How to Cite:
Oghenemaro, E. F., Jemikalajah, J. D., Oghenerhivwe, M. P., & Uruemu, I. S. (2022). Gastrointestinal tract microbiome: The effect of Cnestis ferruginea Vahl ex DC (Connaraceae) root bark extract on albino Wistar rats. *International Journal of Health Sciences*, *6*(S2), 5420–5431. https://doi.org/10.53730/ijhs.v6nS2.6362

Gastrointestinal tract microbiome: The effect of Cnestis ferruginea Vahl ex DC (Connaraceae) root bark extract on albino Wistar rats

Enwa Felix Oghenemaro
Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmacy, Delta State University, Abraka

Johnson Daniel Jemikalajah
Department of Medical Laboratory Science, Faculty of Science, Delta State University, Abraka, Nigeria

Monioro Philip Oghenerhivwe
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka, Nigeria

Ikpama Sephora Uruemu
Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmacy, Delta State University, Abraka

***Abstract*---**Objective: To determine the effect of *Cnestis ferruginea Vahl ex DC (connaraceae)* root bark extract on gastrointestinal tract (GIT) microbiome of Albino wistar rats. Materials and Method: A total of 15 wistar rats were used for this study. They were randomly divided into 5 groups. Fecal samples were collected, a 10 fold serial dilution was done and plated using a spread plate method. The powdered root bark of *Cnestis ferruginea* was extracted with 80% methanol using a soxhlet extractor at 60°c. The resulting extract was administered to the rats in different doses, 200 mg/kg body weight (low dose) and 400 mg/kg body weight (high dose) for 14 days. Results: The study revealed that at both low and high doses, the root bark extract of *C. ferruginea* was able to reduce the population of the bacteria present in the GIT of the Wistar rats with only *providencia, Aeromonas spp,* and *Proteus spp* as remaining organisms when compared to the control group, thus confirming the antimicrobial effect of the root extract. Comparing the pretreatment and post treatment weight of the tests group, there was a slight decrease in weight i.e from 141.0±1.63 to 137.0±5.72 (low dose) and 188.0±3.27 to 155.5±20.82 (high dose) while the control group had a slight mean increase in weight.Conclusion: From this study, the root bark extract of *C.*

ferruginea was beneficial to the Wistar rats. It was able to reduce pathogenic microorganisms from the GIT.

***Keywords*---**GIT, microbiome, pathogenic, weight loss, cnestis ferruginea.

Introduction

Plants have been used by humans for thousands of years because they contain vital therapeutic components and aid in the treatment of chronic ailments. Because pharmaceutical companies commercially produced a wide range of synthetic medications in the past, the period was known as the synthetic age (Nair *et al.,* 2013)[1]. Continuous usage of synthetic medications resulted in significant side effects and microorganism resistance over time. Furthermore, synthetic medications are expensive, and vast populations cannot afford to benefit from them. Green medicines have been the subject of a global movement in recent decades because to their low side effects and cost effectiveness. Many ailments, such as cancer, liver disease, and arthritis, have no complete solution in allopathy, thus medicinal plants play an important part in the creation of modern herbal remedies. Where modern drugs fail to provide a good solution, medicinal plant bioactive chemicals are utilized as anti-diabetic, chemotherapeutic, anti-inflammatory, and anti-arthritic agents (Tanko *et al.*, 2012)[2].

These bioactive substances are employed as deterrents against microbes, insects, and herbivores. Some of these compounds, however, are involved in pigmentation (tannins and quinines) as well as the synthesis of plant odor (terpenoids) and flavor (capsaicin). These defense chemicals provide plants with their therapeutic value, which is valued by humans due to their usefulness in individual and community health care (Akharaiyi and Boboye, 2010; Akharaiyi *et al.*, 2012)[3][4]. Although the modern pharmaceutical industry still relies heavily on the diversity of secondary metabolites in plants and secondary metabolites of which at least 12,000 have been extracted; a figure believed to be less than 10% of the total (Akharaiyi et al., 2012)[4].

