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Antibacterial activity of phytochemicals from *Acacia nilotica* ,*Entada africana* and *Mimosa pigra L.* on *Salmonella typhi*

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1 SUMMARY

The plants *Acacia nilotica*(Nile or scented-pod acacia), *Entada africana*(Dorot),and *Mimosa pigra L.*(Bashful plant) are combined and used to treat diarrhea, typhoid fever and genitourinary tract infections by the people of Timonde, a village under Zebilla in the Bawku West District of the Upper East region of Ghana.

The objective of this research is to identify solvents with optimal ability among diethylether, chloroform and methanol solvents which can fractionate bioactive phytochemicals from ethanol extracts of *Acacia nilotica, Entada africana* and *Mimosa pigra L.* The project is geared towards identifying classes of phytochemicals present in ethanol extracts and other solvent fractions of the three plants, and testing of their anti-typhoid activity on *Salmonella typhi* in comparison to ciprofloxacin standard antibiotic. It is the aim of the research to combine ethanol extracts from the three plants and test for growth inhibitory activity on *Salmonella typhi* so as to provide a scientific back-up for the traditional medicinal use of plants in the treatment of typhoid fever. Extraction process was carried out using 96% ethanol on the stem-bark of both *Acacia nilotica* and *Entada africana,* and the whole of *Mimosa pigra L.* Fractionation process was conducted on the ethanol extracts obtained from the extraction process using diethyl ether, chloroform and methanol solvents in the trend of increasing polarity indices of solvents. Ethanol extracts and solvent fractions obtained from the extraction and fractionation processes were tested for the presence of phytochemicals, and for growth inhibitory activity on *Salmonella typhi.* Ciprofloxacin, a standard antibiotic was also tested on the bacterial isolate. Phytochemical analysis revealed the absence of cardiac glycosides in *Mimosa pigra L.* only, and the presence of alkaloids, amino acids, anthraquinones, flavanoids, general glycosides, saponins, steroids, tannins and terpenoids in all the three plants. The diethyl ether and methanol solvents were the optimal solvents for the fractionation process. Their fractions revealed the presence of 70 % and above of the tested classes of phytochemicals. Ethanol extracts and other solvent fractions from *Acacia nilotica, Entada africana* and *Mimosa pigra L.* demonstrated growth inhibitory effect on *Salmonella typhi.* Also, the combined ethanol extracts from the three plants demonstrated more growth inhibitory effect on the bacterial isolate than the singly tested ethanol extracts. These results supported the use of the three plants combine for the treatment of typhoid fever by the people of Timonde. The phytochemicals present in ethanol extracts and other solvent fractions of the three plants were responsible for the antibacterial activity demonstrated on *Salmonella typhi.* This is an indication that the plants

employed in the research contained bioactive compounds or anti-typhoid agents which supported their ethno-medicinal use in combination to treat typhoid fever.

2 INTRODUCTION

Plants have not only provided mankind with food, clothing, flavors, cosmetic, ornamental, fumigants, insect deterrents and fragrance, but have also served humanity in the treatment of ailments. According to World Health Organization (WHO) report in 1993, about three-quarters of the world's population which live in developing countries rely on plants for the treatment of many illnesses (Akerele, 1993). Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi, wild fires and sometimes flood. Plants protect themselves through a chemical defence system (Van Wyk and Gericke, 2000). Therefore, it is expected that biological active compounds are produced by plants as chemical defense measures against their enemies. The search for biologically active agents is part of a wider renaissance of scientific significance to bring into being new chemotherapeutics (Moundipa et al., 2001). Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with new modes of action (Houghton, 1996). Several useful drugs have been developed from medicinal plants used in traditional medicine in the treatment of a variety of illnesses. According to Gilani and Atta-ur- Rahaman (2005), the use of plant extracts or plant-derived pure chemicals to treat diseases is a therapeutic modality which has stood the test of time. Many studies indicated that medicinal plants contain substances like peptide, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds. Most of the clinical drugs that are currently in use were derived from plants and developed because of their use in the traditional medicine. Atropine from *Atropa belladonna* (Deadly nightshade), ephedrine from *Ephedra sinica* (Pine tree), reserpine from *Rauwolfia serpentine*(Snake root), benzocaine – a molecular modified drug from cocaine (cocaine was derived from *Erythroxylon coca* (Coca plant), quinine (from Cinchona tree), and Artemeter, an anti malarial drug from *Artemesia annua* (Annual wormword), are examples of drugs, which were discovered through the study of ethnobotany.

