In Vitro Antibacterial Potentials and Synergistic Effect of South-Western Nigerian Plant Parts Used in Folklore Remedy for *Salmonella typhi* infection

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Abstract: In the present study, antibacterial potency and synergistic effect of crude aqueous and methanolic extracts of nine plant parts against multi-drug resistant *S. typhi* were investigated and compared. *Salmonella typhi* isolated from patients suffering from typhoid fever in University of Ado-Ekiti Teaching Hospital, Nigeria was tested against nine plant parts: unripe C*arica papaya* fruit, *Citrus aurantifoliia*, *Anana sativus*, *Citrus paradisi*, *Cymbopogon citratus*, *Cocos nucifera* chaffs, brown leaves of *Carica papaya*, leaves *of Euphorbia heterophylla* and *Gossypium* spp. The antibacterial activities of the extracts, individually and in combination were determined using agar diffusion method and the minimum inhibitory concentration (MIC) carried out by agar dilution technique. Both the aqueous and methanol extracts of each plant material and mixture showed appreciable antimicrobial activities on *S. typhi.* Antimicrobial activity increased with increasing concentration of the extracts. Synergistic activity of crude aqueous and crude methanolic extracts of the plant parts, in various combinations of two to nine against the test organism ranged from 10-33mm zone of growth inhibition. The antibacterial efficacy of the mixture of extracts from plant parts increased considerably compared to the low activities recorded with the extract of individual plant parts ($P>0.05$). Methanolic extracts of each plant material and mixture produced greater antimicrobial activity than the aqueous extracts at all concentrations. The minimum inhibitory concentration (MIC) of the individual plant parts ranged between 0.1 and 1.0mg/ml in aqueous extracts and 0.01 and 0.1mg/ml in methanol extracts while the MICs of the combined extracts ranged between 0.1 and 0.01mg/ml in aqueous extracts and 0.01 and 0.0001mg/ml in methanolic extracts. The combined or synergistic activity of the plant parts compared favorably with the standard antibiotics of choice for salmonella-infections therapy, and contained two or more phytochemicals responsible for their antimicrobial activities. The plant materials possessed antimicrobial activity with greater efficacy when used synergistically on the test organism. There is the need therefore to develop effective combination of antimicrobial agents in purified form from higher plants and their parts for clinical trials. [Nature and Science 2010;8(9):52-59]. (ISSN: 1545-0740).

Keywords: Antimicrobial activity, aqueous extract, methanolic extract, *Salmonella typhi,* typhoid fever, phytochemicals.

INTRODUCTION

The use of plants for medicinal purposes dates back to antiquity (Oluma, 2004) and has been very important in the health care delivery of every nation at one stage or another. Recent research has focused on natural plant product as alternatives to the existing drugs for disease remedy in developing countries (Aiyegoro *et al.,* 2007). Plant–derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources of agents to fight microbial diseases (Mohana *et al.*, 2008: Ghaleb *et al.*, 2009; Ajayi and Akintola, 2010).

The development of multiple antibiotic resistance organisms has constituted a global problem as far as treatment of some infectious diseases is concerned. Typhoid fever caused by *Salmonella typhi* has since 1989 developed simultaneously, resistance to conventional antibiotics of choice in several endemic areas (Greenwood *et al.*, 2009). Vehicle of transmission of this etiologic agent are mainly food and water. Many disease-causing organisms of medical importance have developed resistance to antibiotics.

Infectious disease still remains an important cause of morbidity and mortality in man, especially in developing countries. Today, in Africa, many resort to the use of locally made herbal preparations (infusion, decoction or concoction and tincture) as an alternative therapy for salmonella-infections. Thus, the study sought to evaluate the phytochemistry and synergistic antibacterial potential of some herbal preparations from nine different plant Parts used in south-western Nigeria for the traditional treatment of typhoid fever. The plant parts discussed in this study

include unripe C*arica papaya* (Pawpaw), *Citrus aurantifolia* (lime), *Ananas sativus* (Pineapple), *Citrus paradisi* (Grape), *Cymbogogon citratus* (lemon grass), *Cocos nucifera* (coconut) chaffs, brown leave of *Carica papaya*, *Euphorbia heterophylla* (commonly called Jèbbá) and leave of *Gossypium* spp (Cotton wool plant).