Root and Bark: The roots are used to treat dysmenorrhea, as a purgative, and to treat skin infections, frequently as an ointment. The powdered bark is used to treat pyorrhea. To alleviate headaches, the root bark is ground into a paste and administered topically to the forehead. As an appetizer, the ash of the bark of Calpocalyx aubrévillei is served with the root-bark. The root decoction is used as an aphrodisiac and as an enema for gynecological diseases, diarrhea, and urethral discharge (Ahmed, 2017)[5]. Antimicrobial activity of *Cnestis ferruginea* ethanolic stem extracts on multidrug resistant bacteria isolated from raw meat was evaluated. The antibacterial properties of ethanolic extracts of C. ferruginea stem on multidrug resistant Escherichia coli, Staphylococcus aureus, and Salmonella spp isolated from raw meat were studied using the agar well diffusion method and microbroth dilution methods. The antimicrobial activity of *C. ferruginea* ethanolic stem extracts on these multidrug resistant pathogens was measured using the mean inhibition zone diameter (IZD), minimum inhibitory concentration (MIC), and Minimum Bactericidal Concentration (MBC) (MBC). The

microbes were shown to be susceptible, with E. coli and Salmonella being less susceptible (Enemor *et al.,* 2015)[6].

Materials and Methods

Digital weighing balance (KERO BLG 300), binocular light microscope (OLYMPUS), refrigerator (Haier Thermocol®, Model: HRF-250E), incubator Uniscope SM9052 laboratory incubator®), autoclave, hot air oven (Leader® Model: GP/50/CLAD/250/HYD).

Reagents

Ethanol (BDH Chemical Ltd, England), methylated spirit (BIOLAB), distilled water, sterilized water, Kovas's reagent, hydrogen peroxide 3%, disinfectant, chloroform, lactose (Alvis Chemical), sucrose (Kermel), glucose, Lugol's iodine (BEMA Scientific and Chemical, Nigeria), crystal violet (AFIS Biochemicals, Nigeria), safranine (BEMA Scientific and Chemical, Nigeria), immersion oil(cedar wood oil), paraffin wax, hydrogen sulphide

Bacteriological Media

Nutrient Agar, Peptone Water, MacConkey Agar, Simmon citrate Agar, Mannitol salt agar (All from Titan Biotech Ltd., India), MR-VP Medium (Buffered glucose broth), Motility (MIU) agar

Collection and Identification of Finger Millet (Cnestis ferruginea) Root bark

The root bark of Cnestis ferruginea was obtained from mature tree located in Ugono Abraka, Delta State, Nigeria on the 22nd of March, 2021. Identification and authentication of the plant sample was done at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University by the Head of Department; Dr. O.E. Ikpefan.

Preparation of Plant Materials

On collecting the plant sample of *C. ferruginea*, the bark was girdled from the root using a sharp knife after which it was air dried at room temperature for 7 days in the laboratory. After drying, the bark was pulverized to coarse powdered form using a mechanical blender. The blender was properly washed with distilled water cleaned to avoid influx of any adulterant or foreign particles. After blending, the coarse powdered plant sample was stored in an air tight labelled container prior to extraction. (Enwa *et al* 2016)[7]

Extraction of Plant Material

The powdered root bark of C. ferruginea was extracted with 80% methanol using a soxhlet extractor at 60°C. The resulting extract was then concentrated too dryness in an evaporating dish using a rotary evaporator to remove the extracting solvent (80%) methanol. The resulting extract was weighed and stored in a refrigerator at a temperature of 4°C prior to further use.

Experimental Animals

Fifteen (15) healthy Wistar albino rats were purchased from the animal house of the Department of Pharmacology, Delta State University, Abraka and maintained at room temperature under 12hour dark cycle for one week to acclimatize. The animals were weighed and allowed to acclimatize for one week with free access to food pallets and water and libitum. The albino Wistar rats were randomized into four (4) groups of three (3) rats each as group 1, 2, 3, and 4. Group 1 served as standard group administered with 400 mg/kg body weight of metformin, group 2 was the control group sub divided into two,(negative control, was not treated with the root bark extract and was administered only normal saline) and (positive control, was treated with the plant extract). While group 3 and 4 were administered 200 mg/kg body weight and 400 mg/kg body weight respectively of the methanolic extract of *Cnestis ferruginea* root bark. All treatments were administered via oral route with the aid of a needle less syringe for fourteen (14) days.