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity, and there are marked differences between different regions of this continent when it comes to healing practices (Gurib-Fakim, 2006). Medicinal and poisonous plants, including a diverse array of woody plants, have always played an important role in African life. The traditions of collecting plants, processing herbal remedies and applying them have been handed down from generation to generation.

Acacia nilotica is a medium to large tree that can reach a height of 10 m, with an average of 4-7 m in height. The stem-bark is blackish grey or dark brown in mature trees and deeply grooved, with longitudinal fissures. The young branches are smooth and grey to brown in colour with its twigs covered by short hairs (Van Wyk and Van Wyk, 1997). It bears single to several, bright, golden yellow, globose, scented inflorescences between the leaves. The flower stalks are hairy and it flowers mainly from September-January, but it depends on the rainy season. The pods are very characteristic, resembling a beaded necklace which are flat, straight or slightly curved, and fleshy when young with reddish hairs, becoming dark blackish when mature. The pods are sweetly scented when crushed and contain a sticky fluid (Swarbrick, 1997).The hard, reddish wood of this plant is used as firewood and for fencing posts. In Libya, the wood is used to treat smallpox. Most of the plant browsers eat the leaves. The bruised leaves are poultice (a warm moist preparation placed on an aching or inflamed part of the body to ease pain, improve circulation, or hasten the expression of pus) onto ulcers. The leaves, and young pods are strongly astringent due to tannin, and are chewed in Senegal as antiscorbutic (treatment of scurvy); in Ethiopia as lactogogue. The stem-bark exudes; an edible gum is also used medicinally according to Van Wyk et al (2000). In South Africa, the Zulus consume the stem-bark for cough; the Chipis use the root to treat tuberculosis. In Kenya, the Masai are intoxicated by the stem-bark and root decoctions which according to them impart courage, increase sexual urge (Aphrodisia), and cure impotence. Its astringent stem-bark is used to treat diarrhea, dysentery, and leprosy. In Lebanon, the resin is mixed with

orange-flower infusion for typhoid convalescence. Egyptian Nubians believe that diabetics may eat unlimited carbohydrates as long as they also consume powdered pods (Duke, 1983a). Extracts from pods of plant have demonstrated growth inhibition of at least four species of pathogenic fungi. The plant is reported to contain l-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyldjenkolic acid, sulphoxides pentosan, saponin and tannin (Pande et al., 1981).

Entada africana is a small tree of 4 to 10 meters in height and 90 cm in girth; branching low down, with a wide crown; bark brown-grey to black, very rough, transversely striped and scaly The Leaves are bipinnate with a glabrous common stalk of length 15 to 45 centimeters (Keay, 1989).The leaves of *Entada africana* make good fodder and its stem-bark fiber are used for ropes and bands. The bark of the root and stem yields a long fiber used for cordage, commonly for roof binding and grass matting. The wood is light red, soft and easy to work with. *Entada africana* yields a low-quality gum. An infusion of the leaves at a concentration of 1:1 000 kills *Carassius auratus* (goldfish) in 12 hours. The stem-bark is said to have abortive effects, while a root decoction is a stimulating agent and tonic. The plant is said to have antidote effects against various toxic agents because of its emetic properties. Healing and fever-reducing beverages are prepared from leaves, stem-bark, roots and shoots. In Northern Nigeria and Ghana, an infusion of the leaves or of the stem-bark is taken as a tonic and for stomach ache. The leaves also constitute a good wound dressing, preventing suppuration (Oliver-Beyer, 1986).

