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ANTIBACTERIAL EFFICACY OF SOME NIGERIAN HERBS

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ABSTRACT

The effect of cold and hot water extracts of the medicinal plants *Euphorbia hirta* and *Mormodica charantia* on the growth of some bacterial isolates was assessed using agar diffusion technique and broth dilution method. All the extracts showed varying degrees of inhibition depending on the organism tested, the plant species, method of extract preparation and extract concentration. The six pathogenic organisms tested are *Bacillus cereus*, *Salmonella typhi, Shigella dysenteriae, Escherichia coli, Staphylococcus aureus* (local) and *Staphylococcus aureus* (NCIB 8588). The combinations of the two extracts was more active on the test organisms than when used separately. The MIC of the single extract with cold water was in range of between 130-145µg/ml while it was in the range of between 115-130µg/ml in the mixed extract. In the hot water of single extracts, it was in the range of between 100-145µg/ml and between 100-130µg/ml in the mixed extracts. With cold water of single extracts, MBC was in the range of 145-160µg/ml and between 115-145µg/ml in the mixed extract. With hot water of single extract it was between 130 –160 µg/ml and between 100-145µg/ml with mixed extract. *Escherichia coli* was most susceptible to the extracts while *Bacillus cereus* was the least susceptible.

Keywords: Antibacterial efficacy, extract and medicinal plants.

INTRODUCTION

The use of medicinal plants for treatment of microbial diseases is well known and documented since ancient times (Dahannaker *et al.,* 2000) Plants synthesize, many defensive compounds to protect themselves and predators. These compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstances (Karban and English Loeb, 1997) Several plants species have been tested for anti microbial properties (Arora and Ohlan, 1997) but the vast majority have not yet been adequately evaluated. In Nigeria, it has been found that even when people are close to hospital, traditional healers are consulted as a first choice. This is because traditional medicine blend readily into the socio culture life of the people (Kela and Kufeyi, 1995). *Euphorbia hirta* (Linn) is a herb and common all the year round in the tropical and subtropical countries world wide. For wounds not to turn septic, *Euphorbia hirta* leaves are used for the dressing of fresh wounds (Dalziel, 1955 Kokwaro, 1976). It is also used for the cure of guinea worm and as well useful for dysentery and stomach upset. (Watt and Marria, 1962) *Mormodica charantia* is a small climber and common weed that is wide spread all over Nigeria throughout the year round. In the Midwest and Western parts of Nigeria, it is used for stomach-ache and dysentery. The plant can also be collected, burnt on clay pot and grind into powder with little salt to taste and can be taken orally for the cure of pile and acute diarrhoea. Information obtained from literature regarding the antimicrobial activities of *E. hirta* appears to be inconsistent. Where some authors reported negative antimicrobial activity Karel and Roech, others reported positive activity at concentrations of 1:5 to 1:6 (Watt and Marria, 1962). Although there is no scientific report on the antibacterial activity of *Mormodica charantia,* ethno-medical report claimed that it is used for enteric organism infection.

These two plants are common in Nigeria and are very high in their antibacterial content. However, much research work has not been down to ascertain their application for therapeutic potency. Therefore, this study is aimed at evaluating the antibacterial activities of *Euphobia hirta* and *Mormodica charantia* on some pathogenic bacteria.

MATERIAL AND METHODS

Processing of plant samples

Fresh, healthy-looking leaves of *Euphorbia hirta* and *Mormodica charantia* authenticated by Dr. M. B. Oyun of Forestry and wood Technology Department, FUTA, were harvested from the local plants in Akure, Ondo State. A 10g portion of each plant samples was rinsed in saline solution, homogenized in mortar and then mixed with 20ml of sterile distilled water. Another 10g portion of the leave samples was soaked in 20ml of hot water to obtain hot water extract. In each case the mixture was centrifuged at 3000 rpm for 5 minutes to remove particulate debris and the extract stored in sterile McCartney bottles for use.

Sources and Maintenance of Bacterial Isolates

Pure isolates of the bacteria used in this study were obtained from Health Center and Microbiology Laboratories of the Federal University of Technology, Akure. The isolates were maintained through the period of study by aseptically sub-culturing into freshly prepared nutrient agar medium at every 2 weeks for nutrient replenishment. Prior to use, the isolates were inoculated into separate 50ml nutrient broth in a cotton plugged and aluminum foil sealed conical flask and incubated at 37^0C for 24 hours. The bacteria organisms used in this work includes. *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Bacillus cereus, Staphylococcus aureu*s (local) and a typed *Staphylococcus aureus* (NC IB8588).

Antibacterial Screening of the Extracts

Using well-in agar method of Alade and Irobi (1991), the sterilized nutrient agar was aseptically poured into sterile glass petri plates and were allowed to gel. To obtain concrete growth of the test organisms on the surface of agar plates in other to be able to evaluate actual zones of inhibition demonstrated by the extracts, 2ml of know bacterial population was pour plated. The plates were left for about 1 ½ hours to allow the test organisms fully embedded before wells were dug in the seeded plates with number 4 cork borer that was sterilized by burning with absolute ethanol. 0.1 milliter each of the leave extract concentrations were pipetted into the wells. Run over and splashes were avoided, labeled carefully and incubated at 37°C for 24 hours. The sensitivity of the test organisms to each of the extracts is indicated by clear zones of inhibition around the well and diameter of the clear zones of inhibition was taken as an index of the degree of sensitivity by measuring with caliper.

