Journal of Scientific Research & Reports
4(5): 441-449, 2015; Article no.JSRR.2015.046
ISSN: 2320-0227

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Antibacterial and Phytochemical Properties of some Nigerian Medicinal Plants on *Salmonella typhi* and *Salmonella paratyphi* Isolated from Human Stool in Owo local Government, Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OTO research into the antimicrobial and phytochemical properties of various medicinal plants in Nigerian and Africa and design the materials and methods used in the course of the research work. Author FAO helps in proof reading, constructive criticism of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2015/12021
Editor(s):
(1) Luis H. Alvarez Valencia, Laboratory of environmental biotechnology and microbiology, CIIBAA Instituto Tecnológico de Sonora (ITSON), Obregon Sonora, México.
Reviewers:
(1) Anonymous, Industrial Research, Karachi, Pakistan.
(2) Coulibaly Kalpy Julien, Department of Bacterioly and virology; Pasteur Institute of Côte d'Ivoire; Cote d'Ivoire.
(3) Anonymous, India.
(4) Anonymous, University of Porto, Portugal.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=744&id=22&aid=6691

Original Research Article

Received 13th June 2014
Accepted 16th September 2014
Published 24th October 2014

ABSTRACT

Medicinal plants are geared toward the development of the new antibiotics and the use of the medicinal plant in the treatment of typhoid fever. Typhoid fever is a bacterial infection caused by Salmonella species namely, *Salmonella typhi* and *Salmonella paratyphi*. The disease is an important menace to public health in Nigeria and other developing country. The main objective of the study was to test the potency of some Nigerian medicinal plants against *Salmonella typhi* and *Salmonella paratyphi* and to determine the phytochemical constituents of the plants' extracts on test organisms isolated from infected human stool in Owo Local Government. The plants were obtained from Oke-Ako in Ikole Local Government of Ekiti State, Nigeria and prepared for

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extraction with four extracting medium with a simple distillation procedure. Test organisms were isolated from infected patients in the Federal Medical Centre, Owo, Ondo State and were identified using morphological and biochemical characteristics. Antibacterial assay (using the Agar diffusion method) and phytochemical assays were carried out on the plants extract. The test organisms were observed to be sensitive to all four plant extracts (*Alchornea laxiflora, Ageratum conyzoides, Spondia mombin* and *Cymbopogon citarus*). Phytochemical screenings show that the plants contained some classical compounds namely tannin, phenol, alkaloid, flavonoids, oxalate, saponin and phytate. Minerals present in plant extracts (mg/100.0g) were sodium, potassium, calcium, magnesium, zinc, iron, lead, copper, manganese and phosphorus. This research showed that *Alchornea laxiflora, Ageratum conyzoides, Spondia mombin* and *Cymbopogon citarus* were effective against *Salmonella typhi* and *Salmonella paratyphi* and treatment against typhoid fever infection using medicinal plants should be encouraged.

Keywords: Salmonella species (Salmonella typhi and Salmonella paratyphi); antibacterial assay; phytochemical screening; minerals composition; nutrient and anti-nutrient composition.

1. INTRODUCTION

Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. Roughly 50,000 species of higher plants (about 1 in 6 of all species) have been used medicinally. Around 100 plant species have contributed significantly to modern drugs. The use of medicinal plants is increasing worldwide, in direct relation to the persistence, expansion and growing interest in traditional medicine and herbal treatments (Al-Bakri et al. [1]). Many plants produce special substances in their roots, leaves, flowers or seeds that help them to survive. For example, some plants make nasty-tasting substances to defend themselves against plant-eating animals. Since earliest times, people have gathered these substances to create herbal medicines to treat certain diseases. Many of the powerful drugs used in modern medicines are of plant origin. Today's plant-based drugs treat a range of diseases, from headaches to cancer (Wisegeek, [2].

2. *SALMONELLA*

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enteric bacteria with diameters around 0.7 to 1.5µm, lengths from 2 to 5µm and flagella that move in all directions. They are facultative anaerobic bacteria and obtain their energy from oxidation and reduction reactions using organic sources. Most species of *Salmonella* produce Hydrogen Sulphide (Jantsch et al. [3]) which can readily be detected by growing them on media containing ferrous sulfate.

Free-ranging birds, flies and rats may serve as vectors of *Salmonella* species. The incidence of various *Salmonella* species seems to vary with geographic location and the types of food consumed. Imported animals may introduce different species of *Salmonella* to new areas with the possibility of causing devastating outbreaks.

