Research Article

Antibacterial Properties of Selected Medicinal Plants on *E.coli*, Salmonella sp and Shigella sp

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ABSTRACT

The antibacterial activities of the leaves of *Ficus carpensis*, *Newbouldia laevis*, *Vitex doniana*, *Spondias mombin* and *Psidium guajava* on *Escherichia coli, Salmonella* sp. and *Shigella* sp which are common causes of infectious diarrhea were studied. Ethanolic and aqueous extracts of *Ficus carpensis, Newbouldia laevis, Vitex doniana, Spondias mombin* and *Psidium guajava* were obtained using cold extraction at room temperature. The filtrates were dried at 40oC to obtain the crude extracts and stored at 4oC until use. Phytochemical analysis revealed the presence of tannins, flavonoids, glycosides, saponins, alkaloids, steroids, terpenoids. In vitro antimicrobial studies were investigated using macrobroth dilution method to determine the minimum inhibitory concentration (MIC) with ethanol and aqueous extracts of Psidium guajava possessing the lowest MIC of 50 mg/ml. The antimicrobial assay revealed that ethanolic extracts showed more antimicrobial activity than the aqueous extracts. Ethanol and aqueous (hot and cold water) extracts of *Newbouldia laevis, Spondias mombin* and *Psidium guajava* exhibited varying degrees of antimicrobial activity with diameters of zone of inhibition [(21, 18, 19) mg/ml, (20, 17, 17) mg/ml, (18, 15, 17) mg/ml; (23, 19, 20) mg/ml, (20, 18, 18) mg/ml, (22, 19, 20) mg/ml; (26, 23, 24) mg/ml, (24, 21, 20) mg/ml, (24, 21, 21) mg/ml] respectively while ethanol and aqueous extracts of *Ficus carpensis* and *Vitex doniana* showed no activity against the diarrheagenic strains of *E. coli, Salmonella* sp. and *Shigella* sp. The synergistic combination of different plant extracts and also plant extracts with ciprofloxacin showed increased antibacterial effect.

Keywords: Antibacterial, Phytochemicals, Psidium guajava, rotary evaporator.

INTRODUCTION

All parts of the world and mostly in developing countries, enteric bacteria are major causes of food borne illness and gastrointestinal disturbances. Symptoms of food borne illness range from stomach upsets to more serious symptoms including diarrhea, vomiting, abdominal cramps and fever. Diarrhea caused by intestinal pathogens is a global health concern [1] and the second leading cause of mortality and morbidity throughout the world in children less than 5 years[2]. Despite available methods for managing diarrhea, an about 4.6 million person which includes 2.5 million children die yearly from diarrhea[3].The major bacterial groups causing infectious diarrhea are pathogenic *E. coli*[4] and invasive bacterial pathogens like salmonella, shigella, campylobacter and *Vibrio cholerae* [5]. Many studies have validated the use of traditional medicinal plants in treating diarrhea[6,7,8]. However, the curative and remedial potentials of some of these local medicines have not been evaluated scientifically. Among these plants, *Ficus carpensis* (Cape fig, known as akpuru in Igbo), *Newbouldia laevis* (African border tree, known as ogirisi in Igbo), *Vitex doniana* (Black plum, known as uchakuru in Igbo), *Spondias mombin* (Hog plum, known as ichikara in Igbo) and *Psidium guajava* (Guava) which are spread throughout tropical Africa enjoys a lot of ethnomedicinal applications in Nigeria. Therefore, it is important to form the scientific basis for the therapeutic actions of these medicinal plants as they may serve as the basis to develop more effective antibiotics. The aim of this research was to evaluate the antibacterial properties of ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* (cape fig, akpuru), *Newbouldia laevis* (African border tree, ogirisi), *Vitex doniana* (black plum, uchakuru), *Spondias mombin* (ichikara) and *Psidium guajava* (guava) on diarrhea-causing organisms; *Escherichia coli,* salmonella specie and shigella specie.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of plants were collected from Nwakpu village in Ndufu-Alike Community in Ikwo L.G.A of Ebonyi State. The plant materials were examined and authenticated in the Department of Botany, University of Nigeria, Nsukka. Identified samples were transported to the Biology Laboratory of Federal University Ndufu-Alike Ikwo where the plant materials were air dried at room temperature, ground and kept in air tight bottles in a cool, dark and dry place until use.