Mimosa pigra is a species native to Neotropics, but has been listed as one of the world's 100 worst invasive species. It forms dense, thorny impenetrable thickets particularly in wet areas. *Mimosa pigra* is a leguminous shrub, which can reach up to 6m in height. The stem is greenish in young plants but becomes woody as the plant matures. It is armed with broad-based prickles up to 7mm long. The leaves are bright green and bipinnate, consisting of a central prickly rachis 20 to 25cm long with up to 16 pairs of pinnae. Leaves are sensitive and fold up when touched. Flowers are mauve or pink. Each flower head produces a cluster of 10 to 20 seedpods, which then mature and break into segments, each containing an oblong shaped seed. Ripe seeds are light brown to brown or olive green. *Mimosa pigra* is hard seeded, and seeds can germinate year round if the soil is moist but not flooded. (Lonsdale et al.,1995). The plant is a noxious weed which has received international recognition because of its existing and potential impact on biological diversity. Traditional methods of food-gathering by Aborigines are threaten by the weed through its effects on the fauna and flora of the wetlands, which are otherwise rich in traditional food such as fish, turtles and water birds. Tourism is also affected through reduced area and access for tourism activities, reduced wildlife attractions and reduced access to fishing, hunting and scenic areas. Despite its detrimental impacts, *Mimosa pigra* does have uses. It fixes nitrogen and, in areas of Australia where it grows profusely, it increases soil fertility and redistributes nutrients from the lower soil profile to the surface. It is used for firewood, bean-poles and as temporary fences, and has been tested as a medium for growing mushrooms. The harvest of *Mimosa pigra* to extract vegetable tannins and to provide biomass to generate electricity has been proposed under controlled conditions. It has been used as green manure and against snake bites in Thailand (Janzen, 1983).

Acacia nilotica, Entada africana, and *Mimosa pigra L.* have been used by the Asapio's family of Goriga-Timonde in the Bawku West District of the Upper East Region of Ghana to treat diarrhea, typhoid fever and gonorrhea infections for the past fifty years. The purpose of this research is to identify solvents with optimal ability to fractionate bioactive phytochemicals from ethanol extracts of *Acacia nilotica, Entada africana* and *Mimosa pigra L.* The project is geared towards investigating the presence of phytochemicals in the three plants and testing of their antibacterial activity on a typhoid causative agent in comparison to ciprofloxacin, a standard antibiotic.

3. MATERIALS AND METHODS

3.1 Materials/Chemicals: The materials/chemicals used in this study were of analytical grade bought from UK Laboratory Supplies Limited, Kumasi, Ghana. These include 96 % ethanol, chloroform, diethyl ether, methanol, dimethylsulphoxide(D .M .S. O), Sabouraud dextrose and Mueller-Hinton agars, Petri dishes, Whatmann number 1. Filter papers, etc.

The plants and pathogenic bacterium used in the research were *Acacia nilotica*, *Entada africana, Mimosa pigra L.* and *Salmonella typhi* respectively.

3.1.1 Material collection and treatment: The plants *Acacia nilotica*, *Entada africana* and *Mimosa pigra L.* were randomly collected from Tiliga, a forest reserve located at Timonde-Zebilla, a village in the Bawku West District of Upper East Region of Ghana. The stem-bark of both *Acacia nilotica* and *Entada africana* and the entire shrub of *Mimosa pigra L.* were separately air-dried at room temperature (25°C) for two weeks and later pulverized into fine powder.

The pathogenic bacterium, *Salmonella typhi* was collected from the Microbiology Laboratory of Okomfo Anokye Teaching Hospital, Kumasi, Ghana after its characterization and identification using microbiological procedures of Cowan and Steel, 2004.

3.2 Methods

3.2.1 Extraction procedure: The pulverized plant materials; *Acacia nilotica*(200g), *Entada africana*(400g) and *Mimosa pigra L.*(400g), were soaked in 1,000ml, 1,200 ml and 1,200 ml of 96 % ethanol respectively for two weeks with intermittent shaking .The percolates were separately evaporated to dryness at room temperature(25°C) to give crude extracts(ethanol extracts). One point one grams (1.10 g), 4.39 g and 5.65 g of the crude extracts of *Acacia nilotica*, *Entada africana and Mimosa pigra L.* respectively were then transferred into vials(2ml) and kept at 2-4 °C in a refrigerator until required for use .The remaining crude extracts were subjected to the fractionation process.