2.1 *Salmonella* as Disease-causing Agents

Salmonella infections are zoonotic infections which are due to ingestion of contaminated foods (Rothschild et al. [4]). In speaking of other *Salmonella* serotypes, enteritis *Salmonella* and *Salmonella typhoid/paratyphoid Salmonella*, the latter-because of a special virulence factor and a capsule protein (Virulence antigen)-can cause serious illnessess, such as *Salmonella* enteric subsp. enteric serovar typhi. *Salmonella typhi* is adapted to humans and does not occur in other animals (Kerr et al. Jantsch et al. [3]).

2.1.1 Typhoid/ Enteric fever

Infection by *Salmonella typhi* leads to the development of typhoid or enteric fever. The symptoms of typhoid or enteric fever include the sudden onset of a sustained and systemic fever, severe headache, nausea and loss of appetite. Other symptoms include constipation or diarrhea, enlargement of the spleen, possible development of meningitis and general malaise. Untreated typhoid fever may results in the increase of the mortality rates, ranging from 12-30% while treated cases lower the mortality rate up to 99% survival.

2.1.2 Brief history on *Ageratum conyzoides*

Ageratum conyzoides L is an annual herbaceous plant with a long history of traditional medicinal, insecticidal and nematocidal uses in several countries of the world (Jantsch et al. [3]).

2.1.3 Botanical description

Ageratum is derived from the Greek "*a geras*", meaning non-aging, referring to the longevity of the flowers or the whole plant. The specific epithet "*conyzoides*" is derived from "kónyz," the Greek name of *Inula helenium,* which resembles *Ageratum* ranges from Southeastern North America to Central America, but the center of origin is in Central America and the Caribbean. Most taxa are found in Mexico, Central America, the Caribbean and Florida. *Ageratum conyzoides* has now been found in several countries in tropical and sub-tropical regions, including Brazil (Correa et al. Correa, Cruz, Lorenzi, [5,6,7,8]).

Ageratum conyzoides is an erect, herbaceous annual, 30 to 80cm tall; stems are covered with fine white hairs, leaves are opposite composition, pubescent with long petioles and include glandular trichomes. The inflorescences contain 30 to 50 pink flowers arranged as a corymb and are self-incompatible (Kaul and Neelangini, [9]). The fruit is an achene with an aristatepappus and is easily dispersed by wind. In some countries the species is considered a weed and control is often difficult (Johnson, Paradkaretal, Lam et al. Waterhouse, Lorenzi, [10,11,12,8]).

2.1.4 Phytochemical characteristics of *Ageratum conzyoides*

There is high variability in the secondary metabolites of *Ageratum conzyoides* which include flavonoids, alkaloids, cumarins, essential oils and tannins (Edeoga et al. [13]. Many of these are biologically active. Essential oil yield varies from 0.02% to 0.16% (Jaccoud, [14]). These compounds have been shown to affect insect development as anti-juvenile hormones, resulting in sterile adults (Kamal and Mehra, [15]). Ekundayo [16] identified 51 terpenoid compounds, including precocene I and precocene II. Gonzales *et al* (1999)[17] found 11 cromenes in essential oils, including a new cromene, 6-angeloyloky-7-methoxy-2,2-dimethylcromen.

2.1.5 Brief history on *Spondia mombin*

Spondia is a genus of flowery plant in the cashew family, *Anacardiaceae*. The genus consists of seventeen described species, seven of which are native to the Neo-tropics and about 10 are native to tropical Asia. They are commonly named as hog plums, Spanish plums, libas in Bikol and in some cases golden apples (because of their brightly colored fruit which resemble an apple or large plum at a casual glance). They are only distantly related to apple and plum trees, however. A more unequivocal common name is mombin (Aiyeloja et al. [18]).

About ten species of *Spondia* bear edible fruits and have been domesticated for fruit production. These fruits are also consumed by herbivorous mammals such as deer (Ayoka et al. Oladele et al. [19,20]).

2.1.6 Brief historyon *Cymbopogon citarus*

Cymbopogon (*lemongrass*) is a genus of about 55 species of grasses among which is *Cymbopogon citratus* (a natural and soft tea anxiolytic). This tall perennial grass is native to temperate and tropical regions of the Old World and Oceania. Common names include lemon grass, barbed wire grass, silky heads, citronella grass, cha de Dartigalongue, fever grass, tanglad, hierba Luisa or gavati chaha amongst many others (Sauerborn and Kock [21]). Lemon grass oil is used as a pesticide and preservatives. Research shows that lemongrass oil has anti-fungal properties (Shadab et al. [22]).

