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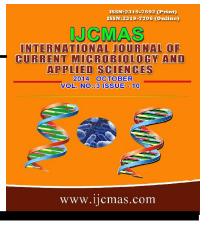

Original Research Article

Potentiating effect of aqueous leaf extract of *Anogeissus leiocarpus* on *Carica papaya* aqueous leaf extract and *Mangifera indica aqueous* stem bark extract - A herbal product used against typhoid fever in Nigeria

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A B S T R A C T

Various aqueous concentrations of (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) a*nogeissus leiocarpus* leaf extract (AL), *carica papaya* leaf extract (CP) and *mangifera indica* stem bark extract (MI) were separately tested for antibacterial activities against *salmonella typhi* (the causative agent of typhoid fever) and six other bacteria using punch hole diffusion method. These three plants' extract showed varying degrees of antibacterial activities as seen in the zones of inhibition of bacterial growth. Equal quantities of the plant extracts were mixed together as follows: AL+ CP, AL+MI, CP+MI and AL+CP+MI to get four herbal preparations. These herbal preparations were separately reconstituted in sterile distilled water to get concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. These were again tested against *salmonella typhi* (*s.typhi*) and six other bacteria by agar gel diffusion method and the zones of inhibition recorded. It was noted that the antibacterial activities of the mixture of the three plant extracts was greater than those of the individual extract and the herbal mixture that does not contain *anogeissus leiocarpus.* This inferred that *anogeissus leiocarpus* aqueous leaf extract had a potentiating effect on the antibacterial activities of the other two plant extracts.

Keywords

Anogeissus leiocarpus, Carica papaya, Mangifera indica, Antibacterial activities, Potentiating effects.

Introduction

Plants have been in use for the management of diverse kinds of man's health conditions all over the world from time immemorial. Man has accumulated an amount of knowledge of drugs derived from various plants. Modern pharmaceutical industries started using crude extracts of medicinal plants for manufacturing drugs because of their established therapeutic efficacy.

Pharmaceutical industries still rely to some extent on the bioactive principle, obtained from plants. For example, the anticancer agent, taxol, Isolated from the pacific yew, *taxus brevifolia* (Wani *et al.*, 1971) and the antimalarial agent artemisinin obtained from the Chinese herb *Artemisia annua* (Willcox 2009).

Due to the relatively high cost and the many adverse side effects of the synthetic drugs, people, all over the world, are drifting back to the use of medicinal plant for their health care needs. 80% of the world population still depends on herbal medicine as their main source of medicinal therapy (Arvigo and Balick 1993). Today many scientists and medical experts around the world are emphasizing the value of herbal remedies for health.

Among phytotherapeutic products, special attention must be given to association of two or more plants with a view to a better therapeutic effect. The associations are widely used in some oriental types of medicine such as Ayurveda (Wu *et al.*, 1998; Williamson, 2002), Chinese medicine (Guo *et al.*, 2004; Kuribara *et al.*, 2004) and have been increasingly acknowledged in western medicine (Williamson 2001; Gilbert *et al.*, 2003). In Nigeria, West Africa, a survey of forest plants used in the traditional treatment of typhoid fever was conducted in Chikun local government area of Kaduna state by Faleyimu *et al.*, (2010). The phyto medication products involve a composition of two or more plants. One product involves the combination of *Anogeissus leoicarpus (marke)* leaf, *Carica papaya (pawpaw)* leaf and *Mangifera indica (mango) stem* bark. This combination is cooked with water. Some of which is used for bathing the patient while some is drunk by the patient.
These plants used in combination are said to be very effective in the treatment of typhoid fever. The combination of these plants, as claimed by the herbalists, totally gets rid of typhoid fever within a space of one to two weeks.