3.2.2 Fractionation of crude extracts: The crude extracts were fractionated using diethyl ether, chloroform and methanol solvents in the order of their increasing polarity indices. For 10g of ethanol extract of *Acacia nilotica* 30ml, 20ml and 15ml of diethyl ether, chloroform and methanol solvents were respectively used. For 40g of ethanol extract of *Entada africana* 80ml, 60ml and 40ml of diethyl ether, chloroform and methanol solvents were respectively used. For 20g of ethanol extract of *Mimosa pigra L.* 60ml, 40ml and 30ml of diethyl ether, chloroform and methanol solvents were respectively used. Solvent solubles of ethanol extracts were filtered and evaporated to dryness at room temperature 25°C) to give diethyl ether fractions, chloroform fractions methanol soluble fractions and methanol insoluble fractions.

3.2.3Qualitative phytochemical evaluation Phytochemical screening was conducted to determine the presence of natural products in the extracts obtained from the stem bark of both *Acacia nilotica and Entada Africana,* and the whole shrub of *Mimosa pigra L.*

3.2.3.1(Wakama test): The plant extract (0.2 g) was re-extracted with 1 % hydrochloric acid (HCl) for 24 hours. A two 2ml portion of the filtrate was taken and tested for alkaloids by adding drops of Meyer's reagent. Alkaloids formed a picric yellow precipitate with the reagent (Adewunmi and Sofowara, 1980).

3.2.3.2 Flavonoids Willistatter test): To a methanol solution of each extract, a piece of magnesium ribbon was added, followed by drop-wise addition of concentrated HCl. Colors ranging from orange to red indicated flavones, red to crimson indicated flavanols and crimson to magenta indicated flavonones(Adewunmi and Sofowara, 1980) .

3.2.3.3 Terpenoids and Steroids (Liebermann Buchart test) : A small quantity of each extract was dissolved in trichloromethane, and a minimum volume of concentrated sulphuric acid was then added to its content. A blue or green color or a mixture of these two shades was taken as positive test for steroidal compounds (Adewunmi and Sofowara, 1980).

3.2.3.4 Test for tannins: The plant extract (0.2 g) was re-extracted with ethanol. The solution obtained was later treated with 5 % ferric chloride. A blue–black or blue-green appearance was taken as positive test for tannins (Adewunmi and Sofowara, 1980).

3.2.3.5 Test for saponins: A small portion of each extract was added to 2 ml of distilled water and boiled for 3-5 minutes .The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins. Froth as high as the aqueous layer was taken as moderate and lower than this as negative for the presence of saponins(Adewunmi and Sofowara, 1980).

3.2.3.6 Anthraquinones(Bonstrater-test Kraus modified): About 0.5 g of each extract was boiled for few minutes with 12 ml of 0.5 M potassium

hydroxide and 2 ml of hydrogen peroxide (10 %). The mixture obtained was then cooled, filtered, acidified and then extracted with a small quantity of ammonium hydroxide solution. A red color formed in the alkaline layer indicated the presence of anthraquinones Adewunmi and Sofowara, 1980).

3.2.3.7 Test for cardiac glycosides: Approximately 0.3 g of each extract from the three plants was hydrolyzed with HCl for few hours on water bath. To the hydrolysate in a test tube, 1ml of pyridine was added and was followed by few drops of sodium nitroprusside solutions. The mixture formed was then made alkaline with sodium hydroxide solution.The appearance of a pink or red colour indicated the presence of glycosides Adewunmi and Sofowara, 1980).

3.2.3.8 Test for glycosides: The crude powder (0.5 g) was dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. The mixture was heated on a water bath for 30 minutes. 5 ml of Fehling's solution was added and the mixture was again boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides (Majaw and Moirangthem, 2002).

3.2.4 Preparation of concentrations of solvents soluble fractions and extracts: Stock solutions of 10mg/ml concentrations of solvent soluble fractions and extracts of the plants were prepared by dissolving 5mg of each fraction or extract in 0.5ml of dimethyl sulphoxide (DMSO). From the stock solution, concentrations of 0.5mg/ml, 1.0mg/ml, and 0.5mg/ml were prepared by serial dilution method. Concentrations of ciprofloxacin standard antibiotic were similarly prepared as those of the plant extracts. They were stored in sterilized vials and kept at the lower compartment of a refrigerator until required for use.