Despite its ability to repel insects, its oil is commonly utilized as a "lure" to attract honey bees. Lemongrass works conveniently as well as the pheromone created by the honeybee's nasonov gland, also known as attractant pheromones. Because of this, lemongrass oil can be used as a lure when trapping swarms or attempting to draw the attention of hived bees.

2.1.7 Brief history on *Alchornea laxiflora* and its uses

In Nigeria, a decoction of the leaves is taken to treat inflammatory and infectious diseases. It is also a common ingredient in herbal antimalarial preparations. In Tanzania, the ground leaves are taken in water to treat hernia (Ayoka et al. [19]).

while the leaves are used as packing and preservation material for kolanuts in Nigeria. The small branches are used as chewing sticks, while the straight stems are used as fence poles (Dalziel [23].

3. MATERIALS AND METHODS

3.1 Collection of Plant Samples

Fresh leaves of the plants were collected from the reserved forest of Ipaho/Oke-Ako, Ekiti State, Nigeria. The samples were authenticated at the Department of Botany, Ekiti State University, Ado-Ekiti. These were washed with distilled water for extraction and then stored in air-tight containers and kept at room temperature until needed (El Astal et al. [24]).

3.2 List of Plant used with their Local Name, Common Name and Botanical Name of Medicinal Plants under Study

Local Name	Common Name	Botanical name	Plant part used
Imiesu	Goat weed	*Ageratum conyzoides*	Leaf
Ewe Tea	Lemon Grass	*Cymbopogon citarus*	Leaf
Ewe Iya	Benth	*Alchornea laxiflora*	Leaf
Iyeye	Hog Plum	*Spondia mombin*	Leaf

3.3 Preparation and Extraction of Plant Materials

Two hundred and fifty gram of each sample was weighed into 1000ml conical flasks, labeling each according to the name of each plant. These were cold exhausted with sterile distilled water, absolute ethanol and ethyl acetate (Olukoya et al. Akinside and Olukoya, Akinyemi et al. [25,26, 27]).

This was done for nine days (9 days), after which each sample was filtered using a Whatman filter paper. The simple distillation procedure was performed on the filterates in order to get the plant extracts from the various extraction media used.

3.4 Preparation of Plants' Extracts (Ethanol, Aqueous and Ethyl Acetate)

Two hundred and fifty grams of each sample was separately soaked in 750ml of ethanol in 1000ml conical flasks for 9 days. The extracts were filtered through Whatman filter paper into different sterile crucibles.

3.5 Procedure of Simple Distillation

Samples were poured into a reaction bottle and placed on water bath. Samples were boiled at different temperatures, depending on the extracting medium used. For ethanol 78ºC, Ethyl acetate 55ºC and distilled water 100ºC. The media used distills out, leaving remains of extracts. The remains were then poured into an open mouth beaker and placed in hot air oven at 105ºC, the extracting media dries off leaving the plant extracts.

3.6 Collection of Samples

Stool samples were collected from patients attending the Federal medical Centre, Owo, Ondo State, Nigeria using swab sticks. Ten samples were collected all together designated by numbering each swab stick from number one to ten and were inoculated on an already prepared media which include MacConkey agar and Chocolate agar. The plates were incubated at 35ºC for 24 hours.

4. BIOCHEMICAL TESTS

Biochemical tests were carried out in order to further identify the isolates. Identification of microorganisms was based on microscopic appearance and biochemical characteristics.

4.1 Antimicrobial Assay (Determination of Zones of Inhibition using Agar Diffusion)

Twenty five milliliters of Kliger iron agar was poured into sterile petri dishes and allowed to set. Standardized test bacterial cultures were inoculated into the sterile Kliger iron agar plate using sterile cotton swab. A sterile cork borer of 6mm diameter was used to punch wells on the agar on each of the petri dishes. Three holes were made on the surface of the plate with one in the center to serve as the control. Each hole was

labeled (A, B, C, D) representing a particular concentration of 20mg/ml, 40mg/ml and 60mg/ml.

The dishes were then filled into the wells with three drop of their respective type of extract according to the labeled format. The central well containing the diluent reagent (DMSO) was used as control. The process was carried out for each extract and the inoculated petri dishes were left for few minutes for extract of diffuse into agar. The plates were incubated at 37ºC for 18-24 hours after which the zones of inhibition (if any) were measured.

