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INVESTIGATION IN TO THE ANTIMICROBIAL PROPERTIES OF *EUPHORBIA HETEROPHYLLA* ON TYPHOID DISEASE CAUSATIVE AGENTS

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ABSTRACT

Different parts of *Euphorbia heterophylla* (florescence, leaf, root, stem and whole plant) were variously screened for antimicrobial activity using disc diffusion method. The in vitro sensitivity test of the ethanol extract of plant parts revealed activity against all the typhoid agents except the root extract which has no activity against any of the organisms except *Salmonella paratyphi* B. The phytochemical analysis of the extract showed the plant as having Tannins and Anthraquinones. These organic substances could be responsible for the antimicrobial properties of plants parts.

KEYWORDS: Antimicrobial, *Euphorbia heterophylla,* Typhoid disease.

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INTRODUCTION

Euphorbia heterophylla is a member of the family *Euphorbiaceae*. It is an annual plant that grow commonly in the surrounding. It has green leaves and bears tiny round seeds. It is one of the medicinal plants used by the local people in the cure of some notable disease.

Medicinal plants contain certain physiologically active principle, which over the years have been exploited in traditional medical practice for the treatment of various ailment[1]. Plants of both lower and higher groups are known to produce chemical substances with which the defend themselves against invading micro organisms. The chemical or substance which are commonly referred to as antimicrobial are develop from the noticeable conditions such as viral disease attack, mammalian predator attack and the development of extra-ordinary array of defences against chemicals and the struggle to survive under intensive competition for resources and nutrients[2]. The against are chemical substances produce by plants and microorganisms which elicit exhibit either inhibitory (bacteriostatic) effect or destructive (bactewricidal) effects on other microorganisms[3].

Antimicrobial agents could be in form of antibiotics, disinfectants or antiseptics. Antibiotic are chemical substances that has the ability to inhibit the growth of or destroy microorganisms.

Disinfectants, on the other hands are germicides which are often use to destroy potentially infectious microorganisms. They are usually too toxic to be applied to tissues, but are suitable for application to inanimate objects.antiseptics are non-toxic antimicrobial substances which may be *applied* to living tissues.

Many phytochemicals have been extracted and isolated from plants based on information provided by local people[4]. Using chewing sticks to clean teeth is a common practice in many African societies. Investigations by[5]. On these chewing sticks showed that they possess antimicrobial activities against oral microbial flora. Ethanol extracts of *Mormodica charantia,Alstonia boone* and *Ocimum bacilicum* according to[6] possess activities against enteric organisms such as *Salmonella typhi, Escherichia coli* and *Shigella dysentriae.* Etracts from the fruit of *Citrus qurantifolia* (lime)have shown activities against a number of gram negative and gram-negative and gram-positive bacteria[7,4] carried out systematic study on the antimicrobial activity of leaf extracts of sodon apple (Calotropis procera) and their study revealed the plant's activity against Clostridia, Salmonella and streptococci.

African people have over the years developed a store of empirical information concerning the therapeutic value of species of local plants.

MATERIALS AND METHODS

Sources and Maintenance of Test Organisms

The test organisms (*Salmonella typhi, Salmonella paratyphi A Salmonella paratyphi B and Salmonella paratyphi C*) were obtained from the Microbiology Laboratory of Federal University of Technology , Minna, Niger state. The stock cultures were maintained on nutrient agar at 4^0c and sub cultured in nutrient broth at 37^0c for 18 hours prior to sensitivity testing.

Preparation of Media

Nutrient agar and nutrient broth were prepared according to the manufacturer's specifications.

Source and preparation of materials

Prior to the collection of the plant material, the was identified by a taxonomist in the department
Lab Laboratory technology, federal polytechnic, Bida, Niger state and confirmed by a Botanist in the
Department Bio Biological sciences, Federal University of Technology, Minna. The seeds of the plant were collected and cultivated on new ridges in the biological gardenof the federal polytechnic, Bida. Each of the parts was then air-dried in the laboratory after which it was crushed and macerated using mortar and pestle. The macerated peel blended into fine powder using electric blender (Mx-491 N electric C.D., (M)Bhd).

Extraction and fractionation procedure

Many methods exist for extraction and fractionation of plant materials. However, the method adopted for the purpose of this work was that of [4].
Fifty gramme of dried powdered sample was percolated in 1.5 litres 0f 95% ethyl alcohol. It was then filtered using filter paper. The residue was discarded while filterate was evaporated and concentrated using a soxhlet extractor (Buchi W 240 N) under reduced pressure at a temperature of 30^0c togive the ethanol extract. This was repeated for all the parts namely leaf, seed, stem, root and the whole plant.

Preparation of sample solution of plant extracts.

The extracts from the various plant parts (leaf, seed, tem, whole plant and root) were dissolved in 10% aqueous solution of dimethyl sulphur oxide (DMSO) at a concentration of 200mg/ml [4;8]. Serial dilutions were prepared from the stock solutions using distilled water as diluent.