3.2.5 Preparation of medium (Mueller-Hinton Agar (Uma et al., 2009 ; Sahoo et al., 2006)**) for culturing microorganisms:** An amount of 19.0g of Mueller-Hinton agar was weighed in a 500ml media bottle. 500ml of distilled water was measured, added to it and agitated for a while. The content was boiled on a hot plate fitted with magnetic stirrer until the powder completely dissolved to ensure uniformity. The pH of the media was adjusted to 7.0±0.1. The bottle was well corked and autoclaved at 121^{o}C for 15minutes at 1atm.

3.2.6 Antibacterial sensitivity test: The bacterium was cultured on nutrient agar and incubated at 37^{o}C for 24hours. The bacterium was repeatedly cultured to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4^{o}C. The agar diffusion method was used (Cruickshanks et al., 1980). Mueller-Hinton agar plates were inoculated with standard test inocula by direct streaking, and plates were properly labeled. A sterile cork borer (5mm in diameter) was used to make wells in the plates for the extracts. 0.1ml of the fractions was dispensed in to each well. The plates were left to allow diffusion of extracts before being placed in the incubator at 37^{o}C for 24hours (Karou et al., 2006).

The relative susceptibility of the organism to the extracts was indicated by zones of inhibition produced after incubation which was measured and recorded in millimeters. But the resistant strains grew up to the edges of the wells (Cheesbrough, 2000).Experiment was conducted in triplicates and values for diameter of growth inhibition were used to calculate the sample standard deviation which gave standard error mean values as those shown in tables 5, 6, 7 and 8.

4 RESULTS AND DISCUSSION

Table 1: weight, texture and colour of extracts and fractions obtained from *Acacia nilotica, Entada africana* and *Mimosa pigra L.*

Plant	Extract/Fraction	Weight	Texture	Colour
	Ethanol	12.10g	Gummy	Brown
	Diethyl ether	0.20g	Oily	Yellow
Acacia nilotica	Chloroform	0.10g	Oily	Yellow
	Methanol soluble	6.00g	Gummy	Dark brown
	Methanol insoluble	2.30g	Gummy	Brown
	Ethanol	45.65g	Gummy	Reddish brown
Entada africana	Diethyl ether	2.90g	Oily	Violet
	Chloroform	0.20g	Gummy	Yellow

Mimosa pigra L.	Methanol soluble	14.70g	Gummy	Deep dark red
	Methanol insoluble	17.20g	Very gummy	Dark red
	Ethanol	24.39g	Gummy	Green
	Diethyl ether	3.60g	Oily	Green
	Chloroform	0.60g	Oily	Green
	Methanol soluble	11.40g	Gummy	Dark brown
	Methanol insoluble	3.90g	Gummy	Dark brown

Table 2: Qualitative phytochemical content of the stem bark extracts of *Acacia nilotica*

Phytochemical	Ethanol extract	Diethyl Ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol Insoluble fraction
Alkaloids	+++	0	0	+++	+++
Amino acids	+	0	0	++	+++
Anthraquinones	+	0	+	++	+++
Cardiac glycosides	+++	0	0	+++	+++
Flavanoids	+++	+++	+	+++	+++
Glycosides	+	0	+	++	+++
Saponins	0	0	0	+++	+++
Steroids	+++	+++	++	+++	+++
Tannins	+++	+	0	+++	+++
Terpenoids	+++	+	0	+	0

Table 3: Qualitative phytochemical content of the stem bark extracts of *Entada africana*

Phytochemical	Ethanol extract	Diethyl Ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol Insoluble fraction
Alkaloids	+++	+++	0	+++	+++
Amino acids	++	++	0	++	++
Anthraquinones	++	+++	0	+++	+++
Cardiac glycosides	+++	0	++	+++	+++
Flavanoids	0	+	+++	+	++
Glycosides	0	++	+	+	+++
Saponins	++	+++	0	+++	+++
Steroids	+++	+++	+++	+++	+++
Tannins	+++	+++	+	0	0
Terpenoids	+++	+++	+++	+++	+++