Typhoid fever is a systemic infection with the bacterium *salmonella enteric serotype typhi*. This disease occurs mostly in developing countries like Nigeria where sanitary conditions are very poor. It is estimated that there is at least sixteen million new cases of typhoid fever each year, with six hundred thousand deaths (Ivanoff, *et al.*, 1995). Typhoid fever is usually contracted by ingestion of food or water contaminated by the faeces or urine of carriers excreting *Salmonella typhi*. In endemic areas identified risk factors for the disease include eating food prepared outside home and consuming ice cream or flavoured iced drinks from street vendors (Black *et al.*, 1985;Luby *et al.*, 1998), drinking contaminated water (Mermin., *et al.*,1999), having a close contact or relative with recent typhoid fever (Black *et al.*, 1985; Luxemburger *et al.*, 2001), poor housing with inadequate facilities for personal hygiene (Gasem *et al.*, 2001) and recent use of antimicrobial drugs (Luby *et al.*, 1998). The treatment of typhoid fever with chloramphenicol in 1948, transformed a severe, debilitating, and often fatal disease into a readily treatable condition (Woodward *et al.*, 1948). The emergence of resistance to chloramphenicol and other antimicrobial agents has been a major setback (Mizra *et al.*, 1996). We now face the very real prospect that untreatable typhoid fever will reemerge (Parry *et al.*, 2002). Hence the more reason why other types of treatment like the use of herbs should be greatly explored. This prompted the investigation of these plants for their antibacterial effects and to ascertain whether or not using them in combination has any added potency/effects.

Anogeissus leiocarpus is a fodder tree occurring in most of savanna areas from the driest region to the borders of forest zones (Ibrahim *et al.*, 2005). It belongs to the family combrataceae. The plant is very common in central and western Africa where it has a wide range of use (Burkill, 1985). It is commonly called the African

Birch. In Nigeria, it is known as Otra in Idoma, Marke (or kwankila) in Hausa, Atara in Ibo and Orin-odan in Yoruba (Agaie and Amali, 2007). It has numerous medicinal applications all over Africa (Adigun *et al.,* 2000). In traditional medicine its infusion and decoction is used as cough medicine, the powdered root is applied to wounds and ulcers while powdered bark is rubbed on gums to reduce toothache (Ibrahim *et al.,* 2005). The decoction is used as vermifuge and for fumigation while leprotic, laxative and antihelmintic properties of the leaf extract have also been reported in man and animals (Burkill, 1985). A leiocarpus is also used as an emulsifier, treatment for diarrhea, syphilis chancres, stimulant, aphrodisiac and tanicide for horses and donkeys (Adigun *et al.,* 2001). This plant has been shown to be active as antimicrobial agent against gram positive and gram negative bacteria (Adeleye *et al.,* 2003; Maichido and Ado 1999), antimycobacterial activity (Malcolm and Sofowora, 1969; Johnbull and Abdu, 2006; Uba *et al.,* 2003), trypanacidal activity (Atawodi *et al.,* 2003) and demonstrated activity against *Candida albicans* (Chaabi *et al.,* 2006; Sanogo *et al.,* 1997, Sanogo 2005).

Carica papaya is a native to the tropics of the Americas. It is distributed throughout Asia and Africa (Afolayan, 2003). It belongs to the family caricaceae. It has the following common names; pawpaw tree, papaya and papayer. The different parts of *Carica papaya* plant (the leaves, fruits, seeds and latex) can be eaten and are used for the treatment of different ailments including wound healing (Wiart 2002, Nor Suhada *et al.,* 2008). The efficacy of some of the traditional claims to this plant has been validated scientifically (Indran *et al.,* 2008, Imaga *et al.,* 2009). The medical folk use the leaves poultice on to nervous pains and elephantoid growths. The leaf is smoked for asthma relief in various remote areas (Reed, 1976). The aqueous leaf extract showed pronounced inhibition demonstrating a high activity against the test bacteria (Anibijuwon and Udeze 2009). The young leaves and to a lesser extent other parts of the plant contain carpain, an active bitter alkaloid which has a depressing action on the heart. The plant is also a strong amoebicide (Reed 1976). The leaf extract is also used as a profilaxis against malaria (Satrija *et al.,* 1994). The leaves are also used as soap substitute which are supposed to remove stains.

