA KAISERANA* ROOT.**

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ABSTRACT

In this project efforts have been made to experimentally examine the effect of crude extracts of *Terminalia kaiserana* on fungal and bacteria micro organisms.

The crude drug extracts were tested for their inhibitory activity on growth of some selected bacteria and fungi.

A total of ten micro organisms (i.e. 2 fungal organisms and 8 bacteria) were tested.

The result showed activity on only the fungal organisms (ie.*Candida albicans* and *Cryptococcus neoformans*). But could not show any antibacterial activity.

INTRODUCTION

The family Combretaceae has about 150 species (Watt and Breyer-Brandwijk,1962;Hutchings et al 1996) several of which are used extensively in traditional medicine for the treatment of various diseases, including stomach disorders, schistosomiasis,diabetes, gynecological diseases, venereal diseases, diarrhea and several other diseases

Terminalia kaiserana is widely distributed in tropical and warm temperate regions especially in Africa. (Arnold and Gulumian, 1984). In Tanzania is found mainly in south western highlands regions where climate favors the growth.

It is a small tree or shrub with long leaves (7.5-12.5cm long), white flowers and purplish brown fruits. (R.M. Polhill 1973)

Decoctions of terminalia kaiserana roots is used in the coastal regions of Tanzania for the treatment of headache and backache (chhabra et al.,1989).

Methanolic leaf extracts of *Terminalia kaiserana* was found to inhibit the growth of *S. aureus. E. coli* and *Candida albicans.*

PROBLEM STATEMENT

Respiratory infections, HIV/AIDS, diarrhea and various fungal infections are the main problems among infectious diseases. Resistance to first line drugs has been observed in all these diseases.

A multitude of traditional plant treatments for bacteria and fungal infections are used throughout the world. In areas of underdeveloped world in which conventional therapy is not readly available, traditional treatment remains the major form of therapy.

Attempts are made every day to search for new antibacterial and antifungal both synthetically and from the natural sources.

LITERATURE REVIEW

There is no any literature review on antibacterial and anti fungal activity of the roots of *Terminalia kaiserana.*

However the root extracts of the other species of terminalia such as *T. mollis* and *T. sericea* have shown antibacterial and antifungal activities.

OBJECTIVES.

BROAD OBJECTIVES.

To investigate the antibacterial and antifungal properties of the extracts of *Terminalia kaiserana.*

SPECIFIC OBJECTIVE.

- To extract *T.kaiserana* roots with solvents of increasing polarity; petroleum ether, dichloromethane, methanol, ethanol and water.
- To test the extracts against standard strains of bacteria and fungi using the disc diffusion method.
- To test the extracts for toxicity against brine shrimp larvae.

METHODOLOGY

Dry powdered root of T.kaiserana was percolated in the successive solvent extractions of their increasing polarity from petroleum ether to water for 24 hours at room temperature. The extracts were then dried under reduced pressure.

Testing for the inhibitory activities of the extracts on the growth of selected fungus and bacteria.

This was done using the agar disc diffusion method. *C. albicans* and *C.neoformans* were grown on saboraud agar and the bacteria were grown on Tryptone soy agar.

500 mg of the extracts were dissolved in 1ml of DMSO except for water extract which was dissolved in a 1ml of the distilled water. Ten micro liters of the solutions were transferred onto the filter paper disc to make 500µg/disc.

BRINE SHRIMP LARVAE LETHALITY TEST

3.8 gm of brine salt was dissolved in 1 liter of distilled water. The saline water was filtered and put into the hatching tank, the former was 75% covered with alminium foil and plastic partition with pin holes was used to separate the covered side from uncovered one. Brine shrimp eggs were put into the darkened side. The uncovered side was illuminated and the tank left at room temperature.

40mg of each crude drug extract was dissolved in 1ml of DMSO except for water extract which was dissolved in a 1ml of distilled water and diluted in saline water that was prepared by dissolving 3.8 gm of brine salt in a liter of distilled water.

Serial dilutions were carried out to obtain final concentration of 240µg/ml, 120µg/ml, 80µg/ml, 40µg/ml and 16µg/ml.

10 brine shrimps larvae were transferred using tit suckers from the hatching tank to each of the vial containing extract or test solution. After 24 hours the surviving larvae in each vial was counted and recorded.

RESULTS.

The result of antibacterial and antifungal activities of the extracts and standard drugs Gentamycin (GT) and clotrimazole (CT) are as shown below.

Bacteria.	Average inhibition zone diameter (mm)					
	Pet.ether	DCM	methanol	ethanol	water	GT
Proteus. sp.	-	-	-	-	-	20
S.aureus	-	-	-	-	-	10
S.typhi.	-	-	-	-	-	23
B.cereus	-	-	-	-	-	20
Pseudomonas sp.	-	-	-	-	-	20
E.coli	-	-	-	-	-	18
Klebsiella sp.	-	-	-	-	-	18
B.anthracis	-	-	-	-	-	20
Fungi.						CT
C. albicans	10.5	12.5	12	12.5	13	25
C.neoformans	12	12.5	13	13.5	14	30

The fig. P. computer program was used to analyse the brine shrimp larvae test. The data were plotted as the mean % mortality of brine shrimps against logarithms of extracts concentrations and regressions equations were obtained.

Y= mlogx+C, where Y=%mortality and X=concentration. The equations were used to calculate the LC50 values.

EXTRACTS.

Petroleum ether.	LC50=3.3µg/ml(1.75-6.20) 95%CI
Dichloromethane	LC50=13.58µg/ml(8.70-21.18)95%CI
Methanol.	LC50=49.85µg/ml(34.86-71.18)95%CI
Ethanol.	LC50=18.18µg/ml(10.96-30.17)95%CI
Water.	LC50=11µg/ml(6.74-17.26)95%CI

DISCUSIONS

Ethinomedical report indicate that *Termnalia kaiserana* is used for the treatment of diarrhea, gonorrhea, and bacteria infections.

Anti bacteria and anti fungal activities were recently reported in ethanol extract of the roots of *terminalia* collected from Tabora, Tanzania.

The results of the present study agree with the traditional uses by the Tabora people with the exception that I did not find any bacteria activity.

CONCLUSIONS

Extracts pf Terminalia kaiserana have shown antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. They also exhibited *cytotoxic* activity on brine shrimp, which is stronger than that of the standard anticancer drug *cyclophosphamide*. Further work should be done to isolate and identify active compounds in the extracts.

RECOMMENDATION

With the development of simpler and in expensive bio assays, it is expected that in future many hither to untested natural products isolated from plants such as ***Terminalia kaiserana*** will be put through a variety of biological tests.

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