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Full Length Research Paper

Screening for anti-typhoid activity of some medicinal plants used in traditional medicine in Ebonyi state, Nigeria

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Ethanol, hot and cold crude water extracts of five medicinal plants namely *Vitex doniana* (root) , *Cassia tora* (Leaf), *Alstonia boonei* (bark), *Stachytarpheta jamaicensis* (leaf), and *Carica papaya* (leaf) used as traditionally medicine for anti *Salmonella typhi* activity in Ebonyi state were evaluated. These plants were screened *in-vitro* for anti-typhoid activity against 10 clinically selected isolates of *S. typhi* using the hole-plate diffusion method. The Minimum inhibitory concentration (MIC) of Ethanol, hot and cold water extract of each herbal plant was determined by broth dilution method. Ethanol extracts of *Vitex doniana* exhibited anti-typhoid activity against 9(90%) of the test organisms, *A. boonei* exhibited activity against 8(80%) of the test organisms, *C. papaya* against 2(20%), *C. tora* against 6(60%), and *S. jamaicensis* against 6(60%). Hot water extract of *Vitex doniana* showed anti-typhoid activity against 7(70%) of the test organisms, *A. boonei* against 9(90%), *C. papaya* against 1(10%), *C. tora* against 8(80%) and *S. jamaicensis* against 7(70%). Cold water extract of *V. doniana,* had anti-typhoid activity against 6(60%) of the test organisms, *A. boonei*, against 6(60%), *C. papaya* against 0(0%), *C. tora* against 6(60%) and *S. jamaicensis* against 4(40%). MIC of ethanol, hot and cold water extracts of *V. doniana*, *A. boonei, C. papaya, C. tora and S. jamaicensis,* fall within 0.4 -128, 0.8 -128, 64 -128, 32 – 128 and 32 – 128. MIC of hot water extracts were within 16 -128, 0.8 – 128, 128 -512, 0.8 – 512 and 0.8 – 128 while MIC of cold water extract are within 64 – 128, 64 – 512, 64 – 512, 64 – 512 and 128-512 respectively. Our findings showed that ethanol and hot water extracts of *V. doniana* and *A. boonei* had the best anti-typhoid activity followed by *C. tora* and *S. jamaicensis* while *C. papaya* showed no activity.

Key words: *Salmonella typhi,* medicinal plants extracts, anti-typhoid activity, minimum inhibitory concentration.

INTRODUCTION

Infectious diseases account for a high proportion of health problems in Africa. Many plants are used in African continent for the treatment of different diseases such as fever, dysentery, cholera, diarrhea etc and others which are typical disease of a tropical country (Ayogu and Amadi, 2009; Ajayi and Akintola, 2010).

Typhoid fever is a systemic infection caused by the bacterium *Salmonella enterica* subspecies *enterica* serotype *typhi*, which is acquired by ingestion of contaminated food and water. Each year the disease affects at least 16 million persons world-wide, most of whom resides in the developing countries of Southeast Asia and Africa (CDC, 2003).Typhoid fever is uncommon in industrialized regions such as the USA, Canada, Europe, Australia and Japan and new cases of the disease in these countries are related to travel to the developing countries (Papadimitropoulos et al., 2004; Taylor et al., 1983). Mortality rates associated with typhoid fever vary from region to region, with highest reported from Indonesia, Nigeria and India (Miller et al., 1994).

According to World Health Organization (WHO) more

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than 80% of the world's population relies on traditional medicine for their primary healthcare, majority of which use plants or their active principles (Gupta et al., 2005). Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. Use of plant resources mainly for herbal medicine, food, forage etc in Nigeria represents a long history of human interaction with the environment and their *in vitro* and *in vivo* properties to microbial pathogens have been widely reported (Okafor, 2001; Iwalokun et al*.,* 2004; Hashish, 2003). Also the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). Today, it is estimated that plant materials are present in or have provided the models relatively for 50% Western drugs (Rodders, 1996).