Imaga *et al.,* (2009) and Indran *et al.,* (2008) have proven that *Carica papaya* leaf extract is a potential anti sickling agent and has protective effect against gastric ulcer in rats. *Carica papaya* flowers have antibacterial activities (Zakaria *et al.,* 2006). Oral administration of the seed extract could induce reversible male infertility and could be used for pharmaceutical development of a male contraceptive (Udoh *et al.,* 2005). Medical research in animals models has confirmed the contraceptive and abortifacient capability of papaya and also found that papaya seeds have contraceptive effects on adult male langur monkeys and possibly in adult male humans as well (Lohiya *et al.,* 2002). Unripe papaya is especially effective in large amounts or high doses but the ripe ones are not teratogenic and will not cause miscarriage in small amounts. Javanese believe that eating pawpaw prevent rheumatism. Dietary papaya reduces urine acidity in humans while the flowers are used against jaundice (Reed, 1976). Papaya seeds may be nephroprotective in toxicity induced kidney failures (Olagunju *et al.,* 2009). The juice of the leaf extract has an antiproliferative effect on in vitro liver cancer cells probably due to its component of lycopene (Rahmat *et al.,* 2006) or immune system stimulation (Dang,

2010). Red flesh papaya fruit is also rich in lycopene (chandrika *et al.,* 2001; Chandrika *et al.,* 2002) a potent antioxidant. Lycopene is reported to be beneficial in cardio vascular ailments and cancer (Kohimeier *et al.,* 1997; Rao and Agarwal, 2000). Papaya seed extract has antibacterial activity against Escherichia Coli, *Staphyloccus aureus* and *Salmonella typhi* (Yismaw *et al.,* 2008).

Mangifera indica (mango) is a large ever green tree, with a strong trunk and heavy crown, native of Asia, it is now completely naturalized in many parts of the tropics and subtropics (Ross, 1999). *Mangifera indica* belongs to the family anacardiaceae. In Nigeria *M. indica* is commonly used as herbal preparations in the treatment of toothache, gastrointestinal disorders, dysentery, diarrhea, gastrointestinal tract infections, respiratory and urinary tract infections, sore gums and sore throats.

In a different study Agoha (1981) and Madunagu *et al.,* (1990) reporteded that *Mangifera indica* is used against asthma, cough, diarrhea, dysentery, jaundice pains and malaria. In all the region of *Mangifera indica* distribution, one of the main organs used is the bark. Based on ethnopharmacological knowledge, a standardized aqueous extract of the plant's stem bark antioxidant, anti-inflammatory and immunomodulatory properties has been developed in Cuba. This extract is proposed as both a nutritional supplement (antioxidant) and an anti-inflammatory, analgesic and immunomodulatory treatment to prevent disease progress or increase the patient's quality of life in gastric and dermatological disorders, Aids, cancer and asthma (Nuñez-selles, 2005).

Mangiferin is proposed as the bioactive principle in the stem bark and leaf extracts and possess several pharmacological activities including antioxidant, analgesic, antidiabetic, anti inflammatory, antitumor, immunomodulatory and anti-HIV effects (Nuñez-Selles, 2005). Antimicrobial (Zhu *et al.,* 1993; Zheng *et al.,* 1990 ; Guha *et al.,* 1996), antibacterial, antifungal (Stoilova *et al.,* 2005) and antiparasitic activities (Perrucci *et al.,* 2006).

There is evidence for several different types of positive interactions between different components of medicinal plants. Pharmacodynamic synergy has been demonstrated between various plants' extracts traditionally combined. Pharmacokinetic interactions occur such that one phytochemical is more rapidly absorbed because of the presence of another phytochemical. Some plant extracts may have an immunomodulatory effect as well as a direct antibacterial effect. Several extracts contain multidrug resistance inhibitors. Some plant constituents are added mainly to attenuate the side-effects of others, for example the addition of ginger in herbal preparations to prevent nausea.

Materials and Methods

Plant Material

Leaves of *Anogeissus leiocarpus, Carica papaya* and bark from the stem of *Mangifera indica* trees were freshly collected from Vom in Jos south L.G.A, and were identified at the Federal Department of Forestry Jos.