Animal Monitoring

The animal were administered with the millet extracts once daily. The fecal of the rats from each group was collected and then cultured in a broth medium in an incubator at 37°C for 24hours. On day 14, the animals were then sacrificed and processed for different biochemical parameters. The gastrointestinal tracts of the sacrificed animals from each group was excised and the internal section swabbed and cultured in a broth medium in an incubator at 37°C for 24hours, and bacterial growth observed, colonies identified and counted to determine the microbial load. Body weights of the Albino Wistar rats were recorded before and after administration of the plant extract.

Serial Dilution

A serial dilution is the step wise dilution of a substance solution. Usually dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A 10 fold dilution of each culture broth was done. 9ml of Peptone water was transferred aseptically into different test-tubes. Then 1ml of the broth was added into the first test-tube. 1ml was then taken from it and added to the next test-tube and so on as forth until the last test-tube. 1ml was taken from the last test-tube and discarded. Then 0.1ml of the mixture was taken from the last test-tube and aseptically transferred into each plate containing solidified nutrient agar and MacConkey agar respectively, and then spread with a sterile spreading rod. The plates were incubated at 37°C for 24hours and the colonies were counted. This is done using both feaces and gastrointestinal tract. Bacteriology and biochemical test were carried out as described by Oghenemaro *et al* (2021)[8]

MR-VP Test

MR-VP aids in the identification of enteric gram negative bacilli, the broth is used for both the methyl red and the Voges Proskauer test. About 1.7 g of MR-VP broth was weighed and dissolved in 100 ml water, 5 ml was measured into different test

tubes, after which the sample organism in the overnight broth was inoculated into each test tubes and incubated for 48hrs and after the incubation period, the test tubes were vortexed and a few drops of methyl red indicator was added. On addition of few drops of methyl red into the sample, the formation of a reddish colour indicates positive for mixed acid fermentation (one/more organic acid formed during the fermentation of glucose). The reddish or pinkish interface indicates positive for acetoin, a precursor of 2/3-butanediol (CLSI, 2017)[9].

Results

Table 3.1: Weight of Wistar rats before and after administration of *Cnestis ferruginea* plant extract

Groups	Treatment period	
	Before (g)	After (g)
1	148.5±1.23	169.0±19.60
2	204.0±16.33	181.5±11.02
3	141.0±1.63	137.0±5.72
4	188.0±3.27	155.5±20.82

mean±SEM values of weight of Wistar rats (n = 2); Group 1: Standard; Group 2: Control; Group 3: Low dose of extract; Group 4: High dose of extract

Table 3.2: Colony Count before Administration of *Cnestis ferruginea* Plant Extract

	Number of Colony		
	T1	T2	Control
R1	TNTC	TNTC	TNTC
R2	TNTC	TNTC	TNTC
R3	TNTC	TNTC	TNTC
R4	TNTC	TNTC	TNTC

TNTC: Too numerous to count

Table 3.3: Colony Count after Administration of *Cnestis ferruginea* Plant Extract

	Number of Colony					
	T1		T2		Control	
	Fecal	GIT	Fecal	GIT	Fecal	GIT
R1	360	356	419	400	450	TNTC
R2	370	247	470	450	480	TNTC
R3	393	370	478	430	TNTC	TNTC
R4	390	359	247	200	390	TNTC

Key:
R1 – R4: Code for Wistar rats
TNTC: Too numerous to count

Table 3.4a: Biochemical Test Result for Microorganisms isolated from the Wistar rats before the Administration of *Cnestis ferruginea* Plant Extract (Negative control)

Code for the bacteria Identified	Catalase	Urease	Citrate	Indole	H2S	MR-VP	MIU	Oxidase	Coagulase	Gram-staining		Fermentation			Name of suspected organisms
												S	G	L	
Nutrient agar															
1	+	-	+	+	+	+	+	+	-	-		A	A	A	*Aeromonas*
2	+	+	+	+	+	+	+	-	-	-		A	A	A	*Proteus* spp
3	+	+	+	+	+	+	+	-	-	-		A	A	A	*Proteus* spp
4	+	-	+	+	+	+	+	+	-	-		A	A	A	*Aeromonas*
5	+	-	+	+	+	+	+	+	-	-		A	A	A	*Aeromonas*
6	+	-	+	+	+	+	+	+	-	-		A	A	A	*Aeromonas*
7	+	+	+	+	+	+	+	-	-	-		A	A	A	*Proteus* spp
8	+	+	-	+	+	+	+	-	-	-		A	A	A	*Proteus* spp
Mannitol Salt Agar															
1	+	+	-	-	-	-	-	-	-	+		A	A	A	*S. aureus*
2	+	+	-	-	-	-	-	-	+	+		A	A	A	*S. aureus*
3	+	+	-	-	-	-	-	-	+	+		A	A	A	*S. aureus*
4	-	+	-	-	-	-	-	-	-	+		A	A	A	*S. aureus*
5	+	+	-	-	-	-	-	-	-	+		A	A	A	*S. aureus*
6	-	+	-	-	-	-	-	-	-	+		A	A	A	*S. aureus*
7	+	+	-	-	-	-	-	-	+	+		A	A	A	*S. aureus*
8	-	+	-	-	-	-	-	+	+	+		A	A	A	*S. aureus*

