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Determination of in Vitro Susceptibility of the Typhoid Pathogens to Synergistic Action of Euphorbia Hirta, Euphorbia Heterophylla and Phyllanthus Niruri for Possible Development of Effective Anti-Typhoid Drugs

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***Abstract*—**Studies were carried out to determine the in vitro susceptibility of the typhoid pathogens to combined action of Euphorbia hirta, Euphorbia heterophylla and Phyllanthus niruri. Clinical isolates of the typhoid bacilli were subjected to susceptibility testing using agar diffusion technique and the minimum inhibitory concentration (MIC) determined with tube dilution technique. These isolates, when challenged with doses of the extracts from the three medicinal plants showed zones of inhibition as wide as 26±0.2mm, 22±0.1mm and 18±0.0mm respectively. The minimum inhibitory concentration (MIC) revealed organisms inhibited at varying concentrations of extracts: E. hirta (S. typhi 0.250mg/ml, S. paratyphi A 0.125mg/ml, S. paratyphi B 0.185mg/ml and S. paratyphi C 0.225mg/ml), E. heterophylla (S. typhi 0.280mg/ml, S. paratyphi A 0.150mg/ml, S. paratyphi B 0.200mg/ml and S. paratyphi C 0.250mg/ml) and P. niruri (S. typhi 0.150mg/ml, S. paratyphi A 0.100mg/ml, S. paratyphi B 0.115mg/ml and S. paratyphi C 0.125mg/ml). The results of the synergy between the three plants in the ration of 1:1:1 showed very low MICs for the test pathogens as follows S. typhi 0.025mg/ml, S. paratyphi A 0.080mg/ml, S. paratyphi B 0.015mg/ml and S. paratyphi C 0.10mg/ml with the diameter zone of inhibition (DZI) ranging from 35±0.2mm, 28±0.4mm, 20±0.1mm and 32±0.3mm respectively. The secondary metabolites were identified using simple methods and HPLC. Organic components such as anthroquinones, different alkaloids, tannins, 6-ethoxy-1,2,3,4-tetrahydro-2,2,4-trimethyl and steroids were identified. The prevalence of Salmonellae, a deadly infectious disease, is still very high in parts of Nigeria. The synergistic action of these three plants is very high. It is concluded that pharmaceutical companies should take advantage of these findings to develop new anti-typhoid drugs from these plants.

***Keywords*—**A Prevalence, Susceptibility, Synergistic, Typhoid pathogens.

I. Introduction

TYPHOID fever is an enteric infection caused by four strains of Salmonella, Salmonella typhi, Salmonella paratyphi A, S. paratyphi B and S. paratyphi C. It is one of the communicable diseases currently ravaging quite a number of people in the tropics besides malaria fever. The typhoid bacilli could be transmitted from food stuff to man or from animal to man making it one of the zoonotic diseases. Salmonella is a genus of rodshaped,Gram-negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 μm, lengths from 2 to 5 μm, and flagella which project in all directions (peritrichous). They obtain their energy from oxidation and reduction reactions using organic sources and are termed facultative anaerobes; which can readily be detected by growing them on media containing ferrous sulfate, such as TSI [1]. Salmonella are closely related to the Escherichia genus and are found worldwide in warm – and cold – blooded animals, in human and in non-living habitats. They cause illness in humans and many animals, such as typhoid fever, paratyphoid fever, and the food borne illness (Salmonellosis) [2].

The consumption of plant materials as alternative medicine has been encouraged because they are cheap and they could significantly contribute to the improvement of human health in terms of cure and prevention of diseases as discussed by Okoko and Oruambo [3]. Plants have been useful as food and medicine as shown elsewhere [3,4,]. They contain vitamins needed by human body for healthy living [5,6].

II. Materials and Methods

A. Sample collection: Plant materials (whole plant) were collected from Bida, Niger State, Nigeria. Identification was carried out by local people and confirmed by a Botanist and Taxonomist in the Department of Botany, University of Ilorin, Nigeria.