Hot Water Extraction of the Crude Plants' Materials

The leaves and the barks of the above mentioned plants were washed with clean water to get rid of dust and dirt. The leaves were air dried to get rid of the water droplets. The leaves were dried in an oven at

60^0C for five days. The barks were also oven dried at 60^0C for seven days. The dried plant materials were pulverized into coarse powder in a mortar with a pestle. They were separately, ground into fine powder with an electric blender. 100g of each plant sample were extracted separately in hot water. The solutions were filtered and the filterate evaporated off under reduced pressure in a rotary evaporator to obtain the crude extract.

Phytochemical Screening of the Crude Plants' Extracts

The extracts were subjected to phytochemical screening to detect the presence of the following secondary metabolites; Resins, Alkaloids, Saponins, Tannins, Glycosides and Flavonoids following standard procedures (Trease and Evans, 1989).

Reconstitution of the Crude Plants' Extracts

The crude extracts of the different plants were separately reconstituted in sterile distilled water to get the following concentrations: 200mg/ml, 100gm/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Also, equal weights of the various crude extracts of the plants were combined as follows:-

- 10g of pawpaw leaves extract and 10g of mango barks extract
- 10g of pawpaw leaves extract and 10g of *Marke* leaves extract
- 10g of mango bark extract and 10g of *Marke* leaves extract.
- 10g each of pawpaw leaves extract, mango bark extract and *Marke* leaves extract.

The above combination of the plant extracts were separately reconstituted in sterile distilled water to also get 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml.

Screening Of Antibacterial Activities Punch Hole Diffusion Method

An inoculum size of 10^8 cfu/ml of the clinical isolates of the different gram-positive and gram-negative bacteria was prepared according to the method of Bauer *et al.,* (1966). Five gram-negative bacteria, *Escherichia coli Pseudomonas aeruginosa, Proteus vulgaris, Shigella* and Salmonella *typhi*. Also *Staphylococcus aureus* and *Strephtoccoccus faecalis* (gram-positive bacteria) were used.

The nutrient agar plates were each inoculated by flooding with the inoculum of the different isolates. The plates were dried in an incubator at 37°C and seven holes were bored on each of the seeded plates, using a 6mm diameter sterile cork borer. A drop of molten nutrient agar was put into each hole to seal off from the bottom of the plate. The different concentrations of the various plants crude extracts and their combinations were used to fill the holes corresponding to their respective labels, avoiding overflowing. Sterile distilled water and nalidixic acid (5µg/ml) were used as negative and positive controls. All the plates were incubated aerobically at 37°C for 24hrs. Diameters of the zones of inhibition were measured in millimeters (mm) and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using freshly prepared nutrient broth. 9mls of the freshly prepared nutrient broth was added to each of the sterile test tubes labeled 2-6. 1ml of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml of the various plants' crude

extract were added to tubes 2-6 respectively, tube 1 is the positive control containing 10mls of nutrient broth. Using a sterile pipette, 50µl of the test organism previously adjusted to1 × 10^8 CFU/ml was added to each of the six tubes. The contents of the tubes were thoroughly mixed and incubated at 37°C for 24hrs. The tubes were then inspected for turbidity. A 0.5 McFarland standard was used for visual comparison . Turbidity shows bacterial growth. The minimum concentration without bacterial growth (i.e. without turbidity) is the minimum inhibitory concentration. The results are shown in tables 8-14.

Result and Discussion

Phytochemical screening of the various plant extracts

The phytochemical screening of the aqueous crude extract of carica papaya (pawpaw) leaf, anogeissus leiocarpus (marke) leaf and mangifera indica (mango) stem bark shows that all the extracts contain resin, alkaloids, saponins and flavonoids, only marke and mango contain tannins. Glycoside was seen in only marke and pawpaw. (Table 1).