The therapeutic use of some of these plants in certain ailments has been reported. The use of leaves and flowers of *Gossypium* species against intestinal parasites and worms (George and Pamplona-Roger, 1998); antibacterial and anti-inflammatory effect of *Cymbopogon citratus* (George and Pamplona-Roger, 1998); therapeutic use of *Cocos nucifera* chaffs decoction against various bacterial, viral and protozoal disease; and the use of leaves and latex of unripe *Carica papaya* for the treatment of chronic gastroenteritis or pancreatitis and against intestinal parasites especially tapeworm (George and Pamplona-Roger, 1998). The antiseptic, diuretic and antibacterial properties of unripe *Citrus Paradisi* and *Ananas sativus have* been equally reported (Alanis *et al.,* 2005).

The present study therefore investigated and compared the antibacterial activities of aqueous and methanolic extracts of nine different plant parts against *S. typhi,* the causative agent of typhoid fever.

Materials and Methods

Plants materials

The plant materials used for the study include *Cymbopogon citratus* (Lemon grass), unripe *Carica papaya* fruit (Pawpaw), unripe *Ananas sativus* (Pineapple), *Citrus paradisi*, (grape), *Cocus nucifera* chaffs (coconut chaff), leaves of *Gossypium* spp (cotton plant) and *Euphorbia heterophylla (*vernacularly called Jèbbá leaves). Plant materials were all collected from the nearby farm around the All Souls Cathedral Church, University of Ado-Ekiti, Nigeria in the month of November, 2008. The plant materials were identified and confirmed at the herbarium unit of the Department of Plant Science, University of Ado-Ekiti, Nigeria.

Preparation of Extracts

Collected Plant materials were washed with sterile water and allowed to drain. Extracts from 10, 20 and 30g of each of the plants materials were obtained separately using 60% methanol (methanolic extraction) and 100ml of water (aqueous extraction). Obtained extracts were filtered using Whatman No. 2 filter paper under suction. Extracts obtained were then concentrated in vacuum using a rotary evaporator.

One hundred miligrammes of the obtained residue were further dissolved in 1ml of the appropriate diluents.

Microorganism

The test organism, *Salmonella typhi* was isolated from faecal samples of patients who were clinically diagnosed of typhoid fever at the University of Ado-Ekiti Teaching Hospital, Ado-Ekiti, Nigeria. The organism was cultured on Salmonella/ Shigella agar and characterized according to Barrow and Feltham, (2004).

Susceptibility of bacteria to plant extracts and standard antibiotics.

Susceptibility of the test isolate to the plant extracts was determined using agar well diffusion method described by Chen *et al* (1997).The bacterial isolate was first enriched in nutrient broth for 18h before use. Using sterile swab sticks, plates were seeded with 1ml of suspension of the test isolate containing approximately 10^5 cells. Seeded plates were allowed to stand for a while at room temperature before wells were bored on them using cork borer (6mm diameter). Each of the bored wells was filled with 5µl of each plant extract. To demonstrate synergism, a combined volume of 5µl mixture of plant extracts was added into each well. Ccommercially available disks of standard antibiotics commonly employed in clinical treatment of salmonella infections (amoxicillin-25µg, ofloxacin-5µg, chloramphenicol-30µg, ciprofloxacin-10µg, perfloxacin-5µg and gentamycin 5µg) were placed and pressed firmly on another set of seeded plates

The plates were allowed to stand on the laboratory bench for I hour to allow proper diffusion of the extract into the media. The two sets of plates were incubated at 37^0C for 24h and the diameters of zones of inhibition measured.

Minimum Inhibitory Concentration (MIC).

The MIC of the aqueous and methanol extracts of each plant material and mixture was determined as described by Akinpelu and Kolawole (2004). Two-fold serial dilutions of the plant extracts were prepared, from which 2mls aliquots was taken and added to 18ml of pre-sterilized molten nutrient agar at a temperature of 40^0C.

The media were then poured into sterile Petri dishes and allowed to solidify. The surfaces of the media were allowed to dry before streaking with 18h old cultures of the test bacterium. The plates were later incubated in an incubator at 37^0C for up to 72hours after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented bacterial growth.

Phytochemistry of the plant extracts.

The extract of individual plant materials was screened for the presence of some phytochemical compounds as described by Trease and Evans (1983) and Harborne (1998).

Statistical Analysis

Data were subjected to analysis of variance and Students't-tests using the Statistical Package for Social Sciences (SPSS version 10).