Table 4: Qualitative phytochemical content of *Mimosa pigra L.*

Phytochemical	Ethanol extract	Diethyl Ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol insoluble fraction
Alkaloids	+++	+	0	+++	++
Amino Acids	+	++	0	0	+
Anthraquinones	++	+	0	++	+
Cardiac Glycosides	0	0	0	0	0
Flavanoids	+++	+++	+++	++	+++
Glycosides	+	+	++	+	0
Saponins	+	0	0	+++	+++

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Steroids	0	+++	0	+++	+++
Tannins	+++	+++	+	+++	+++
Terpenoids	++	++	+	0	0

KEY: +++ □ Appreciable amount ++ □ Moderate amount + □ Trace amount 0 □ Absent

Table 5: Zones of Growth Inhibition Exhibited by Stem-bark Extracts of *Acacia nilotica* on *Salmonella typhi*

PLANT	Concentration (mg/ml)	ZONES OF INHIBITION MEAN VALUES (mm)					
		Ethanol extract	Diethyl Ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol insoluble fraction	Ciprofloxacin
Acacia nilotica	0.5	0	0	0	0	0	5.3±2
	1.0	3.7±0.7	0	0	0	0	8.0±1
	1.5	4.3±0.6	0	0	9.0±0.5	2.5±0.5	15.0±3
	2.0						15.3±1
	10.0	7.0±1	23.0±1	0	10.8±0.8	3.3±0.5	

Table 6: Zones of Growth Inhibition Exhibited by Stem-bark Extracts of *Entada africana* on *Salmonella typhi*

PLANT	Concentration (mg/ml)	ZONES OF INHIBITION MEAN VALUES (mm)					
		Ethanol extract	Diethyl ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol insoluble fraction	Ciprofloxacin
Entada Africana	0.5	0	0	0	0	0	5.3±2
	1.0	0	0	0	9.2±0.3	0	8.0±1
	1.5	0	0	0	12.0±2	3.8±0.3	15.0±3
	2.0						15.3±1
	10.0	11.2±0.3	1.5±1	0	26.7±2	5.2±0.3	

Table 7: Zones of Growth Inhibition Exhibited by Extracts of *Mimosa pigra L.* on *Salmonella typhi*

PLANT	Concentration (mg/ml)	ZONES OF INHIBITION MEAN VALUES (mm)					
		Ethanol extract	Diethyl ether fraction	Chloroform fraction	Methanol soluble fraction	Methanol insoluble fraction	Ciprofloxacin
Mimosa pigra L	0.5	0	0	0	0	0	5.3±2
	1.0	0	0	0	0	0	8.0±1
	1.5	0	0	0	0	12.0±0	15.0±3
	2.0						15.3±1
	10.0	9.0±1	0	0	0	14.5±0.5	

Table 8: Zones of Growth Inhibition Exhibited by combined Ethanol Extracts of the three plants *on Salmonella typhi*

Plant	ZONES OF INHIBITION MEAN VALUES (mm)		
	Concentration (mg/ml)	Combined ethanol extract	Ciprofloxacin
Mimosa pigra L *Entada africana*	0.5 1.0	8.8±0.8 9.9±0.8	5.3±2 8.0±1

Acacia nilotica	1.5	10.3±0.6	15.0±3
	2.0		15.3±1
	10.0	11.2±1	

Key: Standard errors mean (±)