Traditional medical practitioners in Nigeria use herbal preparations to treat microbial infections such as typhoid and para-typhoid infections and they claimed that the primary benefit of using plant –derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. In recent past, attention has been directed towards medicinal plant research to substantiate the claims of cure made by traditional healers thus providing scientific basis for their efficacy (Olukoya et al., 1993). These medicinal plants; *V. doniana* (root) *, C. tora* (Leaf)*, A. boonei* (bark)*, S. jamaicensis* (leaf)*,* and *C. papaya* (leaf) have been claimed by traditional medical practitioners in Ebonyi State to be effective when used for the treatment of fevers, particularly typhoid fever. However, no detailed reports on anti-typhoid activity of these plants exist in literature in Nigeria, therefore; the present study investigates the anti-typhoid activities of extracts of these five medicinal plants.

MATERIALS AND METHODS

Collection of bacteria strains

A total of 10 clinical isolates of *S. typhi* were isolated from patients attending Boromi General Hospital located at Onitsha, Anambra State, Nigeria. All Isolates was identified and characterized using standard microbiology technique (Chessbrough, 2006).

Sources of plants

All plant materials were collected from traditional medicine practitioners in Ebonyi State of Nigeria and were identified by Professor J.C. Onyekwere of Applied Microbiology Department, Ebonyi State University Abakaliki and voucher specimen were kept in the University herbarium.

Preparation of plant extracts

Fresh leaves, bark and the roots of each medicinal plant were collected and properly washed and rinsed in sterile distilled water. It was allowed to dry under room temperature, pulverized using laboratory mortar and pestle and further with manual hand grinding machine and was stored in air tight containers for further analysis.

Extraction of plant materials

Plant materials were extracted with ethanol, cold and hot water, briefly 20 g of each pulverized samples was weighed into 100 ml of the solvent (ethanol, hot and cold water). It was allowed for 24 h in cold water and ethanol, 2 h in hot water with occasional shaking. Each preparation was filtered using Watt man No 1 filter paper and was evaporated to dryness in a steady air current for 24 h in a previously weighed crucible.

Determination of anti-typhoid activity

Ethanol, hot and cold water extracts of each herbal plant was spot checked for anti-typhoid activity using the hole-plate diffusion method. Exactly 0.5 MacFarland equivalent standards of test organisms was inoculated on the surface of sterile Mueller- Hinton agar plate, it was allowed for 10 to 15 min to pre-diffuse, an 8 mm sterile cork borer was used to bore holes on the agar plates. The dried aqueous and ethanol extract was reconstituted in sterile water to a concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml and each extract was used to fill the holes made in the agar plate using a sterile glass pipette. This was allowed for 20 to 30 min to pre-diffuse and then incubated at 37°C for 18 to 24 h. After incubation the radial zones of inhibition was measured around each of the extract. Ciprofloxacin and chloramphenicol was used as control antibiotics.

Determination of minimum inhibitory concentration (Mic µG/ML) of plant extracts

The minimum inhibitory concentration (MIC g/ml) of each extract was determined against test isolates in triplicates at varying concentrations starting from 712 – 0.2 g/ml. The test utilized the lowest concentration of an antimicrobial extract to inhibit the visible growth of a micro- organism after overnight inoculation. Each potential extract was determined by micro-broth dilution technique. These concentrations were obtained by first making serial dilution of the stock concentration of the extracts in double fold and from there a two-fold dilution of the extracts was carried out to obtain the dilutions above. Exactly 0.5 MacFarland standard suspensions of the test organisms were inoculated in a sterile tube of nutrient broth containing different two fold dilutions of each plant extracts. This was incubated at 37°C for 18 to 24 h and MIC was determined by observing for growth or no growth in each of the test tube with different concentrations of the extracts by observing for turbidity. Range of MIC for each extract was determined by observing the lowest concentration of each extract that inhibit growth of each organism.

RESULTS

Profiles of the five medicinal plants used are presented in Table 1. Four out of five plants screened namely *V. doniana*, *A. boonei*, *C. tora* and *S. jamaicensis* showed anti-typhoid activity against *S. typhi* strains tested at different rates. The ethanol extracts of *V. doniana* and *A. boonei* were the most active inhibiting 9(90%) and 8(80%) of test organism, followed by *S. jamaicensis* and

Table 1. Profile of the five Medicinal plants used.