Preparation of Plant Extracts

Twenty grams (20g) of the blended samples were dispensed into a conical flask and 100 m1 of ethanol and sterile distilled water (hot and cold) added separately to each of the conical flasks. Each of the conical flasks were covered with a cork, properly mixed together and allowed to stand for 24 h. Extract from each solvent was decanted and then filtered with Whatman No.1 filter paper. The filtrates were concentrated using a rotary evaporator to obtain crude extracts. The different crude extracts were stored at 4 °C for further uses.

Sterility Test of the Plant Extracts

Each of the plant extract was tested for microbial growth or contaminant by plating them on Nutrient agar and incubated at 37°C for 24 h [9].

Test Strains Used

Three clinical isolates, *Escherichia coli*, salmonella specie and shigella specie isolated from stool samples were used as test organisms for antibacterial activity of the leaves of the plant extracts. Standard strains of *Escherichia coli*, *Salmonella sp*., and *Shigella sp*. were obtained from Federal Teaching Hospital, Abakaliki (FETHA).

Collection of Stool Samples

Sterile universal bottles were used to collect stool samples from patients. The stool samples were transported to Biology Laboratory of Federal University Ndufu-Alike Ikwo maintaining cold chain and processed the same day.

Isolation of Bacterial Isolates

Feacal specimens were processed according to the methods described by[10]. A loopful of feacal sample was streaked on Mac Conkey Agar (MLA) and Salmonella-Shigella Agar (SS) and incubated for 24 h at 37°C. Convex, pink or colourless colonies with or without black centers on MacConkey agar plates were considered for further identification. Also, colourless colonies with or without black centers on Salmonella-Shigella agar (SS) plates were also selected for further identification. Finally, colonies that showed the desired morphology and colour were again re-streaked on the above media to obtain pure culture.

Maintenance of Suspected Bacterial Organisms

The bacterial isolates, *Escherichia coli*, salmonella specie and shigella specie obtained from stool samples were sub-cultured unto fresh plates of Nutrient agar and incubated for 24 h at 37°C.

Characterization of Bacterial Isolates

Colonies that showed characteristic appearance on selective media were used for characterization of isolated bacteria. Characterization was done using the following tests: Gram staining, catalase test, hydrogen sulphide production test, motility test and indole production test.

Preparation and Standardization of Suspected Bacterial Inoculum

The bacterial inoculum size was standardized according to the guideline of[9]. This was achieved by transferring a pure bacterial isolate growing on Nutrient agar into sterile nutrient broth. The nutrient broth was incubated at 37°C for 24 h until it achieves a turbidity equivalent to 0.5 McFarland standard. The final inoculum size was standardized to 10^6 CFU/ml using a spectrophotometer.

Screening for antibacterial Activity of Ethanol and Aqueous (Hot and Cold Water) Extracts Using Agar Well Diffusion Method

The antibacterial properties of ethanolic and aqueous (hot and cold) plant extracts were determined using agar well diffusion method as stated in [9]. A sterile cotton swab was used to collect standardized clinical isolates and plated on sterile Mueller Hinton agar plates. Wells were made using a 6 mm sterilized stainless cork borer on the plate under aseptic conditions and filled with 400 mg/ml each of the respective plant extracts, solvent blanks and standard drug, ciprofloxacin. The inoculated Petri dishes were left to stand for one hour at room temperature. This enabled the diffusion of the extract into the media before the growth of organism commenced. The inoculated petri dishes were incubated for 24 h at 37^0C. The diameters of zones of inhibition around the wells were measured and recorded.

Screening for Synergistic Activities of Ethanol Plant Extracts on *E. coli*, *Salmonella* sp. and *Shigella* sp.