Table.1 Phytochemical screening of the aqueous extract of the various plants

	Mangifera indica (mango)	*Carica papaya* (pawpaw)	*Anogeissus leoicarpus* (marke)
Resin	+	+	++
Alkaloids	+	+++	+
Saponins	+	+	+++
Tannins	+	-	+
Glycosides	-	+	++
Flavonoids	++	++	++

KEY:

- = absent
+ = slightly present
++ = present in moderate quantity
+++ = present in high quantity

Antibacterial Studies

The results of the antibacterial studies show that aqueous *marke* extract inhibited the growth of *Salmonella typhi*, *Shigella*, *Proteus vulgaris*, *Staphylococcus aureus* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis* and was only able to exert a minimal effect on *Pseudomonas aeruginosa* at higher concentrations of 200mg/ml and 100mg/ml. (Table 2)

Table.2 Antibacterial activities of hot aqueous extract of *Anogeissus leiocarpus* (*Marke*)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	10	12	16	18	18	-	25
Shigella	9	9	14	16	16	-	20
Proteus vulgaris	10	12	12	14	15	-	25
Staphylococcus aureus	10	12	12	16	16	-	24
Streptococcus faecalis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	-	-	4	6	6	-	20
Escherichia coli	9	9	12	16	16	-	24

Key: +ve = positive control (5µ/ml nalidixic acid)
-ve = negative control (sterile distilled water)
- = no zone of inhibition

Table 3 shows the results of the antibacterial studies of *pawpaw* leaf extract. This inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis.*

Table.3 Antibacterial activities of hot aqueous extract of *Carica papaya* (pawpaw)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	13	13	14	15	17	-	20
Shigella	10	10	12	13	17	-	24
Proteus vulgaris	10	15	16	16	16	-	20
Staphylococcus aureus	10	11	11	12	12	-	18
Streptococcus faecalis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	6	6	6	6	6	-	18
Escherichia coli	10	12	13	13	15	-	20

The results of this antibacterial studies show that aqueous *mango bark* extract inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis.* (Table 4)

Table.4 Antibacterial activities of hot aqueous extract of *Mangifera indica* (mango)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	-	10	10	11	13	-	30
Shigella	8	9	9	9	10	-	26
Proteus vulgaris	10	11	13	13	13	-	26
Staphylococcus aureus	10	11	13	13	13	-	25
Streptococcus faecalis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	9	10	10	11	11	-	30
Escherichia coli	8	9	10	10	10	-	28

The results in table 5 shows the antibacterial studies of aqueous *mango and pawpaw* extract. This extract inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis.*

Table.5 Antibacterial activities of hot aqueous extract of *Carica papaya* (pawpaw) and *Mangifera indica* (mango)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	10	10	13	15	15	-	10
Shigella	10	10	10	12	15	-	24
Proteus vulgaris	10	15	18	18	20	-	22
Staphylococcus aureus	6	8	10	15	18	-	25
Streptococcus faecalis	-	-	-	-	-	-	18
Pseudomonas aeruginosa	6	6	10	10	10	-	20
Escherichia coli	10	12	15	18	18	-	24

The results of this antibacterial studies show that the mixture of aqueous *marke* and pawpaw extract inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis.* (Table 6)

Table.6 Antibacterial activities of hot aqueous extract of *Carica papaya* (pawpaw) and *Anogeissus leoicarpus* (*Marke*)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	20	20	22	22	22	-	24
Shigella	5	10	15	15	18	-	25
Proteus vulgaris	15	20	20	24	24	-	24
Staphylococcus aureus	12	12	12	16	16	-	25
Streptococcus faecalis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	10	11	11	12	13	-	18
Escherichia coli	10	14	16	16	18	-	24

The results of the antibacterial studies show that acqueous *marke and mango* extract inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis*. (Table 7)

Table.7 Antibacterial activities of hot aqueous extract of *Mangifera indica* (mango) and *Anogeissus leoicarpus* (*Marke*)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	20	20	22	24	24	-	24
Shigella	18	18	20	20	20	-	20
Proteus vulgaris	14	15	18	20	22	-	23
Staphylococcus aureus	12	12	13	18	20	-	25
Streptococcus faecalis	-	-	-	-	-	-	20
Pseudomonas aeruginosa	9	10	11	11	12	-	22
Escherichia coli	18	18	20	22	22	-	20