Botanical name	Family name	Local igboname	Plant part used
Vitex doniana	Verbenaceae Vitex	Ucha koro	Root
Alstonia boonei	Apocynaceae Alstonia	Egbu	Bark
Carica papaya	Caricaceae Carica	Mgbimgbi	Leaf
Cassia tora	Caesulpinaceae Cassia	Baka egbe	Leaf
Stachytarpheta jamaicensis	Verbenaceae Linn	Albaka	Leaf

Table 2. Anti-typhoid activity of ethanol, hot and cold water plant extracts.

Medicinal plants	Sensitivity (%)	Resistance (%)
Ethanol extract		
Vitex doniana (Root)	9(90)	1(10)
Alstonia boonei (Bark)	8(80)	2(2)
Carica papaya (Leaf)	2(20)	8(80)
Cassia tora (Leaf)	6(60)	4(40)
Stachytarpheta jamaicensis (Leaf)	6(60)	4(40)
Hot water extract		
Vitex doniana (Root)	7(70)	3(30)
Alstonia boonei (Bark)	9(90)	1(10)
Carica papaya (Leaf)	1(10)	9(90)
Cassia tora (Leaf)	8(80)	2(20)
Stachytarpheta jamaicensis (Leaf)	7(70)	3(30)
Cold water extract		
Vitex doniana (Root)	6(60)	4(40)
Alstonia boonei (Bark)	6(60)	4(40)
Carica papaya (Leaf)	0(0)	10(100)
Cassia tora (Leaf)	6(60)	4(40)
Stachytarpheta jamaicensis (Leaf)	4(40)	6(60)
Control antibiotics		
Ciproloxacin	8(80)	2(20)
Chloramphenicol	6(60)	4(40)

C. tora with 6(60%) while *C. papaya* were active against 2(20%) . Hot water plant extract of *V. doniana* showed activity against 7(70%), *C. tora* showed activity against 8(80%), *A. boonei* against 9(90%), *S. jamaicensis* against 7(70%), *C. papaya* against 1(10%). Cold water extract of *V. doniana* showed activity against 6(60%) of test organism, *A. boonei* 6(60%), *C. tora* 6(60%), *S. jamaicensis* against 4(40%), *C. papaya* against 0 (0%). Control antibiotics, ciprofloxacin have activity against 8(80%) of the test organisms while chloramphenicol had activity against 6(60%) (Table 2). The Minimum Inhibitory Concentration (MIC) of ethanol extract of *V. doniana*, *A. boonei*, *C. papaya, C. tora* and *S. jamaicensis,* and fall within the following ranges: 0.4 -128, 0.8 -128, 64 -128, 32 – 128 and 32 – 128. MIC of hot water extract were within 16 -128, 0.8 – 128, 128 -512, 0.8 – 512 and 0.8 – 128 g/ml while MIC of cold water are within 64 – 128, 64 – 512, 64 – 512, 64 – 512 and 128-512 (Table 3). MIC of ciprofloxacin was between 2-16 g/ml while that of chloramphenicol was between 4 - 64 g/ml. Our result showed that *V. doniana* and *A. boonei* showed the highest anti-typhoid activity with ethanol extract, *A. boonei, S. jamaicensis* and *C. tora* showed the highest activity with hot water extract and *A. boonei, C. tora* and *V. doniana* showed the same activity with cold water extract. In general, our findings showed that four of the plant extracts namely *V. doniana, A. boonei, S. jamaicensis* and *C. tora* tested had good anti-typhoid

Table 3. Minimum inhibitory concentration (mg/ml) of ethanol, hot and cold water extracts.