Minimal inhibitory concentration (MIC) of the extracts was determined by broth dilution method(Akinyosoye and Oladunmoye 2000). Decreased concentration of the extracts was prepared (175-100µg/ml) by evaporating the leave extracts at 45°C to semisolid using rotary evaporator (Rosona, England). The concentrates were weighed and reconstitute appropriately in sterile distilled water. In test tubes containing 8ml of sterile nutrient both, 1ml of the different concentration of extracts and 1ml broth culture containing known population of the test organisms were introduced separately into the various tubes. The tubes were rolled between two palms for even mix up and were incubated at 37°C for 24 hrs.

Turbid tubes after incubation indicate negative and the least extract concentrations where clearity in medium starts in the test tubes, determine the MIC of extract. Meanwhile, the bacterial population of the broth cultures of the test organism used for the MIC analysis was same for the antibacterial screening of the extracts as the experiments were performed side by side. The bacterial population are $4.21x10^6$, 4.32×10^6, $3.65x10^6$, $3.76x10^6$ $5.31x10^6$ and 6.03×10^6 cfu/ml respectively for *S. typhi, E. coli, S. dysenteriae, B. cereus, S. aureus* (local) and *S. aureus* NCIB 8588.

The Minimal Bactericidal Concentration (MBC) was also determined by plating 1ml of the MIC positive tubes of 24 on nutrient agar to determine the bacteriostatic and bactericidal activities of the leave extracts.

RESULTS

Effects of extracts on the test organisms

The *E. hirta* extract inhibited the growth of all the test organisms except *Bacillus cereus* that it exhibited a low antibacterial effect. *M. charantia* had various degrees of inhibitory effect on all the test organisms, while the mixture of the two extracts in equal volume of ratio 1:1 demonstrated a more therapeutic capacity. M. *charantia* had a higher therapeutic affinity over *E. hirta* as the inhibitory properties exhibited on the test organisms were higher (Table -1). The MIC of the cold water extract was between the range of 115-145µg/ml while it was between 100-145µg/ml with the hot water extract (Table -2). Comparing the bacterial load of the reinoculated MIC positive tubes for 24 hours treatment to the initial load of the test organisms, reduction in bacterial load was notice at 130µg/ml.

Table -1: Sensitivity of Bacterial Isolates to the Extracts

Extract	Test organisms	Zones of inhibition (mm)	
		Cold water	Hot water
E. hirta	*E. coli*	20	22
	B. cereus	7	10
	S. typhi	13	20
	S. dysenteriae	20	24
	*S. aureus (*Local)	18	21
	S. aureus (NCIB 8588)	23	25
M. charantia	*E. coli*	27	25
	B. cereus	10	14
	S. typhi	22	30
	S. dysenteriae	27	30
	*S. aureus (*Local)	20	23
	S. aureus (NCIB 8588)	29	32
E. hirta + M.charantia.	*E. coli*	25	29
	B. cereus	18	22
	S. typhi	21	26
	S. dysenteriae	27	35
	*S. aureus (*Local)	26	28
	S. aureus (NCIB 8588)	33	35

Table - 2: Concentration of Extracts (µg/ml) at which MIC and MBC are valuable on the test organisms

Extract	Test organisms	Cold water			Hot water		
		MIC	MBC	Action	MIC	MBC	Action
E. hirta	*E. coli*	145	145	C	130	130	C
	B. cereus	145	160	S	145	160	S
	S. typhi	130	145	S	130	140	S
	S. dysenteriae	145	160	S	130	130	C
	*S. aureus (*Local)	130	145	S	115	130	S
	S. aureus (NCIB 8588)	130	145	S	115	115	C
M. charantia	*E. coli*	130	130	C	100	100	C
	B. cereus	145	160	S	130	140	S
	S. typhi	130	145	S	115	130	S
	S. dysenteriae	145	145	C	130	130	C
	*S. aureus (*Local)	130	160	S	130	145	S
	S. aureus (NCIB 8588)	130	145	S	115	115	C
E. hirta + M.charantia.	*E. coli*	115	115	C	100	100	C
	B. cereus	130	145	S	130	145	S
	S. typhi	130	130	S	100	100	C
	S. dysenteriae	130	130	C	100	100	C
	*S. aureus (*Local)	115	130	S	100	100	C
	S. aureus (NCIB 8588)	115	115	C	100	100	C

Key:

MIC = Minimal Inhibitory Concentration
MBC = Minimal Bactericidal Concentration
S = Bacteriostatic
C = Bactericidal

Though clarity were observed in the positive MIC tube cultures, the MBC test revealed the concentrations at which the extracts were bactericidal and bacteriostatic on the test organisms (Table -2).

However, the hot and cold water method of extraction of the leave samples was valuable and much differences were not observed in their antibacterial evaluations. It highlights that the active principles of the two leaves are easily extractable without the use of chemicals solvents.

DISCUSSION

The findings in this work showed that the leaves juice of *E. hirta* and *M. charantia* have inhibitory effect on the six bacteria isolates used for the test. The combination of the two extracts were found to be very effective on the test organisms. This could be as a result of the active principles in the two leave extracts coming together to boost each others ability to inhibit the test organisms than when used in single form. This suggests that the two extracts can be used together in equal volume for the suppression of the test organisms at a specific given time because reduction in bacterial populations in both the cold and hot water extract when reinoculated after treatment. Despite the fact that the two leave extracts acted on the test organisms, the satisfactory result obtained on their efficacy was greatly highlighted by the variations in the extract concentrations and time of treatment.

It was observed that *Bacillus cereus* was not totally eliminated in both the single and combined forms of the two extracts. However, further treatment time is required to conclude a final interpretation of the therapeutic potency on this organism. Meanwhile, the active ingredients of these extracts are not known but the satisfactory therapeutic potency exhibited even with water extraction indicates that the concentration of active component contained is high and easily extractable. The search for more plants with antimicrobial agents should continue, to guide against organisms that often evolve into new genetic variants, which may subsequently be resistant to the existing therapeutic agents.

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