B. Test organisms: Clinical strains of the organisms Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B and Salmonella paratyphi C were isolated from clinical specimens obtained from patients visiting designated Hospitals for this research work in North Central Nigeria. Isolates were identified using their physical and Biochemical characteristics [7].

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Open Science Index, Pharmacological and Pharmaceutical Sciences Vol:5, No:10, 2011 publications.waset.org/618.pdf

C. Isolation of Test Organisms

- A total of 15.96g Selenite F broth was weighed into a flask bottle and covered tightly.
- About 60ml of sterile water was added and shacked gently.
- It was warmed to dissolve evenly, and continually swirled to mix well.
- It was sterilized in a boiling water bath for 10 minutes, after which it was removed and allowed to cool to about 47oC before dispensing into slant bottles.
- About 14ml each was dispensed into 40 slant bottles.
- Using inoculating needle under flame, the stool samples were inoculated into the slants corresponding as labeled age 1 to 40 respectively.
- The slants were then incubated at 370C for 18-24 hours.
- After this, the isolates were kept sterile in the refrigerator for further analysis.

D. Biochemical Identification

- In order to keep the organisms viable and fresh, 18 hours broth culture was prepared from each isolate and used for this test.
- A total of 55.25g of Triple Sugar Iron (TSI) agar was weighed into a flask and covered tightly [8].
- About 850ml of de ionized water was added and then allowed to soak for 10 minutes, swirled to mix well while boiling.
- It was then sterilized by autoclaving at 121oC for 15 minutes.
- About 20ml each was dispensed into sterile petri dishes, and then incubated at 37oC for 48 hours.
- After which results were read and recorded.

Extraction and preparation of plants' materials: Voucher specimens of plant with number MFT1675 were deposited in the herbarium in Federal University of Technology, Minna and room dried for two weeks until well dried. The dried plant materials were pound in laboratory mortar and pulverized to powdered form using blender. This was then followed by extraction of materials with alcoholic solvent. Ethanol was used as solvent for the extraction of the plant materials. The method of Silva [9] was adopted. Fifty (50) grams of ground sample of each plant part was suspended in 250ml of 95% ethanol for a period of about 120 hours. The extract was decanted and filtered and the filtrate evaporated in vacuo at 45oC. The yield of each plant extract was calculated accordingly and then reconstituted in 95% ethanol and reserved as stock concentration then stored.

E. Antibacterial Assay

Each of the microorganisms was subjected to the action of the extracts from the tree shrubs using the agar diffusion technique as described by Silva [9] and Abalaka [10]. Using cork borer No.4, three holes were bored on the surface of the agar medium equidistant from one another. The bottom of each hole was sealed with molten agar to avoid seepage. When solidified, each of the wells made was filled with 1ml of the .prepared extract solution at various test concentrations and allowed to fully diffuse. The surface of the agar was inoculated for confluent growth with an 18 hour culture of the test organism which has been standardized to 106cfu/ml and incubated at the temperature of 37oC in the incubator for 24 hours.

F. Minimum Inhibitory Concentration (MIC)

Using tube dilution method, the least concentration of plant extract in which there was no turbidity was taken as the minimum inhibitory concentration (MIC) [11]. The MIC of the plant extracts was determined by serially diluting extract from 101 to 1010. 1ml of each of the extracts was introduced into 9ml of nutrient broth in the test tube. This mixture was then inoculated with 0.1ml culture of the test organism which was standardized to 106cfu/ml. This was then incubated at 37oC for 24 hours. The least concentration of plant extract in the test tube with no turbidity was taken as the Minimum Inhibitory Concentration (MIC).

G. Minimum Bactericidal Concentration (MBC)

This was an offshoot of the previously determined MIC. The least concentration of plant extract in the test tube with no turbidity was taken as the Minimum Inhibitory Concentration (MIC). Subsequently, those tubes that showed no turbidity were plated out on nutrient agar plates and absence of growth on incubation for 24 hours was confirmatory for Minimum Bactericidal Concentration (MBC).