RESULTS

Both aqueous and methanolic extracts of each plant material showed antimicrobial activity on the test inoculum (Table 1).The test pathogen was more susceptible to the methanol extract of each plant material than the aqueous extract at all concentrations. The highest susceptibility was recorded with the methanol extract of *Citrus aurantifolia* at 30mg/ml of the extract, followed by the extracts of unripe *Carica papaya* and *Euphorbia heterophylla*, and the least, being recorded with the extract of unripe *Citrus paradisi.* The susceptibility of the test inoculum to the extract of individual plant material increased with increasing concentration of the extract (Table 1).

The antibacterial efficacy of the extracts of mixture of the plant materials was very high compared to low activities recorded with the extract of each plant material (P>0.05) (Table 2). Similarly, the methanol extract of mixture of different plant parts produced more appreciable activity on the test pathogen than the aqueous extract at all concentrations. The antimicrobial activities vary with the number and type of plant materials in the mixture. A zone of growth inhibition of 19mm was produced with the extract of mixture containing three different plant parts (unripe *Carica papaya* fruit, *Euphorbia heterophylla* and *Carica papaya* brown brown leaves) and 24mm with the mixture of four different plant materials (Unripe *Carica papaya* fruit, *Citrus paradisi, Gossypium* spp and unripe *Citrus paradisi*) at 30mg/ml of the extract.

The susceptibility of the test pathogen to the extracts of mixture increased radically with increase in the number of the plant parts, with the highest susceptibility recorded in methanol extract of mixture containing all the nine plant materials (Table 2). The minimum inhibitory concentration (MIC) of the plant materials ranged between 1.0 and 0.1mg/ml in aqueous extracts and between 0.1 and 0.001mg/ml in methanol extracts (Table 3a). Meanwhile, the MICs of the mixture ranged between 0.1 and 0.01mg/ml in aqueous extracts and between 0.01 and 0.0001mg/ml in methanol extracts (Tables 3b). Table 4 reveals the antibacterial effectiveness of the plant materials as was compared with the choicest commercially prepared antibiotics routinely used for salmonella-infection therapy.

Phytochemical screening of the extracts of the plant materials revealed the presence of one or more of the following phytochemical components; alkaloids, tannin, saponin, phytates, cardiac glycosides, anthraquinones and flavonoids (table 5).

Table 1: Antibacterial activities of aqueous and methanolic extracts of each plant material against Salmonella typhi.

Plant part Concentration	**Inhibition zone(mm)**					
	Aqueous extract			**Methanolic extract**		
	10mg/ml	**20mg/ml**	**30mg/ml**	**10mg/ml**	**20mg/ml**	**30mg/ml**
Unripe *Carica papaya* fruit	4	5	10	7	13	16
Citrus aurantifolia	6	8	11	9	10	18
Gossypium spp leaves	4	7	10	6	8	15
Cocus nucifera chaffs	3	6	9	7	9	14
Unripe *Ananas sativus*	5	6	8	7	10	14
Unripe *Citrus Paradisi*	3	7	9	5	8	12
Cymbopogon citratus	5	6	11	8	9	15
Carica papaya brown leaves	5	6	10	9	11	15
Euphorbia heterophylla	4	5	9	11	13	16

Table 2: Synergistic antibacterial effects of the aqueous and methanolic extracts of mixture of the plant materials on *S. typhi*

Plant part (ratio1:1) Concentration	**Inhibition Zone(mm)**					
	Aqueous Extract			**Methanolic Extract**		
	10mg/ml	**20mg/ml**	**30mg/ml**	**10mg/ml**	**20mg/ml**	**30mg/ml**
Unripe Carica papaya fruit and Citrus aurantifolia.	10	13	16	15	17	21
Unripe Carica papaya fruit and Euphorbia heterophylla.	10	13	15	15	18	21