(+) stands for positive result, (-) stands for negative result, A; acid and gas

Table 3.4b: Biochemical Test Result for Microorganisms isolated from the Wistar rats before the Administration of *Cnestis ferruginea* Plant Extract

Code for the bacteria Identified	Catalase	Urease	Citrate	Indole	H2S	MR-VP	MIU	Oxidase	Coagulase	Gram-staining		Fermentation			Name of suspected organisms
												S	G	L	
Centrimide															
1	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas*

															spp
2	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
3	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
4	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
5	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
6	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
7	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
8	+	-	+	-	-	-	+	+	-	-		-	-	-	*Pseudomonas spp*
Mc-Conkey Agar															
1	+	+	+	+	+	+	+	-	-	-		A	-	-	*Proteus* spp
2a	+	+	+	+	+	+	+	-	-	-		A	-	-	*Proteus* spp
2b	+	-	+	-	+	+	+	-	-	-		A	-	-	*Citrobacter*
3	+	-	+	+	+	+	+	+	-	-		A	-	-	*Aeromonas spp*
4	+	+	+	-	-	-	+	+	-	-		A	-	-	*Pseudomonas spp*
5	+	-	+	+	+	+	+	+	-	-		A	-	-	*Aeromonas spp*
6	+	+	+	+	+	+	+	-	-	-		A	-	-	*Proteus* spp
7	+	-	+	+	+	+	+	+	-	-		A	-	-	*Aeromonas spp*
8	+	-	+	+	+	+	+	+	-	-		A	-	-	*Aeromonas spp*

(+) stands for positive result, (-) stands for negative result, A; acid and gas

Table 3.5: Biochemical Test Result for Microorganisms isolated from the Gastrointestinal Tract of Wistar rats after Administration of *Cnestis ferruginea* Plant Extract

Growth	Catalase	Urease	Citrate	Indole	H2S	MR-VP	Gram staining	Oxidase	Coagulase	Motility		Fermentation			Name of suspected organisms
												S	G	L	
Standard															
1a	+	+	+	+	+	+	-	-	-	+		A/G	A/G	A/G	*Providentia*

1b	+	+	+	+	+	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
2a	+	-	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
2b	+	-	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
+ve Control															
1	+	-	+	+	-	+	-	-	-	+		-	-	-	*Providentia*
2	+	-	+	+	-	+	-	-	-	+		-	-	-	*Providentia*
3	+	-	+	+	+	+	-	-	-	+		-	-	-	*Aeromonas spp*
High															
1a	+	-	+	+	+	+	-	-	-	+		A/G	A/G	A/G	*Aeromonas spp*
1b	+	+	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
2	+	-	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providencia*
3	+	-	+	+	+	+	-	-	-	+		A/G	A/G	A/G	*Aeromonas spp*
Low															
1a	+	-	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
1b	+	+	+	+	-	+	-	-	-	+		A/G	A/G	A/G	*Providentia*
2	+	+	+	+	+	+	-	-	-	+		A/G	A/G	A/G	*Proteus* spp.
3	+	-	+	+	-	+	-	-	-	+		A/G	A/G	A/G	Providencia

High and Low= Dose of plant extract; A/G= acid and gas

Table 3.6: Percentage of Incidence of Identified Bacteria

Groups	Organisms	No of organisms	% incidence
Negative Control	*Aeromonas*	8	25
	Proteus spp	7	21.9
	Citrobacter	1	3.1
	Pseudomonas	9	28.1
	Staphylococcus aureus	8	25
Standard	*Providencia*	4	100
Positive Control	*Providencia*	2	66.7
	Aeromonas	1	33.3
Extract (low and high)	*Providencia*	4	66.6
	Aeromonas	1	16.7
	Proteus spp.	1	16.7