Using Clinical and Laboratory Standards Institute guideline (2007), a sterile cotton swab was used to collect standardized suspected isolates and plated on sterile Mueller Hinton agar plates. Wells were made using a 6 mm sterilized stainless cork borer on the plate under aseptic conditions and a combined volume of 400 mg/ml mixture of plant extracts were added into each well. The inoculated petri dishes were incubated for 24 h at 37^0C. The diameters of zones of inhibition around the wells were recorded. Parallel experiment was carried out and the results represented the average of three independent experiments. Synergism effect was considered when combined zone of inhibition was increased by 5mm compared to the individual plant extract or drug [11].

Determination of Minimum Inhibitory Concentration (MIC)

Macro broth dilution method was used as described by [9]. Varying concentration of the extract (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml were prepared. A 2 ml of each concentration was added to each of 2 ml of Nutrient Broth containing 0.1 mL of standardized test organism of bacterial cells. The tubes were incubated aerobically at 37 °C for 24 h.The tube with the lowest concentration of extracts which showed no growth after incubation was reported as the MIC.

Phytochemical Analysis

The ethanol and aqueous (hot and cold water) extracts of the plants were subjected to qualitative phytochemical screening for the identification of the flavonoids, steroids, saponins, glycosides, alkaloids and tannins using methods described by [12,13].

Statistical analysis

Data was analyzed using mean of three replicates.

RESULTS

The Result of Antibacterial Inhibitory Activities of Ethanol and Aqueous (Hot and Cold Water) Plant Extracts on bacterial isolates is shown in Table 1.

The ethanol leaf extract of *Psidium guajava* had the highest inhibitory effect on *E. coli,* salmonella specie and shigella specie than the other plant extracts with diameter of zone of inhibition 24.33 ± 0.58 mm, 22.00 ± 0.00 mm, 23.33 ± 0.58 mm respectively. *Spondias mombin* ethanol extract and *Newbouldia laevis* ethanol extract exhibited moderate inhibitory action on the bacterial isolates with diameter of zone of inhibition (22.00 ± 1.00 mm, 18.33 ± 0.58 mm, 18.00 ± 0.00 mm) and (19.33 ± 0.58 mm, 18.00 ± 1.00 mm, 18.00 ± 1.00 mm) respectively. Ethanol extracts of *Psidium guajava* inhibitory activity was highest on *E. coli* and the least effect on salmonella specie. Ciprofloxacin a positive control, was also effective in inhibiting the growth of the bacterial isolates with diameters of zone of inhibition (20.00 ± 0.00 mm, 18.00 ± 0.00 mm, 18.67 ± 0.71 mm) respectively. *Psidium guajava* ethanol extract was more effective in inhibiting the growth of the test bacterial isolates when compared with the standard drug, ciprofloxacin. Negative control (ethanol) and ethanol extracts of *Ficus carpensis* and *Vitex doniana* showed no visible zone of inhibition against the suspected isolates.

The results of preliminary tests for antibacterial activities of the aqueous hot water plant extracts of *Ficus carpensis, Newbouldia laevis, Spondias mombin* and *Psidium guajava* revealed that the following plant extracts, *Newbouldia laevis, Spondias mombin* and *Psidium guajava* showed antibacterial activity against the three suspected bacterial isolates, *E. coli,* salmonella specie and shigella specie tested, with diameter of zone of inhibition ranging from 17.00 ± 0.00 – 23.33 ± 0.58 mm. *Psidium guajava* hot water extract appeared to exhibit the greatest antibacterial activity against the test bacterial isolates, *E. coli,* salmonella specie and shigella specie with diameters of zone of inhibition (23.33 ± 0.58 mm, 20.67 ± 0.71 mm, 20.00 ± 0.00 mm) respectively when compared with the standard drug, ciprofloxacin. The negative control (hot water) and hot water extracts of *Vitex doniana* and *Ficus carpensis* showed no visible zone of inhibition against the suspected bacterial organisms.