5 DISCUSSION

Results in table 1 show the weight, texture and color of extracts and fractions obtained from the stem-bark of both *Acacia nilotica* and *Entada africana*, and the entire plant, *Mimosa pigra L.* The ethanol extract of *Acacia nilotica* weighed 12.10g, and its highest weighed fraction was soluble methanol (6.0g). The ethanol extract of *Entada africana* weighed 45.65g, and its highest weighed fraction was insoluble methanol (17.20g). For *Mimosa pigra L.*, its ethanol extract weighed 24.39g, and its highest weighed fraction was also soluble methanol (11.40g). Despite methanol being the least solvent used in terms of volume in the fractionation process, results indicated that it was the best suitable among diethyl ether and chloroform solvents since it yielded the highest weighed fractions. Ethanol extracts, and both the soluble and insoluble methanol fractions of the three plants were gummy. Their soluble diethyl ether fractions were oily. Soluble chloroform fractions of both *Acacia nilotica* and *Mimosa pigra L.* were also oily, while that of *Entada africana* was gummy. The gummy texture shown by the ethanol extracts and soluble methanol fractions of the three plants, and the soluble chloroform fractions of both *Acacia nilotica* and *Mimosa pigra L* was the same as the texture of similar solvent extracts and fractions obtained from *Hyptis suaveolens LAM* as reported in the work of Mbatchou et al., 2010.The ethanol extract of *Acacia nilotica* was brown in appearance, and both its soluble diethyl ether and chloroform fractions were yellowish. These results revealed that there were some similarities in the texture and color of extracts and fractions of plants under investigation which can also be linked to the classes of phytochemicals present. The presence of a particular phytochemical cannot be revealed by test reagents when it is masked by other phytochemicals. This explains why test results in table 2 revealed the absence of saponins in ethanol extract, and its presence in soluble fractions obtained from the ethanol extract. These results are in conformity with those reported by Mbatchou and coworkers, 2010 on ethanol extracts and other solvent fractions obtained from *Hyptis suaveolens LAM*. Of all the solvent fractions obtained from ethanol extract of *Acacia nilotica* and tested for the presence of phytochemicals, soluble methanol fraction revealed the presence of 100%, while insoluble methanol fraction revealed the presence of 90% with terpenoids being the only class of phytochemicals absent. The soluble diethyl ether and chloroform fractions revealed the presence of the least number of classes of phytochemicals. Diethyl ether fraction revealed the presence of flavaniods, steroids, tannins and terpenoids, while chloroform fraction revealed the presence of anthraquinones, flavaniods, general glycosides and steroids.

In total, all the classes of phytochemicals tested were present in the stem-bark of *Acacia nilotica*. The presence of all the classes of phytochemicals tested in soluble methanol fraction was an indication that methanol solvent was optimal among diethyl ether and chloroform solvents in the fractionation process.

Test results of ethanol extract from *Entada africana* shown in table 3 revealed the presence of 80% of the tested classes of phytochemicals. Flavanoids and general glycosides were absent, but were present in subsequent fractions obtained from this extract. These results can be explained by masking effect.

On the whole, all the classes of phytochemicals tested were present in the stem-bark of *Entada africana*. The soluble diethyl ether and methanol fractions of the plant revealed the presence of 90% of the classes of phytochemicals respectively, whereas the soluble chloroform fraction revealed only 60%. These results indicated that diethyl ether and methanol solvents were optimal among the three solvents used in the fractionation process.

Test results of ethanol extract shown in table 4 revealed the absence of steroids which were present in solvent fractions obtained from the ethanol extract. This behavior can be explained by masking effect. Soluble diethyl ether fraction revealed the presence of 80% of the classes of phytochemicals, soluble methanol fraction revealed the presence of 70%, while soluble chloroform fraction revealed the presence of only 40%.

In total, of all the classes of phytochemicals tested in *Mimosa pigra L.*, only cardiac glycosides were absent. The presence of more classes of phytochemicals in soluble diethyl ether fraction than any other solvent fraction indicated that diethyl solvent was optimal among the three solvents used in the fractionation process.

Of the four solvent fractions obtained from ethanol extract of *Acacia nilotica*, only soluble chloroform fraction did not inhibit growth of *Salmonella typhi* at all its tested concentrations. This can be attributed to its low phytochemical content of only 40% of the classes of phytochemicals tested, and the complete absence of tannins and terpenoids which were present in the other solvent fractions. Masking effect can also be an attribute. The soluble diethyl ether fraction inhibited growth of the bacterial isolate only at its highest concentration tested with a mean value of 23.0±1.0mm. Both the soluble and insoluble methanol fractions demonstrated growth inhibitory effect at concentrations of 1.5mg/ml and above. Results of growth inhibitory activity exhibited on *Salmonella typhi* by the ethanol extract, the diethyl ether, and both the soluble and insoluble methanol fractions of *Acacia nilotica* confirmed that the plant contained bioactive compounds which account for its ethno-medicinal use in the treatment of typhoid fever.