Medicinal plant	Ranges of MIC
Ethanol extract	
Vitex doniana (Root)	0.4 - 128 mg/ml
Alstonia boonei (Bark)	0.8 - 128 mg/ml
Carica papaya (Leaf)	64 -128 mg/ml
Cassia tora (Leaf)	32 - 128 mg/ml
Stachytarpheta jamaicensis (Leaf)	32 - 128 mg/ml
Hot water extract	
Vitex doniana (Root)	16 - 128 mg/ml
Alstonia boonei (Bark)	0.8 - 128 mg/ml
Carica papaya (Leaf)	128 - 712 mg/ml
Cassia tora (Leaf)	0.8 - 712 mg/ml
Stachytarpheta jamaicensis (Leaf)	0.8 - 128 mg/ml
Cold water extract	
Vitex doniana (Root)	64 - 128
Alstonia boonei (Bark)	64 - 712
Carica papaya (Leaf)	128 - 712
Cassia tora (Leaf)	64 - 712
Stachytarpheta jamaicensis (Leaf)	64 - 712
Control antibiotics	
Ciprofloxacin	4 - 16 g/ml
Chloramphenicol	4 - 64 g/ml

activity while *C. papaya* do not.

DISCUSSION

Effort towards drug discovery and prudent use of antimicrobial agents are the mainstay for overcoming the worldwide problems of microbial resistance (Al- Bakri et al., 2005). One resort for drug discovery is natural products, crude or isolated from medicinal plants. Many plants and their structurally diverse compounds are known for their antimicrobial activities that have been routinely assessed against different microbes.

In our study the anti-typhoid activity of ethanol, hot and cold water extracts of root, bark and leaf of *V. doniana, A. boonei, C. papaya, C. tora and S. jamaicensis* were evaluated and the result indicates that four namely *V. doniana, A. boonei, S. jamaicensis* and *C. tora* has activity against *S. typhi* strains tested while *C. papaya* did not. The four plants with anti-typhoid activity are promising to act as potential agent for treating typhoid fever from natural plant source. Crude ethanol and hot water extracts had the same anti- typhoid activity but greater than cold water. *C. papaya* leaf is traditionally used for treating malaria fever although no literature has reported this; some studies in the past have reported the in-effective activity of *C. papaya* against typhoid bacilli which gives support to our findings (Adegoke et al., 1968). It is important to note that these plants are solely used for the treatment of typhoid fever and with our findings we tend to disagree with the traditional used of this plant (*C. papaya*) for the treatment of typhoid fever in Ebonyi State, Nigeria. The *in vitro* inactivity of this medicinal plant against *S. typhi* strains as established in this study does not therefore necessarily translate to their *in vivo* in-activity but may probably be playing an immuno-modulatory role in the body system. This effect has been documented were immuno-modulation (*in vivo*) of chemical compounds from medicinal plants, many of which have been proven to be inactive *in-vitro* against pathogen (Bever, 1986).

Among the tested, ethanol extracts exhibited the highest antimicrobial activity, this is due to the differences in the type and concentrations of the secondary metabolites across different plants, variation in antimicrobial activities are expected. These quantitative and qualitative differences in constituents are influenced by the chemo types and particularly by environmental factors and method of extraction. Also the antimicrobial activity of Hot water extracts is also high compared to cold water extract. Although, we did not determined the actual composition of our ethanol plant extracts since

several studies demonstrated that crude plants extracts bio-activity was superior to that of their purified fractions due to the additive or synergistic activity (Kamatu et al., 2006). The development of bacterial resistance to multiple plant constituents may be slower than to single entities as such this might be considered as another advantage for using extracts rather than pure compounds as antimicrobial agents. Currently, typhoid fever is a problem in African continent with limited success of treatment with antimicrobial agents thereby necessitate for search for other agent in form of herbal product for curing this disease. In comparison of the control antibiotics (chloramphenicol and ciprofloxacin) the activity of *V. doniana* and *A. boonei* is the most encouraging among the plant extracts tested to some extents that it will be necessary to go into more detail studies to fine out the bioactive constituent of these plants.

The result of the present investigation emphasizes the usefulness of *V. doniana*, *A. boonei, S. jamaicensis* and *C. tora* in the treatment of typhoid fever infection and the need to enhance its exploitation in this regard and suggest the immediate stoppage of the use of *C. papaya* in the treatment of *S. typhi* since this plants showed no anti-typhoid activity as claimed by the traditional medical practitioner. Also the remarkable anti- typhoid activity observed with *V. doniana* and *A. boonei* is of particular urgent interest considering the rate of multi-drug resistance strains of *S. typhi* and the fact that typhoid fever ranks high as one of the most common ailments among all age groups in the under developed countries.