The results of the antibacterial studies show that aqueous *marke, mango and pawpaw* extract inhibited the growth of *Salmonella typhi, Shigella, Proteus vulgaris, Staphylococcus aureus* and *Escherichia coli* at all the concentrations used. It had no inhibitory effect on *Streptococcus faecalis.* (Table 8)

Table.8 Antibacterial activities of hot aqueous extract of *Carica papaya* (pawpaw), *Anogeissus leoicarpus* (*Marke*) and *Mangifera indica* (mango)

ZONES OF INHIBITION OF THE VARIOUS BACTERIA (mm)							
Concentration of extract (mg/ml)	12.5	25	50	100	200	-ve	+ve
Salmonella typhi	20	20	22	23	23	-	23
Shigella	19	18	20	20	20	-	23
Proteus vulgaris	16	16	16	18	18	-	20
Staphylococcus aureus	13	15	15	18	18	-	20
Streptococcus faecalis	-	-	-	-	-	-	22
Pseudomonas aeruginosa	11	13	13	13	13	-	24
Escherichia coli	16	16	17	18	18	-	24

Minimum Inhibitory Concentration (MIC)

The 1:10 dilutions of various concentrations of the crude extract of pawpaw leaf against *Salmonella typhi* shows no turbidity at tube 4 (Table 9), indicating that tube 4 is the highest concentration showing no turbidity. This therefore suggests that the MIC for pawpaw leaf extract against *Salmonella typhi* is 50mg/ml.

Table.9 1:10 dilutions of the various concentration of crude extract of pawpaw leaves against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	+	-	-	-

The 1:10 dilutions of various concentrations of the crude extract of mango bark against *Salmonella typhi* shows no turbidity at tube 4 (Table 10), indicating that tube 4 is the highest concentration showing no turbidity therefore the MIC for mango bark extract against *Salmonella typhi* is 50mg/ml.

Table.10 1:10 dilutions of the various concentration of crude extract of mango barks against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	+	-	-	-

The 1:10 dilutions of various concentrations of the crude extract of marke leaf against *Salmonella typhi* shows no turbidity at tube 4 (Table 11), indicating that tube 4 is the highest concentration showing no turbidity therefore the MIC for marke leaf extract against *Salmonella typhi* is 50mg/ml.

Table.11 1:10 dilutions of the various concentration of crude extract of *Marke* leaves against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	+	-	-	-

Key:

- = no turbidity (no bacterial growth)
+ = presence of turbidity (presence of bacterial growth)

The 1:10 dilutions of various concentrations of the crude extract of mango bark and pawpaw leaf against *Salmonella typhi* shows no turbidity at tube 4 (Table 12), indicating that tube 4 is the highest concentration showing no turbidity therefore the MIC for the combination of mango bark and pawpaw leaf extract against *Salmonella typhi* is 50mg/ml.

Table.12 1:10 dilutions of the various concentration of crude extract of pawpaw leaves and mango barks against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	+	-	-	-

The 1:10 dilutions of various concentrations of the crude extract of pawpaw leaf and *Marke* leaf against *Salmonella typhi* shows no turbidity at tube 3 (Table 13), indicating that tube 3 is the highest concentration showing no turbidity therefore the MIC for pawpaw leaf and *Marke* leaf extract against *Salmonella typhi* is 25mg/ml.

Table.13 1:10 dilutions of the various concentration of crude extract of pawpaw leaves and *Marke* leaves against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	-	-	-	-

The 1:10 dilutions of various concentrations of the crude extract of mango barks and *Marke* leaf against *Salmonella typhi* shows no turbidity at tube 3 (Table 14), indicating that tube 3 is the highest concentration showing no turbidity therefore the MIC for mango barks and *Marke* leaf extract against *Salmonella typhi* is 25mg/ml.

Table.14 1:10 dilutions of the various concentration of crude extract of mango barks and *Marke* leaf against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	-	-	-	-

KEY:

- = no turbidity (no bacterial growth)

\+ = presence of turbidity (presence of bacterial growth)

The 1:10 dilutions of various concentrations of the crude extract of mango barks, pawpaw leaf and *Marke* leaf against *Salmonella typhi* shows no turbidity at tube 3 (Table 15), indicating that tube 3 is the highest concentration showing no turbidity therefore the MIC for mango barks, pawpaw leaf and *Marke* leaf extract against *Salmonella typhi* is 25mg/ml.