5. PHYTOCHEMICAL SCREENING METHODS

The extracts were analysed for the presence of alkaloid, glycosides, tannins, saponins, anthraquinones, anthocyanosides, flavonoids and reducing sugars (Sofowora, [28]).

6. RESULTS

The result obtained during the course of the research work were represented Table 1,2,3,4,5,6,7, which is as follows.

7. DISCUSSION

This present finding indicates that there is evidence of antibacterial activities of plants under study (*Alchornea laxiflora, Ageratum conyzoides, Spondia mombin* and *Cymbopogon citarus*) on *Salmonella typhi* and *Salmonella paratyphi.*

The findings from the research indicates that *Ageratum conyzoides*, *Cymbopogon citarus*, *Alchornea laxiflora*, *Spondia mombin* had more pronounced antibacterial activity against *Salmonella paratyphi* in aqueous extraction compared to that of *Salmonella typh*i (Kamatul et al*.* [29]).

Ethyl acetate extracts of *Cymbopogon citarus* were observed to have lesser antimicrobial activity against *Salmonella typhi* and *Salmonella paratyphi*, compared to the other three plants: *Alchornea laxiflora, Spondia mombin* and *Ageratum conyzoides.* This shows that the other three plants are potential agents for the treatment of Salmonella infections like typhoid fever and *salmonellasis* (Kamatul et al. Kilani, [29,30]).

Table 1. Antibacterial effect of aqueous extracts at 20mg/ml, 40mg/mland 60mg/ml concentrations

	Salmonella typhi			*Salmonella paratyphi*		
Extract	**20mg/ml**	**40mg/ml**	**60mg/ml**	**20mg/ml**	**40mg/ml**	**60mg/ml**
Ageratum conyzoides	7.0	13.0	15.0	10.0	15.0	17.0
Cymbopogon citarus	5.0	10.0	13.0	8.0	10.0	15.0
Alchornea laxiflora	6.0	7.0	8.0	7.0	10.0	11.0
Spondia mombin	5.0	11.0	15.0	6.0	14.0	16.0

The unit for zone of inhibition is mm

Table 2. Antibacterial effect of ethyl acetate extracts at 20mg/ml, 40mg/ml and 60mg/ml concentrations

	Salmonella typhi			*Salmonella paratyphi*		
Extracts	**20mg/ml**	**40mg/ml**	**60mg/ml**	**20mg/ml**	**40mg/ml**	**60mg/ml**
Ageratum conyzoides	15.0	17.0	21.0	10.0	13.0	5.0
Cymbopogon citarus	5.0	8.0	11.0	4.0	6.0	9.0
Alchornea laxiflora	12.0	14.0	24.0	9.0	14.0	19.0
Spondia mombin	21.0	23.0	25.0	19.0	16.0	18.0

The unit for zone of inhibition is mm

Table 3. Antibacterial effect of ethanol extracts at 20mg/ml, 40mg/ml and 60mg/ml concentrations

	Salmonella typhi			*Salmonella paratyphi*		
Extracts	**20mg/ml**	**40mg/ml**	**60mg/ml**	**20mg/ml**	**40mg/ml**	**60mg/ml**
Ageratum conyzoides	18.0	19.0	24.0	20.0	22.0	26.0
Cymbopogon citarus	15.0	16.0	17.0	14.0	18.0	20.0
Alchornea laxiflora	4.0	6.0	8.0	6.0	9.0	10.0
Spondia mombin	20.0	24.0	26.0	21.0	26.0	29.0

The unit for zone of inhibition is mm

Table 4. Phytochemical screening results

Plant	Cardiac-Glucoside	Tannin	Phenol	Alkanoid	Saponin	Phibatanin	Steroid	Flavonoids
Alchornea laxiflora	+ve	+ve	+ve	+ve	+ve	+ve	±ve	+ve
Ageratum conyzoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Spondia mombin	+ve	+ve	+ve	+ve	+ve	+ve	±ve	+ve
Cymbopogon citarus	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

KEY:+ve = Presence of Constituents; -ve = Absence of constituents

Table 5. Minerals present in plant extract (mg /100grams) result

Plant used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
Alchornealaxiflora	19.82	24.77	29.49	24.21	36.10	6.53	ND	0.02	5.45	35.78
Ageratum conyzoides	20.33	41.21	15.50	25.37	18.75	4.36	ND	ND	15.33	85.43
Spondiamombin	21.37	30.54	23.55	19.67	17.58	10.21	ND	0.03	25.37	97.65
Cymbopogoncitarus	7.35	5.24	3.55	12.50	8.73	0.50	ND	ND	5.33	6.38