The result indicated that the cold water plant extracts showed antibacterial activities at variable degrees against the bacterial isolates, *E. coli,* salmonella specie and shigella specie. Cold water extracts of *Psidium guajava* displayed the most important spectrum of inhibitory activity with diameters of zone of inhibition (22.67 ± 0.71 mm, 21.33 ± 0.58 mm, 21.00 ± 0.00 mm), followed by the extracts of *Spondias mombin* (21.00 ± 0.00 mm, 19.00 ± 0.00 mm, 18.00 ± 0.00 mm) and *Newbouldia laevis* (17.67 ± 0.71 mm, 16.00 ± 0.00 mm, 17.67 ± 1.15 mm). *Psidium guajava* cold water extract was more effective in inhibiting bacterial growth than the standard drug (Ciprofloxacin). The negative control (hot water) and cold water extracts of *Vitex doniana* and *Ficus carpensis* did not show any antibacterial activity against the suspected organisms.

The Result of the Minimum Inhibitory Concentration (MIC) of the Active Ethanol and Aqueous (Hot and Cold Water) Plant Extracts on bacterial isolates is shown in Table 2.

The result of minimum inhibitory concentration (MIC) of active ethanol plant extracts against *E. coli,* salmonella specie and shigella specie were as follows: *Newbouldia laevis* (200 mg/ml), *Spondias mombin* (100 mg/ml) and *Psidium guajava* (50 mg/ml) on the tested bacterial organisms respectively.

The result of minimum inhibitory concentration (MIC) of active aqueous hot water plant extracts against *E. coli,* salmonella specie and shigella specie were as follows: *Newbouldia laevis* (200 mg/ml), *Spondias mombin* (100 mg/ml) and *Psidium guajava* (50 mg/ml) on the tested bacterial organisms respectively.

The result of minimum inhibitory concentration (MIC) of active aqueous cold water plant extracts against *E. coli,* salmonella specie and shigella specie were as follows: *Newbouldia laevis* (200 mg/ml), *Spondias mombin* (100 mg/ml) and *Psidium guajava* (50 mg/ml) on the tested bacterial organisms respectively.

The Result of the Synergistic Activities of Ethanol Plant Extracts on *E. coli,* Salmonella Specie and Shigella Specie are Shown in Table 3.

The synergistic activities of ethanol leaf extracts in combination of two and also with the standard drug, ciprofloxacin exhibited varying degrees of zone of inhibition as shown in Table 4. For *E. coli,* diameters above 28 mm zone of inhibition were noted in ethanol extract combinations: E+F, D+F, D+E, C+D and B+D. Ethanol extract combinations of C+F, C+E, B+F, B+E and A+F exhibited zone of inhibition ranging from 25-28 mm. Also, zone of inhibition less than 25 mm were exhibited by the following plant extract combinations B+C, A+E, A+D, A+C and A+B. The highest zone of inhibition of 31.67 ± 0.71 mm against *E. coli* was exhibited by ethanolic extract combination of C+D while the lowest zone of inhibition of 17.00 ± 0.00 mm was exhibited in extract combination A+E.

Table 1: Antibacterial Inhibitory Activities of Ethanol and Aqueous (Hot and Cold Water) Plant Extracts on bacterial isolates

Plant extract	Bacterial Isolates/Diameters of Zone of Inhibition in mm								
	Ethanol extract			Hot water extract			Cold water extract		
	Escherichia coli	*Salmonella specie*	*Shigella specie*	*Escherichia coli*	*Salmonella specie*	*Shigella specie*	*Escherichia coli*	*Salmonella specie*	*Shigella specie*
Ficus carpensis	-	-	-	-	-	-	-	-	-
Newbouldia laevis	19.33 ± 0.58	18.00 ± 0.00	18.00 ± 0.00	19.67 ± 1.15	17.33 ± 0.58	17.00 ± 0.00	17.67 ± 0.71	16.00 ± 0.00	17.67 ± 1.15
Vitex doniana	-	-	-	-	-	-	-	-	-
Spondias mombin	22.00 ± 1.00	18.33 ± 0.58	18.00 ± 0.00	21.33 ± 0.58	18.00 ± 0.00	17.67 ± 0.71	21.00 ± 0.00	19.00 ± 0.00	18.00 ± 0.00
Psidium guajava	24.33 ± 0.58	22.00 ± 0.00	23.33 ± 0.58	23.33 ± 0.58	20.67 ± 0.71	20.00 ± 0.00	22.67 ± 0.71	21.33 ± 0.58	21.00 ± 0.00
Standard drug (Ciprofloxacin 10 mg/L)	20.00 ± 0.00	18.00 ± 0.00	18.67 ± 0.71	18.00 ± 0.00	17.00 ± 0.00	17.33 ± 0.58	18.00 ± 0.00	17.00 ± 0.00	17.00 ± 0.00
Negative control (ethanol, hot water and cold water respectively)	-	-	-	-	-	-	-	-	-