In conclusion, *V. doniana* and *A. boonei* showed good anti-typhoid activity against *Salmonella typhi* and we recommend for further exploration on their bioactive compounds and there subsequent development for use in the treatment of multi-drug resistance *S. typhi* strains.

REFERENCES

Al-Bakri AG, Bustanji Y, Yousef A (2005). Common comsumption of antibacterial drugs with the Jordanien population: sources, patterns and appropriateness. Int. J. Antimicrob. Agents, 28: 389-95.

Ajayi AO, Akintola TA (2010). Evaluation of antibacterial activity of some medicinal plants on common enteric food-borne pathogens. Afr. J. Microbiol. Res., 4(4): 314-316.

Ayogu TE, Amadi ES (2009). Studies on the antibacterial activities of medicinal plants on typhoid fever organism. Int. J. Third World Med., 7(2): 1-4.

Adegoke EA, Akinsanya A, Nagri SHZ (1968). Studies of Nigerian medicinal plants: A preliminary Survey of plant alkaloids J. West Afr. Sci. Assoc., 13: 13-17.

Bever BO (1986). Anti-infective activity of chemical components of higher plants. Medicinal plants research of tropical West Africa. Cambridge University Press, Nigeria., p. 53.

Centers for Disease Control and Prevention (CDC) (2003). Travelers health. Preventing typhoid fever: a guide for travelers. Atlanta (http://www.cdc.gov/travel/disease/typhoid.htm).

Chessbrough M (2006). District laboratory practice in tropical countries Part two, 2nd Edn. Cambridge University Press, UK., 123-201.

Erdogrul OT (2002). Antibacterial activity of some plant extracts used in folk medicine. Pharm. Microbial., 40: 269-273.

Gupta MP, Solis PN, Calderon AI, Guionneau Sinclair F, Correa C, galdames C, Guerra C, Espinosa A, Alvenda GI, Robles G, Ocampo R (2005). Medical ethnobotany of the Teribes of Bocas del Toro, Panama. J. Ethanopharmacol., 96: 389-401.

Hashish MN, Gomaa NF (2003). The inhibitory effects of garlic (*Allium sativa*) on growth of some microorganisms, J. Egyt. Pub. Health Assoc., 78(5-6): 361-72.

Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, JIMI-Omojola J (2004). *In-vitro* antimicrobial properties of aqueous garlic extract multi-drug resistant bacteria and *Candida* species from Nigeria. J. Med. Food, 7(3): 327-333.

Kamatu GP, Viljoen A, Van Vuuren SF, Van Zye RL (2006). *In- vitro* evidence of antimicrobial synergy between *Savia chamelaeagea* and *Leontis leonurus*. S. Afr. J. Bot., 72: 634-6.

Miller SI, Hohmann EL, Pegues DA (1994). *Salmonella* (including *Salmonella typhi*). In principles and practice of infectious diseases Vol. 2 4th ed. Edited by: Mandell GL, Bennet JR, Dolin R. New York: Livingstone, pp. 2013-2033.

Okafor GC (2001). Predictive values of plants taxonomy in ethno-medicine: a paper presented at a workshop on medicinal plants of Nigeria organized by the Association for Conservation, Utilization and Preservation of medicinal plants of Nigeria (ASICUMPON), Enugu May.

Olukoya DK, Idika N, Odugbemi T (1993). Antibacterial activity of some medicinal plants from Nigeria. J. Ethanopharmacol., 39: 69-72.

Papadimitropoulous V, Vergidis PI, Bliziotis I, Falagas ME (2004). Vaccination against fever in travelers: a cost-effectiveness approach. Clin. Microbiol. Infect., 10: 681-683.

Rodders J, Speedie M, Tyler V (1996). Pharmacognosy and harmacobiotecknology. Baltimore: Williams and Wilkins pp., 7-12.

Taylor DN, Pollard RA, Blake PA (1983). Typhoid in the United States and the risk to the international travel. J. Infect. Dis., 148: 599-602.