Sensitivity Test

The disc diffusion method was employed in sensitivity test.
A paper perforator was used to prepared the disc from Whatman filter paper size 11cm. one hundred discs were counted in a McCartney bottle and sterilized in an autoclave at a pressure of 15mmHg and temperature of 121^0c for 15minute. Into each bottle, 1ml of the corresponding concentration of extract was pipetted and mixed. The disc were allowed to absorb the eztracts and then left to dry at room temperature. Ten millilitre of the molten agar media (NA) waspipetted into a Petri dish and set aside to gel. After solidifying, 1ml of the test organism was used to seed the agar medium by spreading methods.
After the inoculation, four disc containing different concentrations of the extract were placed equidistance on each plate. Plates were incubated at 37^0c for 24hours.
The antimicrobial activities of plant extracts were expressed as zone of clearing observed round the disc. Controls were set up using standard antibiotic discs (oxoid LTD; Basingstoke, Hamphire, England).

The phytochemical screening of the ethanol extract of *Euphorbia heterophylla*

Phytochemical analysis for the detection of organic components was conducted based on the method of [9] and as cited by [5].

RESULTS

Leaf extracts had activity against all the test organisms while the seed, stem and whole plant extracts were active against at least two of the organisms. The root extract was active against *Salmonella paratyphi B only* (Table 1)
The organisms were sensitive to all the antibiotics in the positive control but in the negative control, some were resistant to some of the antibiotics like gentamycin and streptomycin (Table 2 and 3)
The phytochemical analysis of the plant extract revealed the presence of tannins and arthraquinones as the active compounds (Table 5).
All results are on the tables at the appendix.

DISCUSSION

Clear zones of inhibition indicate high activity against the typhoid bacilli. The leaf extract was active against all the test organisms which show that the active principle may be highly concentrated in the leaf more than any other plant parts. It therefore suggests probably that, the leaf serves as the storage organ of the plant. That the remaining parts of the plant showed activity are an indication of availability of the active components in them. The activity of each of the plant extracts in the crude forms are this active shows that if they are refined they may be more potent than available and used anti_typhoid antibiotics like chloramphenicol and amoxicillin.
The plant as been shown to certain tannins and arthraquinones. These compounds are known to possess antimicrobial capabilities. According to [10] tannin_like

substances are present in the bark and pulp of dicotyledonous plants and that earlier reports by [11]; [12] have shown that they inhibit bacterial growth and are capable of protecting certain plants against bacterial infection. Tannins have been shown to form irreversible complexes with praline_ rich proteins[13] which would lead to inhibition of cell_wall_protein synthesis, a property that may explain the mode of action of this plant extract.

The successful screening of this plant parts in in vitro technique in the laboratory in an indication that if subjected to in vitro laboratory animal testing, we may evolve a drug that will proffer lasting cure to the torments and menace of typhoid fever in the tropics and world at large

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Table 1. Result of the sensitivity test of the plant extracts on the test organisms using disc diffusion method

	Plant extracts				
Organisms	H_L	H_F	H_S	H_W	H_R
S. typhi	10.5	15.0	N.I	N.I	N.I
S. paratyphi A	15.0	N.I	30.0	25.0	N.I
S. paratyphi B	35.0	37.0	37.0	36.0	36.0
S. paratyphi C	12.5	N.I	N.I	N.I	N.I

Diameter of zones of inhibition (mm) by Ethanol extracts of plant.
Results are average of four trials at different test concentrations.

Table 2. Diameter of zones of clearing of standard antibiotic disc against test organisms.

Positive control

Zones of clearing (mm)

Standard	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	AMC	CPY
Antibiotics(ug/ml)	25	20	10	10	10	1.5	10	10	25	10
Test organisms										
Salmonella typhi	3	5	8	9	6	5	10	11	8	7
Salmonella paratyphi A	5	5	10	10	8	8	10	10	10	15
Salmonella paratyphi B	5	4	8	9	7	9	7	8	9	12
Salmonella paratyphi C	4	5	9	11	6	6	9	4	2	6

Table 3. . Diameter of zones of clearing of standard antibiotic disc against test organisms
Negative control Zones of clearing (mm)

Standard	AMP	CHL	CXC	ERY	GEN	PEN	STR	TET	AMC	CPY
Antibiotics(µg/ml)	25	20	10	10	10	1.5	10	10	25	10
Test organisms										
Salmonella typhi	5	8	5	4	2	6	N.I	6	5	N.I
Salmonella paratyphi A	7	8	10	2	N.I	5	1	8	10	N.I
Salmonella paratyphi B	6	7	8	3	N.I	5	2	6	10	N.I
Salmonella paratyphi C	9	9	7	10	5	11	3	9	10	8

Key :-
AMP= Ampicillin
CXC= Cloxacillin
AMC= Amoxycillin
PEN= Penicillin
TET= Tetracycline
N.I= No zone of inhibition.
CHL= Chloramphenicol
ERY= Erythromycin
GEN= Gentamycin
STR= Streptomycin
CPX= Ceproflaracin

Table 4. Weight recovered after percolating 50g of plant powder in ethanol

Plant part	Weight in grammes
Leaf	7.6
Seed	9.2
Stem	10.0
Whole plant	10.8
Root	11.8

Table 5. Results of phytochemical analysis.

Compound screened for	Result obtained
Tannins	+
Alkaloids	-
Arthraquinones	+
Phlobatannins	-
Saponins	-
Glycosides	-

Key :- + = present, - = Absent

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