Keys: (-) = No visible zone of inhibition

Table 2: Minimum Inhibitory Concentration (MIC) of the Active Ethanol and Aqueous (Hot and Cold Water) Plant Extracts on Suspected Organisms.

Plant extract	Suspected Bacterial Isolates/Minimum Inhibitory Concentration (MIC) in mg/ml								
	Ethanol extract			Hot water extract			Cold water extract		
	Escherichia coli	*Salmonella specie*	*Shigella specie*	*Escherichia coli*	*Salmonella specie*	*Shigella specie*	*Escherichia coli*	*Salmonella specie*	*Shigella specie*
Newbouldia laevis	200	200	200	200	200	200	200	200	200
Spondias mombin	100	100	100	100	100	100	100	100	100
Psidium guajava	50	50	50	50	50	50	50	50	50
Standard drug (Ciprofloxacin 10 mg/L)	100	100	100	100	100	100	100	100	100

Table 3: Synergistic Activities of Ethanol Extract of Selected Plant Leaves in Combination of Two against Suspected Organisms

Suspected Organism	Combination of Plant Extracts Tested/ Zone of Inhibition in mm														
	A+B	A+C	A+D	A+E	A+F	B+C	B+D	B+E	B+F	C+D	C+E	C+F	D+E	D+F	E+F
E. coli	18.00 ±1.00	24.00 ±0.00	22.00 ±1.73	17.00 ±0.00	25.00 ±0.00	24.33 ±0.58	28.33 ±0.58	26.67 ±0.71	25.33 ±0.58	31.67 ±0.71	25.00 ±1.00	26.00 ±1.73	29.00 ±1.00	29.33 ±0.58	30.33 ±0.58
Salmonella specie	19.00 ±0.00	18.00 ±1.00	17.67 ±1.15	20.67 ±0.71	23.00 ±0.00	19.00 ± 1.00	24.33 ± 0.58	19.67 ± 1.15	21.00 ± 1.73	20.00 ± 0.00	21.00 ± 1.00	25.00 ± 1.00	21.00 ± 0.00	23.33 ± 0.58	27.00 ± 0.00
Shigella specie	18.00 ± 1.00	19.33 ± 0.58	16.33 ± 0.58	24.33 ± 0.58	21.00 ± 1.00	21.33 ± 0.58	24.33 ± 0.58	23.33 ± 0.58	22.00 ± 1.73	22.00 ± 1.00	22.00 ± 1.00	22.00 ± 0.00	23.33 ± 0.58	23.00 ± 1.00	28.00 ± 0.00

A = *Ficus carpensis*, B =*Newbouldia laevis*, C = *Vitex doniana*, D =*Spondias mombin*, E = *Psidium guajava*

F = Standard drug (ciprofloxacin)

Table 4: Presence of Phytochemicals in the plant extracts.