H. Phytochemical Analysis of Plant extracts for Active Components

Phytochemical screening of the extracts was carried out according to the methods described by Odebiyi and Sofowora [12] and Trease and Evans [13] and used by Abalaka [14] for the detection of active components like saponins, tannins, alkaloids, phlobatanins, glycosides e.t.c

III. Results

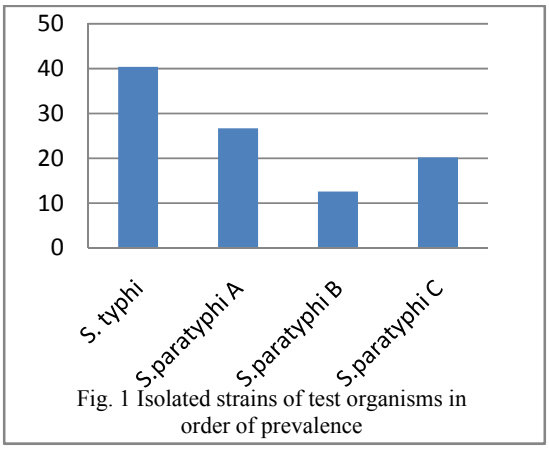

Fig. 1 Isolated strains of test organisms in order of prevalence

Fig. 1 above appeared to be in the order of magnitude as S. typhi>S. paratyphiA>S. paratyphiC>S. paratyphiB.

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TABLE I
ZONES OF INHIBITION PRODUCED AROUND TEST ORGANISMS WHEN CHALLENGED WITH CERTAIN CONCENTRATIONS OF THE PLANTS' EXTRACTS

Plant	Organisms	Concentration (g) 0.5	0.05	0.005	0.0005	0.00005	Amox 0.33mg/ml	Chlor 20mg/ml
E. hirta	*S.t*	35	32	30	24	20	40	38
	S.pA	36	34	32	30	26	35	32
	S.pB	28	26	22	20	18	32	30
	S.pC	38	32	28	26	18	30	26
E. heteroph ylla	*S.t*	32	29	26	24	18	40	38
	S.pA	30	28	26	20	16	35	32
	S.pB	28	24	20	18	14	32	30
	S.pC	34	30	26	20	15	30	26
P. niruri	*S.t*	34	32	28	27	24	40	38
	S.pA	32	30	29	26	24	35	32
	S.pB	30	28	26	24	22	32	30
	S.pC	38	35	36	32	28	30	26
Synergy	*S.t*	40	38	36	32	30	40	38
	S.pA	39	37	34	32	28	35	32
	S.pB	35	33	28	26	22	32	30
	S.pC	40	38	34	32	30	30	26

Key: *S.t=Salmonella typhi, S.pA=Salmonella paratyphiA, S.pB=Salmonella paratyphiB, S.pC=Salmonella paratyphiC,* Amox=Amoxicillin, Chlor=Chloramphenicol
*Results are mean of triplicate trial & have S.D of 0.00-0.004

TABLE II
MINIMUM INHIBITORY CONCENTRATION (MIC) OF EXTRACTS FROM THE THREE PLANTS AGAINST TEST THE ORGANISMS EXTRACTS/CONCENTRATION (MG/ML)

Organisms	E. hirta	E. heterophylla	P. niruri	Synergy
S. typhi	0.250	0.280	0.150	0.025
S. paratyphi A	0.125	0.150	0.100	0.080
S. paratyphi B	0.185	0.200	0.115	0.015
S. paratyphi C	0.225	0.250	0.125	0.010

Key: Synergy=combined action of the three extracts in the ratio of 1:1:1

TABLE III
MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF EXTRACTS FROM THE THREE PLANTS AGAINST THE TEST ORGANISMS
Extracts/Concentration (mg/ml)