Unripe *Carica papaya* fruit, *Citrus aurantifolia*, and *G*ossypium spp leaves.	12	13	15	14	17	20
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, and *Carica papaya* brown leaves.	7	11	15	12	13	19
Carica papaya fruit, Citrus aurantifolia, Gossypium species leaves and unripe Citrus paradisi.	15	16	19	18	19	24
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, *Carica papaya* brown leaves and *Cymbopogon citratus*.	14	17	20	17	19	25
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, *Carica papaya* brown leaves, *Cymbopogon citratus* and *Citrus aurantifolia*.	14	18	21	17	19	24
Unripe *Carica papaya* fruit, *Citrus aurantifolia*, *Gossypium* species leaves, unripe *Citrus paradisi*, *Euphorbia heterophylla* and unripe *Ananas sativus*.	15	18	23	18	21	26
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, *Carica papaya* brown leaves, *Cymbopogon citratus*, *Citrus aurantifolia* and *Gossypium* species leaves.	14	17	23	19	20	26
Unripe *Carica, papaya* fruit, *Citrus aurantifolia*, *Gossypium* species leaves unripe *Ananas sativus*, *Cymbopogoon citratus*, unripe *Citrus paradisi*, *Euphorbia heterophylla*.	14	17	23	20	23	26
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, *Carica papaya* brown leaves, *Cymbopogon citratus*, *Citrus aurantifolia*, *Gosspypium* species leaves and *Cocus nucifera*.	16	20	24	22	24	29
Unripe *Carica papaya* fruit, *Citrus aurantifolia*, *Gossypium* species leaves, unripe *Citrus paradisi*, *Euphorbia heterophylla*, unripe *Ananas sativus*, *Cymbopogon citratus* and *Carica papaya* brown leaves.	17	20	26	24	26	30
Unripe *Carica papaya* fruit, *Euphorbia heterophylla*, *Carica papaya* brown leaves, *Cymbopogon citratus*, *Citrus aurantifolia*, *Gossypium* species leaves, *Cocus nucifera* chaffs and unripe *Citrus paradisi*.	17	21	25	24	26	30
Unripe *Carica Papaya* fruit, *Citrus aurantifolia*, *Gossypium* species leaves, *Cocus nucifera* chaffs and unripe *Citrus paradisi*.	19	22	27	24	28	33

Table 3(a). Minimum Inhibitory Concentration of the aqueous and methanolic extracts of the plant materials.

Plant part	**MIC (mg/ml)**	
	Aqueous extract	**Methanolic extract**
Unripe *Carica papaya* fruit	0.1	0.1
Citrus aurantifolia	0.1	0.01
Gossypium spp leaves	0.1	0.01

Cocus nucifera chaffs	1.0	0.1
Unripe *Ananas sativus*	1.0	0.1
Unripe *Citrus Paradisi*	0.1	0.01
Cymbopogon citratus	0.1	0.01
Carica papaya brown leaves	1.0	0.1
Euphorbia heterophylla	0.1	0.01

Table 3(b). Minimum Inhibitory Concentration of the aqueous and methanol extracts of mixture of the plant materials

Mixture of extract (ratio1:1)	MIC (mg/ml)	
	Aqueous extract	Methanolic extract
Unripe *Carica papaya* fruit and *Citrus aurantifolia.*	0.1	0.01
Unripe *Carica papaya* fruit and *Euphorbia heterophylla.*	0.1	0.01
Unripe *Carica papaya* fruit, *Citrus aurantifolia* and Gossypium spp leaves.	0.01	0.01
Unripe *Carica papaya* fruit, *Euphorbia heterophylla* and *Carica papaya* brown leaves.	0.01	0.01
Carica papaya fruit, *Citrus aurantifolia, Gossypium* species leaves and unripe *Citrus paradisi.*	0.01	0.01
Unripe *Carica papaya* fruit, *Euphorbia heterophylla, Carica papaya* brown leaves and *Cymbopogon citratus*.	0.01	0.01
Unripe *Carica papaya* fruit, *Euphorbia heterophylla, Carica papaya* brown leaves, *Cymbopogon citratus* and *Citrus aurantifolia.*	0.01	0.01
Unripe *Carica papaya* fruit, *Citrus aurantifolia, Gossypium* species leaves, unripe *Citrus paradisi, Euphorbia heterophylla and* unripe *Ananas sativus*.	0.01	0.001
Unripe *Carica papaya* fruit, *Euphorbia heterophylla, Carica papaya* brown leaves, Cymbopogon citratus, *Citrus aurantifolia and Gossypium* species leaves.	0.01	0.001
Unripe *Carica, papaya* fruit, *Citrus aurantifolia, Gossypium* species leaves, unripe *Ananas sativus Cymbopogoon citratus,* unripe *Citrus paradisi and Euphorbia heterophylla.*	0.01	0.001
Unripe *Carica papaya* fruit, *Euphorbia heterophylla, Carica papaya brown leaves, Cymbopogon citratus, Citrus aurantifolia, Gosspypium* species leaves *and Cocus nucifera.*	0.01	0.001
Unripe *Carica papaya* fruit, *Citrus aurantifolia, Gossypium* species leaves, unripe *Citrus paradisi, Euphorbia heterophylla,* unripe *Ananas sativus, Cymbopogon citratus and Carica papaya* brown leaves.	0.01	0.001
Unripe *Carica papaya* fruit, *Euphorbia heterophylla, Carica papaya* brown leaves, *Cymbopogon citratus, Citrus aurantifolia, Gossypium* species leaves, *Cocus nucifera* chaffs and unripe *Citrus paradisi.*	0.01	0.0001
Unripe *Carica Papaya* fruit, *Citrus aurantifolia, G*ossypium species leaves, *Cocus nucifera* chaffs and unripe *Citrus paradisi, Ananas sativus, Euphorbia heterophylla, Carica papaya* brown leaves, *Cymbopogon citratus*.	0.01	0.0001