Discussion

Medicinal plants are regarded as reservoir of various types of bioactive compounds with varied therapeutic and pharmacological activities. The therapeutic potential of medicinal plants has been well researched over the years Africa CDC, (2020)[10]. The knowledge of uses of medicinal plants as drugs in various traditional medical systems of medicine has been of great importance in the discovery of new drugs for orthodox medicine (Ramawat *et al.*, 2009)[11]. Despite the current ascendency of synthesis as a preferred method of drug discovery, the potential of medicinal plants and their extracts to yield new drugs for therapy and prophylaxis remains immense (Ahmed, 2017)[5].

The result as presented in this study showed a significant increase in the body weight of Wistar rats of group 1 (standard group) while those treated with low and high dose of *C. ferruginea* plant extract revealed a significant decrease in the weight of the Albino Wistar rats. Group treated with high dose of the plant extract had a significant decrease in body weight when compared to those treated with low dose of the plant. The study corroborate with the study of Osibemhe *et al.* (2017)[12] who reported a significant decrease in the body weight of wistar rats treated with 400 mg/kg of aqueous extract of the plant *Anisopus manni*, while the standard and control group had a significant increase in body weight.

It is well known that plant extracts have diversified action in relation to various microorganisms. There are extracts from plants that have a prebiotic effect (Bondareva *et al.*, 2017)[13], and also some that on a contrary have an antimicrobial properties (Asimi and Sahu, 2013; Dwivedia *et al.*, 2014)[14][15]. In this case, it was interesting to determine the effect of the methanol extract of *C. ferruginea* leaves on the population of GIT microflora of Albino Wistar rats, taking into account the fact that it is a well-known medicinal plant. Generally, the human body harbors a lot of microbial cells whose coordinated actions are believed to be important for human life. Such microbial cell populations reach their highest density in the intestinal compartment, where they collectively form a complex microbial community known as the gut microbiota which develops over the course of host infancy to eventually reach its adult form (Lozupone *et al.*, 2012)[16].

The present study revealed the effect of the root bark extract of *C. ferruginea* on the number of bacteria colony before and after administration (Table 3.2 and 3.3). The result revealed a large mass of bacteria colony in the GIT of the Wistar rats while on treating the rats with various concentration of the plant extract, there was significant reduction in the number of bacteria colony both in the fecal and GIT of the Wistar rats when compared to the control group. Studies have reported high antimicrobial potency of medicinal plants against different strains of bacteria, this can be ascribed to the presence of bioactive components present in the plants extract (Oloyede *et al.*, 2017; Fodouop *et al.*, 2017)[17][18]. A large number of flavonoids have been reported to possess antimicrobial properties (Olowosulu and Ibrahim, 2006)[19]. Oluwafemi and Debiri, (2008)[20] attributed the antimicrobial activities of flavonoids to their ability to complex with extracellular and soluble proteins as well as their ability to complex with bacterial cell walls.

They suggested that more lipophylic flavonoids exert antimicrobial activity by disrupting microbial cells membranes.

Result presented in Table 3.4a and b respectively highlight the different bacterial isolated from the GIT of the Wistar rats. The study revealed the presence of *Aeromonas hydrophyla*, *Proteus* spp, *Pseudomonas* spp, *Citrobacter freundii*, *Staphylococcus aureus* and *Providencia* spp in the GIT of the alloxan induced diabetes Wistar rats. In the standard group *Providencia* spp was the only bacteria isolated while *Providencia* spp. and *Aeromonas hydrophyla*, were isolated from group administered with normal saline (positive control group). In group treated with low and high dose of the root bark extract of *C. ferruguinea* the bacterial isolated include; *Aeromonas hydrophyla*, *Providencia* spp and *Proteus* spp. respectively.