Organisms	E. hirta	E. heterophylla	P. niruri	Synergy
S. typhi	0.350	0.380	0.250	0.125
S. paratyphi A	0.225	0.250	0.210	0.180
S. paratyphi B	0.285	0.300	0.215	0.115
S. paratyphi C	0.325	0.350	0.225	0.110

Key: Synergy=combined action of the three extracts in the ratio of 1:1:1

TABLE IV
AVAILABLE SECONDARY METABOLITES IN THE THREE PLANTS' EXTRACTS

Organic compounds	E. hirta	E. heterophylla	P. niruri
Alkaloids	+	+	+
Anthraquinones	+	+	-
Cardiac glycosides	-	-	+
Phlobatannins	+	-	+
Polyphenols	+	+	+
Resins	-	-	+
Saponins	-	-	-
Tannin	+	+	+
Steroids	+	+	+

Key: + = present
- = absent
Key: Synergy=combined action of the three extracts in the ratio of 1:1:1

IV. DISCUSSION

The occurrence of the strains of Salmonella in the samples screened appeared to be in the order of magnitude as S. typhi>S. paratyphiA>S. paratyphiC>S. paratyphiB with the percentage occurrence of 40.4, 26.7, 20.2 and 12.6 respectively. This shows that S. typhi is the leading cause of typhoid fever followed by Salmonella paratyphi A. S. paratyphi C is next to S. paratyphi A while the least strain that is responsible for the disease, according to these findings, is S. paratyphi B having the percentage occurrence of just 12.6 (fig 1).

The test organisms showed varying degrees of susceptibility to various concentrations of the extracts with diameter of zones of inhibition (DZI) ranging from 14±0.02 to 35±0.02 and compared to those of standard antibiotics used in this analysis Table I. The results of synergistic actions of the extracts on the organisms revealed far wider diameter zones of clearing compared with those created by each of the extracts. This justifies the reason herbalists usually prescribe the mixture of different herbs to cure various ailments. The Herbalists seldom prescribe one herb for the cure of any disease.

The results of Minimum Inhibitory Concentration (MIC) revealed MIC ranging from 0.010mg/ml- 0.280mg/ml representing 100µg/ml-280µg/ml. The concentration of 100µg/ml is very small with the highest as 280µg/ml. It was observed that the Minimum Bactericidal Concentration (MBC) appeared to be higher than the MIC about 0.010mg/ml or 100µg/ml. The results showed the MBC ranging from 0.110mg/ml- 0.380mg/ml equivalent to 110µg/ml-380µg/ml (Tables II and III). It is a known fact that higher concentrations of antibacterial agents than the concentration of MIC are usually required to lyse bacterial cells. This was evident in this research work as higher concentrations of the extracts were needed to produce cidal effects on the test organisms. Alade and Irobi [15] recorded similar findings in their research work on the antimicrobial activities of crude leaf extract of Acalypha wilkesiana.

The secondary metabolites identified in the extracts include Alkaloids, Anthroquinones, Cardiac glycosides, Resins, Phlobatanins, Polyphenols, Tannins and Steroids (Table 4). These metabolites are known to posses antimicrobial properties and may be responsible for the important antibacterial activities recorded in this experiment. Elsewhere [16,17,18] it was reported that secondary metabolites from

plants are useful sources of novel drugs and antibiotics. Bioactive components of plants are very useful in the development of new antimicrobial agents. The findings that these plants contain bioactive principles that are active against typhoid agents such as Salmonella typhi and the paratyphi strains is very imperative to the development of drugs against typhoid fever which is still ravaging many in the third world countries including Nigeria. Orthodox drugs are developed from plant sources world over [19].

V. Conclusion

We conclude that this research has provided the lead way for the development of very active drugs needed for the control of typhoid fever in Nigeria in particular and Africa in general since the present drugs of choice (Chloramphenicol, Amoxicillin) for the treatment of the disease are now being invaded by the causative organisms. Pharmaceutical Companies are therefore advised to make enquiries about these plants and begin work in ernest to enable the utilization of these plants on a wide scale because these plants are available in abundance in the black world.