Phytochemicals	*Ficus carpensis*			*Newbouldia laevis*			*Vitex doniana*			*Spondias mombin*			*Psidium guajava*		
	ET	HW	CW	ET	HW	CW	ET	HW	CW	ET	HW	CW	ET	HW	CW
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	-	-	+	-	-	-	-	-	+	-	-	+	+	+
Saponins	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Glycosides	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Keys: ET = Ethanol extract, HW = Hot Water extract, CW = Cold water extract, (+) = Present, (-) = Absent

For salmonella specie, diameters above 21 mm zone of inhibition were exhibited by ethanol extract combinations: E+F, D+F, C+F, B+D and A+F followed by other extract combinations. The highest zone of inhibition of 27 ± 0.00 mm against salmonella specie was exhibited by ethanolic extract combination of E+F while the lowest zone of inhibition of 17.67 ± 1.15 mm was exhibited in extract combination A+D For shigella specie, an average diameter between 23-28 mm zones of inhibition were observed in the following ethanol plant extract combinations: E+F, D+F, D+E, B+E, B+D and A+E followed by other extract combinations. The highest zone of inhibition of 28.00 ± 0.00 mm against shigella specie was exhibited by ethanolic extract combination of E+F while the lowest zone of inhibition of 16.33 ± 0.58 mm was exhibited by extract combination A+D.

The Result of the Presence of Phytochemicals in the plant extracts is shown in Table 4.

Qualitative phytochemical analysis of ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* showed the presence of flavonoids, alkaloids, tannins and saponins. Glycosides were not present in both ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* with steroids being present in ethanol extract of *Ficus carpensis*. The preliminary phytochemical analysis of ethanol and aqueous (hot and cold water) extracts of *Newbouldia laevis* showed the presence of flavonoids, alkaloids, saponins, glycosides, and tannins. Steroids were not present in aqueous extracts of *Newbouldia laevis*. The result of the phytochemical analysis of ethanol and aqueous (hot and cold water) extracts of *Vitex doniana* showed the presence of flavonoids, tannins and alkaloids. Glycosides and saponins were present in both ethanol and hot water extracts of *Vitex doniana* with steroids been absent in both ethanol and aqueous extracts of *Vitex doniana*. Qualitative phytochemical analysis of ethanol and aqueous (hot and cold water) extract of *Spondias mombin* showed the presence of flavonoids, alkaloids, saponins and tannins. Steroids were not present in the aqueous plant extracts with glycosides being present in ethanol and hot water extracts of *Spondias mombin*.

Qualitative phytochemical analysis of ethanol and aqueous (hot and cold water) extract of *Psidium guajava* showed the presence of alkaloids, steroids, flavonoids, saponins, tannins and glycosides.