Test results in table 6 revealed that ethanol extract of *Entada africana* exhibited growth inhibitory activity on *Salmonella typhi* only at its highest tested concentration of 10.0mg/ml with a mean value of 11.2±0.3mm. Of the four solvent fractions obtained from this extract, soluble chloroform fraction did not inhibit growth of *Salmonella typhi* at all its tested concentrations just as the soluble chloroform fraction of *Acacia nilotica*. The inactiveness of this soluble chloroform fraction can be attributed to its low phytochemical content of 60% of the classes of phytochemicals tested, and masking effect. The soluble diethyl ether fraction demonstrated growth inhibitory effect on the bacterial isolate only at its highest tested concentration of 10.0mg/ml with a mean value of 1.5 ±1.0mm. Both the soluble and insoluble methanol fractions exhibited growth inhibitory activity on *Salmonella typhi* at concentrations of 1.0mg/ml and1.5mg/ml respectively, and above. The soluble methanol fraction demonstrated higher growth inhibitory effects on the bacterial isolate than all other solvent fractions with growth inhibitory mean values of 9.2±0.3mm, 12.0±2mm and 26.7±2mm respectively which can be compared to those of ciprofloxacin broad spectrum antibiotics. Results of growth inhibitory activity exhibited on *Salmonella typhi* by the ethanol extract, the diethyl ether and both the soluble and insoluble methanol fractions of *Entada africana* indicated that the plant contained anti-typhoidal agents which supported its use in combination with *Acacia nilotica and Mimosa pigra L.* in the local treatment of typhoid fever.

Test results in table 7 revealed that ethanol extract of *Mimosa pigra L.* inhibited the growth of *Salmonella typhi* only at its highest tested concentration of 10.0mg/ml with a mean value of 9.0±1.0mm. Also, the first three solvent fractions obtained from ethanol extract did not inhibit growth of the bacterial isolate at the different concentrations tested. It was only the insoluble methanol fraction that exhibited growth inhibitory effect at concentrations of 1.5mg/ml and above with mean values of 12.0±0.0mm and 14.5±0.5mm respectively. Results of growth inhibition shown on *Salmonella typhi* by the ethanol extract and the insoluble methanol fraction of *Mimosa pigra L.* indicated that the plant contained anti-typhoidal agents which supported its use in combination with *Acacia nilotica and Entada africana* in the local treatment of typhoid fever.

Test results in table 8 for the combined ethanol extracts of *Acacia nilotica, Entada africana* and *Mimosa pigra L.* revealed that there was enhancement of growth inhibition on *Salmonella typhi* which yielded mean values of 8.8±0.8mm, 9.9±0.6mm, 10.3±0.6mm and 11.2±1mm at concentrations of 0.5mg/ml, 1.0mg/ml, 1.5mg/ml and 10.0mg/ml respectively. In some instances, the singly tested ethanol extract of each of the three plants did not demonstrate growth inhibition at concentrations below 10.0 mg/ml, whereas combined extracts did. The growth inhibitory effect demonstrated by the combined ethanol extracts on the bacterial isolate strongly supported the use of the three plants to prepare concoctions for the local treatment of typhoid fever.

6 CONCLUSION

Upon methanol being the least solvent employed in terms of volume in the fractionation process of this research, results indicated that it was the best suitable among diethyl ether and chloroform

solvents since it yielded the highest weighed fractions. Also, the presence of 100 and 90% of the tested classes of phytochemicals in the soluble methanol fractions of *Acacia nilotica* and *Entada africana* respectively can be attributed to the best suitable fractional ability of methanol solvent . Some similarities in textures and colors of extracts and fractions of the three plants under investigation can be linked to the same classes of phytochemicals present in them.

The growth inhibitory activity exhibited on *Salmonella typhi* by the ethanol extracts and other solvent fractions of *Acacia nilotica, Entada africana* and *Mimosa pigra L.* is associated with the classes of phytochemicals present in them which have been reported to consist of antimicrobial therapeutic properties by Gislene and coworkers, 2000. This is a confirmation that the three plants under investigation contained bioactive compounds or anti-typhoidal agents which supported their ethno-medicinal use in combination to treat typhoid fever.

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