References

[1] Minor L., and Popoff, M.Y (1987). Designation of Salmonella Enterica. (465468). Nea M.I. (2002). Fourth Edition Medical Pharmacology at a glance.
[2] Health Protection Report (2009). http://www.hpa.org.uk/hpr/archives/2009/news1409.htm reptiles Okoko, T. and Oruambo, I. F. (2008). The effects of Hibiscus sabdariffa calyx on cisplatin-induced tissues damaged in rats. Biokemistri. 20(2):47-52.
[3] Okoko, T. and Oruambo, I. F. (2008). The effects of Hibiscus sabdariffa calyx on cisplatin-induced tissues damaged in rats. Biokemistri. 20(2):47-52.
[4] Adebooye, O.C. and Opabode, J.T. (2004). Status of conservation of the indigenous leaf vegetables and fruits of Africa. Afr. J. Biotech. 3:700-705.
[5] Szeto, Y.P., Tomlinson, B., Benzie, I.F.F. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: Implications for dietary planning and food preservation. British Journal of Nutrition. 87:55-59.
[6] Jimoh, F.O., Adebayo, A.A., Aliero, A.A., Afolayan, A.J. (2008). Polyphenol contents and Biological activities of Rumex ecklonianus. Pharm. Biol. 45(5):333-340.
[7] Buchanan, R. and Gibbons, N.E. (1974). Bergey's manual of determinative bacteriology. 8th edition,.Baltimore: Williams and Wilkins. 113-136.
[8] Middle Brooks Pharmaceuticals (2009). http://www.middlebrook pharma.com/NEWSROOM antibiotics/news details.
[9] Silva, O., Daurk, A., Pimentel, M., Viegas, S., Barroso, H., Machado, J., Pires, I., Carbrita, J. and Gomes, E. (1997). Antimicrobial Activity of Terminilia macroptera root. Journal of Ethnopharmacology. 57:203-207.
[10] Abalaka, M. E., Onaolapo, J.A., Inabo, H.I. and Olonitola, O.S. (2009). Extraction of Active Components of Mormodica Charantia L (Cucurbitaceae) for Medicinal Use. Afr J Biomed Eng & Sc, (1): 38 -44
[11] Hugo, S. B. and Rusell, A. D. (2003) Pharmaceutical microbiology, 6th edition, Blackwell scientific publishers, Oxford, London Pp. 91-129.
[12] Sofowora, A., (1993) Medicinal Plant and Traditional Medicine in Africa, 2nd Edition, Spectrum Books Ltd. Ibadan, Nigeria. 50 – 58.
[13] Trease, G.E. AND Evans, W.C. (1989). A text book of Pharmacognosy, 13th edition, Bailliere Tindall Ltd., London. 394.
[14] Abalaka, M. E., Daniyan, S. Y and Mann, A (2010). Evaluation of the antimicrobial activities of Two Ziziphus species (Ziziphus mauritiana L. and Ziziphus spinachristi L.) on some microbial pathogens. African Journal of Pharmacy and Pharmacology 4(4),135-139.
[15] Alade, R.I and Irobi, O.N. (2002). Antimicrobial activities of crude leaf extracts of Acalypha wilkisiana. Journal of Ethnopharmacology. 39:171-174.
[16] Martinez, M.J. (2004). Screening of some Cuban Medicinal plants for antimicrobial activity. Journal of Ethnopharmacology. 52:174-178.
[17] Martos, F.J.A., Aguian, L.M. and Silva, M.G. (2002). Chemical constituents and antimicrobial activities of Vatairea macrocarpa. Ducke Acta Amazonia. 18:351-352.
[18] Marwan, A.G. (2003). Quantitative determination of infinite inhibition concentration of antimicrobial agents. Applied Environmental Microbiology. 51(3):559-561.
[19] Wagner, H., Blandts, S.and Zgainki, E.M. (2000). Plant Drug Analysis. Spring-Verlag, New York. 320.

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