DISCUSSION

The evaluation of medicinal plants for antibacterial activity against *E. coli*, salmonella specie and shigella specie showed that some of the medicinal plants tested such as *Newbouldia laevis, Spondias mombin* and *Psidium guajava* exhibited inhibitory activities while some did not exhibit any activity (*Ficus carpensis* and *Vitex doniana*). Of the five plants used, result showed that both ethanol and aqueous (hot and cold water) extract of *Newbouldia laevis, Spondias mombin* and *Psidium guajava* exhibited antibacterial activity with diameters of zone of inhibition [(19.33 ± 0.58, 18.00 ± 0.00, 18.00 ± 0.00) mm, (19.67 ± 1.15, 17.33 ± 0.58, 17.00 ± 0.00) mm, (17.67 ± 0.71, 16.00 ± 0.00, 17.67 ± 1.15) mm; (22.00 ± 1.00, 18.33 ± 0.58, 18.00 ± 0.00) mm, (21.33 ± 0.58, 18.00 ± 0.00, 17.67 ± 0.71) mm, (21.00 ± 0.00, 19.00 ± 0.00, 18.00 ± 0.00) mm; (24.33 ± 0.58, 22.00 ± 0.00, 23.33 ± 0.58) mm, (23.33 ± 0.58, 20.67 ± 0.71, 20.00 ± 0.00) mm, (22.67 ± 0.71, 21.33 ± 0.58, 21.00 ± 0.00) mm] respectively on the suspected isolates, *E. coli,* salmonella specie and shigella specie while ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* and *Vitex doniana* showed no inhibitory activity against the suspected isolates. *Psidium guajava* ethanol extract exhibited highest zone of inhibition against *E. coli* with diameter of zone of inhibition 24.33 ± 0.58 mm, followed by ethanol extracts of *Spondias mombin* and *Newbouldia laevis* with zone of inhibition measuring 22.00 ± 1.00 mm and 19.33 ± 0.58 mm respectively. Antibacterial activity against salmonella specie and shigella specie inhibited mostly the ethanol plant extracts than the aqueous extracts. This result relates to the work done by [14,15].The hot water extracts of the leaves of *Newbouldia laevis, Spondias mombin* and *Psidium guajava* showed inhibitory activities against *E. coli,* salmonella specie and shigella specie with inhibitory zone diameter ranging between 17.00 ± 0.00 – 23.33 ± 0.58 mm while hot water extracts of *Psidium guajava* exhibited the greatest antibacterial inhibitory effect with zone of inhibition (23.33 ± 0.58 mm, 20.67 C 0.71 mm, 20.00 ± 0.00 mm) on *E. coli,* salmonella specie and shigella specie when compared with the standard drug, ciprofloxacin. Cold water extracts of the leaves of *Newbouldia laevis, Spondias mombin* and *Psidium guajava* showed inhibitory activities against *E. coli,* salmonella specie and shigella specie with inhibitory zone diameter ranging between 16.00 ± 0.00 mm – 22.67 ± 0.71 mm. Cold water extracts of *Psidium guajava* exhibited the greatest antibacterial inhibitory effect with zone of inhibition diameter measuring inhibition (22.67 ± 0.71 mm, 21.33 ± 0.58 mm, 21.00 ± 0.00 mm) on *E. coli,* salmonella specie and shigella specie when compared with the standard drug, ciprofloxacin. Ethanol and aqueous extracts of *Newbouldia laevis* showed similar values with their MICs as 200 mg/ml, 200 mg/ml, and 200 mg/ml while ethanol and aqueous extracts of *Spondias mombin* also showed similar values with their MICs as 100 mg/ml, 100 mg/ml, and 100 mg/ml respectively for the bacterial isolates. *Psidium guajava* ethanol and aqueous extracts had the highest inhibitory and bactericidal concentration against *E. coli,* salmonella specie and shigella specie with MIC 50 mg/ml followed by ethanol and aqueous extracts of *S. mombin* 100 mg/ml and then ethanol and aqueous extracts of *Newbouldia laevis* 200 mg/ml. This study thus confirms the traditional claims on these plants as a remedy to treat diarrhea and dysentery. This agrees with the research by [16] after examining the antibacterial activity of some medicinal plants on common enteric food-borne pathogens. This corroborates the work of[17], who evaluated antimicrobial activities of

Croton zambesicus, *Zygotritonia crocea* and *Spondias mombin*.