The percentage of this bacteria colony was also reported in this study. The most prevalence in the negative control group was *Pseudomonas aeruginosa* and *Aeromonas hydrophyla* with percentage incidence of 28.1 and 25% respectively while the least prevalence was *Citrobacter freundii* with a percentage incidence of 3.1%. Those of the standard and positive control group had bacteria percentage of 100%, 66.7%, and 33.3% *Providencia* spp and *Aeromonas hydrophyla* respectively, with *Providencia* spp being the most prevalence in the group. While the group treated with low and high dose of the plant extract was 66.6% for *Providencia* spp and 16.7% each for *Aeromonas hydrophyla* and *Proteus* spp respectively.

The study revealed that the root bark extract of *C. ferruguinea* was able to reduce the population of the bacteria present in the GIT of the Wistar rats, thus confirming the antimicrobial effect of the root bark extract. The high percentage incidence of *P. aeruginosa* infections appear secondary to a breach in host defenses, this organism was inhibited on administration of the plant root bark extract. Although the translocation of endogenous intestinal *P. aeruginosa* extraluminally is an important pathogenic phenomenon and a cause of systemic infections, especially in neutropenic patients with hematological malignancies, thus the plant extract has the potency of alleviating this effect (Gonelimali *et al* 2018)[21]. During the translocation process, bacteria and their products cross the intestinal barrier by traveling between or through the cells of the intestinal epithelium, causing infection and massive inflammation (Chang *et al.*, 2017)[22].

A. hydrophyla which was the second most prevalence of the bacteria found in the GIT of the alloxan induced diabetic Wistar rats possess serious health challenge to the GIT of the specimen as *A. hydrophyla* has been reported to be an important disease causing pathogen responsible for a number of infectious complications in both immunocompetent and immunocompromised persons (Janda and Abbott, 2010)[23]. The occurrence of *C. freundii* found in the negative control group may be ascribed to the presence of water which encourage the growth of the organism and the presence of the organism causes diarrhea in human **(**Janda and Abbott, 2010)[23]. Although the presence of *C. freundii* in the GIT microbiome is associated in healthy species as it acts as an anti-obesity (Zhang *et al.*, 2020)[24]. The effect of this organism can be countered as a result of high percentage incidence of

Proteus spp. in the GIT compared to that of the *C. freundii* (Parvataneni *et al.*, 2017)[25].

Conclusion

From the finding of this study it can be concluded that the methanol extract of *Cnestis ferruginea root bark* extract had effect on the microbiome gastrointestinal tract of alloxan induced diabetic Wistar rats as this was seen in the change of microorganisms present, reduction of the population of the bacterial colonies on administration of the plant extract.

References

1. Nair, S.S., Kavrekar, V. and Mishra, A. Evaluation of In-Vitro Anti diabetic Activity of Selected Plant Extracts. International Journal of Pharmaceutical Science Invention, 2(4): 2013, 12-19.
2. Tanko, Y., Mohammed, A., Musa, K.Y. and Eze, E.D. Evaluation of Effect of Ethanolic Leaf Extract of Mucuna pruriens on Blood Glucose Levels in Alloxan-Induced Diabetic Wistar Rats. Asian Journal of Medical Sciences, 4(1), 2012, 23-28.
3. Akharaiyi, F.C. and Boboye, B. Antibacterial and Phytochemical Evaluation of Three Medicinal Plants. Journal of Natural Products, 3, 2010, 27-34.
4. Akharaiyi, F.C., Boboye, B. and Adetuyi, F.C. Antibacterial, phytochemical and antioxidant properties of Cnestis ferruginea DC (Connaraceae) extracts. Journal of Microbiology, Biotechnology and Food Sciences, 2(2), 2012, 592-609.
5. Ahmed, H.A. Therapeutic Potentials of Cnestis ferruginea: A Review. Journal of Pharmacognosy and Phytochemistry, 6(6): 2017, 1397-1401
6. Enemor, E.C., Akagha, T.N., Ngwoke, K.G., Gugu, T.H., Oli, A.N., Eze, C.O., *et al.* Phytochemical analysis and Antimicrobial Activity of Ethanolic Stem Extracts of Cnestis ferruginea on Multidrug Resistant Bacteria Isolated from Raw Retail Meat Sold in Awka, Nigeria. J. Pharm. Sci. & Res., 7(11): 2015, 1044-1049.
7. Enwa FO, Michael O, Anie CO, Ayeh RA. Antibacterial Screening of the Ethanol and Aqueous Extract of the Fruit Peel of Persea Americana Mill against Selected Enteric Bacteria. Acad. J. Microbiol. Res. 4(3): 2016, 040-046.
8. Oghenemaro E F, Oise I E, Cynthia D. The Effects of Securinega Virosa Leaves on Methicillin-Resistant Staphylococcus Aureus (MRSA). Int j pharm res allied sci. 10(2): 2021, 29-34. https://doi.org/10.51847/HAQZ2xRZFh
9. CLSI, 2017, Performance Standards for Antimicrobial Susceptibility Testing 27th Edition M100
10. Africa CDC, (2020). World Health Organisation, Africa CDC in joint push for COVID-19 traditional medicine research in Africa. Geneva, Switzerland: World Health Organization.
11. Ramawat K G 2009 (ed.), Herbal Drugs: Ethnomedicine to Modern Medicine, 7 DOI 10.1007/978-3-540-79116-4 2, c Springer-Verlag Berlin Heidelberg 2009