Table 6. Anti–nutrients present in plant extracts result in percentage (%)

Parameters	Plant used							
	Alchornea laxiflora		***Ageratum conyzoides***		***Spondia mombin***		***Cymobogon citarus***	
	Sample A	**Sample B**	**Sample A**	**Sample B**	**Sample A**	**Sample B**	**Sample A**	**Sample B**
Tannin	2.20	2.10	2.32	2.37	2.30	2.25	ND	ND
Phenol	3.50	3.55	2.50	2.47	3.42	3.47	ND	ND
Phylate	17.30	17.27	15.65	15.71	12.36	12.42	1.25	1.30
Oxalate	3.69	3.70	6.57	6.55	8.55	8.59	1.50	2.00
Saponin	13.89	14.01	9.71	9.75	7.51	7.60	ND	ND
Flavonoid	8.53	8.55	6.49	6.55	10.32	10.40	ND	ND
Alkaloids	1.23	1.25	4.25	4.31	4.36	4.37	ND	ND

KEY: ND =Not Detected

Table 7. Nutrient Composition percentage of the plant extracts (%)

Plant used	Ash	MC	CP	Fat	Fibre	CHO
Sample A *Alchornea laxiflora*	11.26	5.70	16.25	5.36	8.47	52.96
Sample B *Alchornea laxiflora*	11.24	5.68	16.30	5.37	8.50	52.91
Sample A *Ageratum conyzoides*	10.30	5.72	15.46	3.68	7.33	57.51
Sample B *Ageratum conyzoides*	9.75	6.10	15.49	4.25	6.75	57.66
Sample *A* *Spondia mombin*	7.25	5.36	14.75	6.38	5.32	60.94
Sample B *Spondia mombin*	7.30	5.34	15.32	6.40	5.29	60.35
Sample A *Cymbopogon citarus*	5.27	4.67	5.38	0.25	12.46	72.07
Sample B *Cymbopogon citarus*	5.31	5.03	5.17	0.22	11.89	72.38

KEYS: CP= Crude Protein, MC= Moisture content ,CHO= Carbohydrate

In alcohol extraction, it was observed that plant extracts have a wider range of zone of inhibition, compare to the plant extracts of ethyl acetate and distilled water. *Salmonella paratyphi* is most susceptible in alcoholic plant extraction while *Salmonella typhi* is less susceptible to the alcoholic plant extraction. During this research work, it was observed that the ethanol plant extracts exhibited the highest antimicrobial properties against the tested organisms. This was due to the differences in the type and concentrations of the secondary metabolites across different plants which were extracted, a function of resultant variable in the antimicrobial activity of the plants. The quantitative and qualitative differences in constituents were also influenced by method of extraction and environmental factors such as the availability of water for the plants, relative humidity and type of soil.

The phytochemical screenings of *Alchornea laxiflora, Ageratum conyzoides,* was observed to contain tannin, phenol, alkaloid, saponin, phibatannin and flavonoid. This result shows that these plants are not harmful to the system. Tannins are widely distributed in many species of plants, where they play a role in protection from predation and in plant growth regulation. Tannin helps in blocking the activity of cancer causing agent and helps to inhibit hormone related cancer such as the ovarian cancer, phenol helps to protect the human system against cancer in the stomach, while flavonoid helps in the adsorption of vitamin C and its prevention from oxidation (Lisa, [31]). These are the functions of some of the phytochemicals.

The proximate analysis of the samples *Alchornea laxiflora, Ageratum conyzoides, Spondia mombin, Cymbopogon citarus* showed that ash, moisture content, crude protein, fat, carbohydrate and fibre were present in the four tested plants, which shows that the plant contain some vital nutrient that can support life.

The analysis of mineral compositions of *Alchornea laxiflora, Ageratum conyzoides, Spondia mombin* and *Cymbopogon citarus* showed that Na, K, Ca, Mg and Zn were present in large quantities and Fe, Cu and Mn in lesser quantity, while Pb was not present in any of the plants.

8. CONCLUSION

Medicinal plants are used in many parts of Nigeria for the treatment of infections. The four plants under study showed various degrees of antibacterial and phytochemical properties, making them potential agents for the treatment of *Salmonella*-related infections. Thus, research on other plants' extracts should be encouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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