In this study, synergy effect resulting from the combination of different plant extracts and ciprofloxacin with crude plant extracts were verified for all plants. Synergistic activity of selected ethanol leaf extracts, in combination of two against suspected organisms ranged from 16.33 ± 0.58 – 31.67 ± 0.71 mm zone of inhibition. The highest inhibition zone diameter of 31.67 ± 0.71 mm was exhibited against *E. coli* in ethanolic extract combination C+D while the lowest zone of inhibition 16.33 ± 0.58 mm was observed against shigella specie in extract combination A+D. In our present study, when *Ficus carpensis* and *Vitex doniana* were used singly on the suspected bacterial isolates, no visible zone of inhibition was observed but when used in combination with other plant extracts additive and/or synergistic effects were produced. Combination of *Ficus carpensis* with *Newbouldia laevis*, *Spondias mombin* or *Psidium guajava* resulted in additive effects on all the suspected bacterial isolates because the zone of inhibition produced by the combinations was only 1-4 mm more than that produced by the individual plant extracts (less than 5 mm). On the other hand, combination of *Ficus carpensis* + *Vitex doniana* and the plant-drug combination *Ficus carpensis* + ciprofloxacin resulted in synergistic effects producing additional increase of 5 mm than that produced by either *Ficus carpensis* or *Vitex doniana* alone and ciprofloxacin alone respectively.The combination of *Vitex doniana* + *Psidium guajava* produced an additive effect on *E. coli*. Combination of *Vitex doniana* + *Newbouldia laevis*, *Vitex doniana* + *Psidium guajava* produced additive effects on salmonella specie and shigella specie while synergistic effects were observed in extract combination *Vitex doniana* + *Ficus carpensis* and *Vitex doniana* + ciprofloxacin on both salmonella specie and shigella specie. Combination of *Vitex doniana* + *Ficus carpensis*, *Vitex doniana* + *Newbouldia laevis*, *Vitex doniana* + *Spondias mombin*, *Vitex doniana* + ciprofloxacin produced synergistic effects on *E. coli* producing an additional rise of ≥ 5 mm than that produced by any of the plant or drug alone.Also, synergistic effects were produced by combination of *Newbouldia laevis* + *Spondias mombin*, *Spondias mombin* + *Psidium guajava*, *Spondias mombin* + ciprofloxacin and *Psidium guajava* + ciprofloxacin on *E. coli* and combination of *Newbouldia laevis* + *Spondias mombin* and *Psidium guajava* + ciprofloxacin on both salmonella specie and shigella specie. This is in line with the report that the combination of different plant extracts and also antibacterials and plants produced synergistic antibacterial activity against resistant bacteria [18] and stand out as veritable sources of potential resistance modifying agents. Qualitative phytochemical analysis of ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* showed the presence of flavonoids, saponins, tannins and alkaloids. Absence of glycosides was observed in both ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis* with steroids being only present in ethanol extracts of *Ficus carpensis*. The presence of flavonoids, saponins, alkaloids and tannins in the ethanol extracts of *Ficus carpensis* was in line with the reports of [19]. Several available literature reports are discordant on the phytochemical composition of the plant. [20] did not detect the presence of saponins and glycosides in the ethanol extracts of *Ficus carpensis* in contrast to the report of [21] which revealed the absence of alkaloids, flavonoids and glycosides but showed high quantities of saponins in the ethanol leaf extracts.

The preliminary phytochemical analysis of ethanol and aqueous (hot and cold water extract) extracts of *Newbouldia laevis* showed the presence of flavonoids, steroids, alkaloids, saponins, glycosides and tannins. Steroids were not present in aqueous extracts of *Newbouldia laevis*. [22,23,24] detected the presence of tannins, steroids, flavonoids and glycosides in the leaf extract which was in line with the result gotten from the phytochemical analysis of ethanol and aqueous extracts of *Newbouldia laevis*. This was different with [24] did not detect the presence of alkaloids and saponins in their study while, [25] reported the absence of alkaloids, flavonoids, saponins and steroids on the leaf extract. Differences in these reports could be attributed to environmental factors, time of collection and handling.

Phytochemicals found in plants exert antimicrobial activities via different mechanisms. Tannins for instance act by deprivation of iron or by specific interactions with essential proteins [26]. Tannins are well known for their antioxidant and antimicrobial properties, as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics [27]. Tannins are astringent in nature and plants that possess this component are used in treating gastrointestinal problems such as dysentery and diarrhea [28]. This may therefore explain the use of *Ficus carpensis, Newbouldia laevis, Vitex doniana*, Spondias *mombin* and *Psidium quajava* in folklore remedy for gastrointestinal ailments [14, 15, 19].

CONCLUSION

Phytochemical screening of ethanol and aqueous (hot and cold water) extracts of *Ficus carpensis, Newbouldia laevis, Vitex doniana, Spondias mombin* and *Psidium guajava* showed the presence of tannins, flavonoids, glycosides, alkaloids, saponins and steroids. The ethanol extract of *Psidium guajava* exhibited the greatest antibacterial potency against *E. coli,* salmonella specie and shigella specie. This study showed that all the leaves of plant materials tested possess a measure of antibacterial properties and the antibacterial potency is much greater when used in combination against the suspected bacterial isolates. Hence, there is a possibility of combination of leaves of plant extracts against infections caused by *E. coli*, *Salmonella* sp. and *Shigella* sp. as seen from the results. The synergistic effects of the antibacterial activities of these leaves will be used in the administration of these leaves in the treatment of gastrointestinal ailments.

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