Table 4. Susceptibility of *S. typhi* to Standard antibiotics

Antibiotic	**Zone of inhibition(mm)**
Amoxicillin 25µg	26
Ofloxacin 5µg	28
Chloramphenicol 30µg	30
Gentamycin µg	10
Pefloxacin 5µg	30
Ciprofloxacin 10µg	36

Table 5 Phytochemistry of the plant materials.

Plant parts	Phytochemical components						
	Alkaloids	Tannin	Saponin	Phytates	Cardiac glycosides	Anthraquinones	Flavonoids
Unripe *Carica papaya* fruit	+	+	+	+	+	-	+
Citrus aurantifolia	-	+	-	+	-	-	-

Gossypium spp leaves	+	-	+	+	-	-	-
Cocus nucifera chaffs	+	-	+	+	+	+	-
Unripe *Ananas sativus*	+	-	-	+		-	-
Unripe *Citrus Paradisi*	-	-	+	+	+	-	-
Cymbopogon citratus	+	+	+	+	-	-	-
Carica papaya brown leaves	+	+	-	+	-	-	-
Euphorbia heterophylla	+	+	+	+	-	-	-

Legends: + positive - not present

Discussion

The study shows that all the plants parts studied possess antimicrobial properties, with greater antimicrobial efficacy when used synergistically. This might be due to the resultant effect of the active agents in the plant materials. The antibacterial activities of all the extracts of the plants materials either when used separately or combined were concentration dependent as zone of growth inhibition increased with increasing concentration of the extracts. Ekwenye and Elegalam (2005) and Azu and Onyeagha (2007) reported that the efficacy of most plant extracts is concentration dependent.

The results of the MIC showed that the plant materials are very potent against the test pathogen even at a very low concentration of 0.01mg/ml when used separately and 0.0001mg/ml when used synergistically. Overall, the extracts of each of the plant materials and that of the mixture compared favourably with the choicest modern antibiotics for typhoid fever therapy. The presence of some phytochemicals in the plant extracts understudied is a function of their antimicrobial activities against the test pathogen as they play important roles in bioactivity of medicinal plants.

Phytochemicals exert antimicrobial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen binding or specific interactions with vital proteins such as enzymes in microbial cells. (Scalbert, 1991; Akinpelu *et al.*, 2008). Herbs that have tannins as their component are astringent in nature and are used for the treatment of gastrointestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). This may therefore explain the use of unripe *Carica papaya* fruit, *Citrus aurantifolia, Cymbopogon citratus, Euphorbia heterophylla and Carica papaya* brown leaves in folklore remedy for typhoid fever. Hence, supports the usefulness of the plant materials understudied in the treatment of other ailments caused by microorganisms.

Saponin, which is responsible for numerous pharmacological properties (Estrada *et al.*, 2000) was also tested positive in one or more of the plant materials examined. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects (Liu and Henkel, 2002). Alkaloid is present in all the plant materials used except unripe *Citrus paradisi* and *Citrus aurantifolia and* has been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms (Akinpelu *et al.*, 2008). Similarly, phytates which was present in all the extracts of the plant materials used in this study, posses great inhibitory properties hence, the antimicrobial activity observed against the test pathogen.

Generally, the present study has shown that all the plant materials tested possess a measure of antimicrobial properties and the antimicrobial potency is much greater when used synergistically against the test pathogen and is concentration dependent. The plant materials examined in the present study have been successfully in use among Yoruba tribe of South Western Nigeria in the Preparation of decoction and concoction for

the treatment of typhoid fever caused by the test pathogen particularly, when modern drugs of choice failed in achieving the therapy. This study has confirmed the antibacterial potentials of the plant materials, thus supporting their folklore application as a medical remedy for typhoid fever. With these, there is need for the preparation of different formulations towards ensuring acceptable dosing regime pursuant to clinical trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin for the treatment of salmonella-infections particularly typhoid fever among others.

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