12. Osibemhe M., Lawal, N., Umar, D., Omaji, G.O. and Jibiya, S.A. Effect of aqueous extract of Anisopus manni stem in alloxan-induced diabetic rats. Science World Journal, 12(3): 2017, 7-10
13. Bondareva N.I., Timchenko L.D., Dobrynya Y.M., Avanesyan S.S., Piskov S.I., Rzhepakovsky I.V., Kozlova M.A., Areshidze D.A. and Lyhvar A.V. Influence of the Chlorophytum Comosum Leaves Hydroalcoholic Extract on Some Representatives of Intestinal Microflora of Rats. Journal of Pharmaceutical Sciences and Research, 9(6): 2017, 874-877.
14. Asimi, O.A. and Sahu, N.P. Herbs/spices as feed additive in aquaculture. Science Journal of Pure and Applied Science, 2: 2013, 284- 292.
15. Dwivedia, S., Sahrawat, K., Puppala, N. and Ortiz, R. Plant prebiotics and human health: Biotechnology to breed prebiotic-rich nutritious food crops. Electronic Journal of Biotechnology, 2: 2014, 238-245.
16. Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. and Knight, R. Diversity, stability and resilience of the human gut microbiota. Nature 489: 2012, 220–230.
17. Oloyede, D.D., Odedara, O.O. and Omemu, M.A. Effect of Extracts of Parquetina nigrescens (Afzel.) Bullock on Rat Gastrointestinal Microflora. African Journal of Biomedical Research, 20: 2017, 209- 215.
18. Fodouop, S.P.C., Tala, S.D., Keilah, L.P. Kodjio, N., Yemele, M.D., Nwabo, A.H.K., Nji-kah, B., Tchoumboue, J. and Gatsing, D. Effects of Vitellaria paradoxa (C.F. Gaertn.) aqueous leaf extract administration on Salmonella typhimurium-infected rats. BMC Complementary and Alternative Medicine, 17: 2017, 160.
19. Olowosulu, A.K. and Ibrahim, Y.K. Studies on the antimicrobial screening of Aqueous extracts of five plants used in Folk medicine in Nigeria. West African Journal of Biological Science. 3(5): 2006, 21-26.
20. Oluwafemi, F. and Debiri, F. Antimicrobial Effect of Phyllantus amarus and Parquetina nigrenscens on Salmonella typhi. African Journal of Biomedical Research. 11: 2008, 215-219.
21. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M and Hatab SR. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Front. Microbiol. 9: 2018, 1639. doi: 10.3389/fmicb.2018.01639
22. Chang J, Leong RW, Wasinger VC, et al. Impaired intestinal permeability contributes to ongoing bowel symptoms in patients with inflammatory bowel disease and mucosal healing. Gastroenterology. 2017;153:e1.
23. Janda J.M. Abbott S.L. The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin Microbiol Rev. 23: 2010, 35-73
24. Zhang M-L, Li M, Sheng Y, Tan F, Chen L, Cann I, Du Z-Y. Citrobacter species increase energy harvest by modulating intestinal microbiota in fish: nondominant species play important functions. mSystems 2020, 5:e00303-20. https://doi.org/10.1128/mSystems.00303-20
25. Parvataneni S, Mijalis EM, Kuty Everett GF, Rasche ES, Liu M, Gill JJ. Complete genome sequence of Citrobacter freundii myophage Mijalis. Genome Announc 2017, 5:e00228-17. https://doi.org/10.1128/genomeA.00228-17.