Table.15 1:10 dilutions of the various concentration of crude extract of mango barks, pawpaw leaf and *Marke* leaf against *Salmonella typhi*

No of tubes	1	2	3	4	5	6
Concentrations (mg/ml)		200	100	50	25	12.5
Nutrient broth (ml)	10	9	9	9	9	9
Extract (ml)	-	1	1	1	1	1
Salmonella typhi (µl)	50	50	50	50	50	50
Turbidity	+	+	-	-	-	-

Key:

- **=** no turbidity (no bacterial growth)

\+ = presence of turbidity (presence of bacterial growth)

The results of the antibacterial studies of these plants' extracts showed that the aqueous extracts of all the plants studied had varying degrees of antibacterial activities on the various bacteria tested (Tables 2-7). Only *streptococcus faecalis* was resistant to all the plant extracts. Antibacterial efficacy of these plants extracts have been reported by previous authors (Adeleye et al 2003, Anibijuwon and Udeze 2009, Stoilova et al 2005). The datas contained in this present study confirms the antibacterial efficacy of these plants extracts. The antibacterial activities of these plants extracts could be due to their rich contents of secondary metabolites. Many secondary metabolites belonging to alkaloids, flavonoids, saponins, tannins, glycosides and resins were detected in the tested plant extracts (Table 1). These phytochemicals have been reported for their antibacterial activities (2012, Adigun et al 2006,Cowan 1999,Kuete 2010). The antibacterial activities of these plants extracts are dose dependent. The extracts are more effective at higher doses suggesting that the bioactive agents are present in small quantities in the extracts. It could also mean that water is a poor extracting medium of the bioactive components in these plants. Many reports show that solvent systems play significant roles in the solubility of the bioactive components and hence influence the antibacterial activities (El-Mahmood 2009). The differences in susceptibility of the various bacteria to the extracts may be either due to the differences in cell wall composition and/or genetic content of their plasmids (Karaman *et al* 2003) and also the bacteria' reaction to the composition and the mechanism of action of the bioactive compounds (Ono *et al* 2004).

MIC is a quantitative measurement of in vitro activity of potential antibacterial agents. The low values of MIC obtained for these plants (Tables 9-15) shows that these plants extracts have medicinal properties and therefore justifies their uses by traditional healers.

The zones of inhibition of bacterial growth produced by these plants extract show that the crude marke (anogeissus leiocarpus) extract and any of the herbal mixtures

containing marke have wider zones (Tables 2, 6, 7 and 8). This suggests that some compounds in marke have more antibacterial activities and enhanced the bioactive components of mango and pawpaw. It should be noted that marke aqueous extract possesses more classes of phytochemicals than the extracts from mangifera indica and carica papaya (Table 1). *Anogeissus leiocarpus* contains all the secondary metabolites tested. However, antibacterial activity does not depend only on the number of classes of detected bioactive compounds, but mostly on their concentration (Noumedeem *et al* 2013).

Synergy between different constituents of extracts has been reported (Williamson 2001, Houghton 2009) and there appear to be positive interactions between the bioactive substances in marke and those in mango and pawpaw. Though the mechanism of action of marke's synergistic effect is not yet known, It could be that marke crude extract has a number of substances that act at different receptor targets in the bacteria to enhance overall therapeutic effect (pharmacodynamic synergy). It is also a known fact that substances with little or no activity on the causative agent assist the main active principle to reach the target by improving bioavailability (pharmacokinetic synergy). It could be that some of the bioactive agents in marke made available the bioactive constituents of the other plants extracts by making the bacterial cell wall more permeable to these antibacterial agents.

In conclusion, this study has corroborated with the traditional use of the combination of these herbs in the management of typhoid fever. The combination of these plants has greater antibacterial actions than any single one of the plants and marke appears to have a potentiating effect on the other two plants.

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