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Effect of Extracts of *Parquetina nigrescens* (Afzel.) Bullock on Rat Gastrointestinal Microflora

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ABSTRACT

Plants with antibacterial activity have gained increasing importance due to the development of antimicrobial drug resistance organisms and the occurrence of undesirable side effects of some antibiotics. This study is aimed at the evaluation of the in-vivo and in-vitro activities of water and ethanol extracts of *Parquetina nigrescens* (PN) on gastrointestinal microflora of small mammals. Twenty-one Wistar rats were grouped into 7 of 3 rats each. Group A serves as the control, the other groups were separately administered extract in the following ratio: Group B-50 mg/kg, Group C-100 mg/kg, Group D-200 mg/kg of aqueous extract respectively and Group E-50 mg/kg, Group F-100 mg/kg, Group G-200 mg/kg of ethanol extract respectively. The experiment was conducted for 21days. Caecum samples were collected and serially diluted in peptone water for microbial analysis. The mean total bacteria counts, Enterobacteriaceae and Lactobacilli counts showed a significant ($p \leq 0.05$) decrease in Group C (51.67×10^6 CFU/g, 41.67×10^6 CFU/g and 40.67×10^6 CFU/g), Group D (37.3×10^6 CFU/g, 34.3×10^6 CFU/g and 35.0×10^6 CFU/g), Group F (38×10^6 CFU/g, 34×10^6 CFU/g and 37.33×10^6 CFU/g) and Group G ($37.0 .x 10^6$ CFU/g, 25.33×10^6 CFU/g and 19.33×10^6 CFU/g) respectively when compared with the control (98.33×10^6 CFU/g, 57.33×10^6 CFU/g and 71.33×10^6 CFU/g). The *in vitro* analysis showed ethanol extracts exhibiting the highest inhibitory potentials (90%) on the isolated microorganisms, while the least effective (30%) was 50 mg/ml of the water extract. The phytochemical screening of Parquetina nigrescens showed the presence of anthraquinones, saponins, phlobatannis, ascorbic acid, alkaloids, flavanoids and terpenoids

Keywords: Gastrointestinal microflora, *Parquetina nigrescens*, In-vivo, *In-vitro*, phytochemicals.

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INTRODUCTION

Medicinal plants are plants that possess therapeutic properties and despite the widespread use of modern medicine, herbal products are still in use in most developing countries of Africa and Asia for the management of ailments. A considerable percentage of medicinal plants identified around the World are from tropical Africa (Sofowora, 2008). One of such medicinal plants is *Parquetina nigrescens*. Also known as bullock, it is a shrub found in equatorial West Africa and has been in traditional medicine practice for centuries with its leaves, roots and latex all in use (Owoyele *et al*., 2011). It is perennial with twinning stem and woody base shortly tapering 10-15 cm long, 6-8 cm broad with a smooth long stem on the leaves. In Oyo State, Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm), while the roots are used for the management of rheumatism (Owoyele *et al*., 2011). Over the years, P. nigrescens has been used as an ingredient in the medications for insanity, as well as an aphrodisiac in East Africa. Other uses include the decoction of the stem bark been given as cardiac tonic while the leaf and root decoctions have been used for the treatment of gonorrhoea and menstrual disorders (Odetola *et al*., 2006). As a constituent of a commercial herbal preparation (Jubi formular), *P. nigrescens* is used in the treatment of anaemia in humans in Nigeria.

In Nigeria, as in most developing countries, about 80% of the populations still use traditional medicines for the treatment of a wide variety of diseases including gastro-intestinal

disorders (GIT) which are very common. Traditionally, diagnoses of the different types of gastrointestinal diseases are sometimes indistinct as most disorders of the GIT are simply described as stomach ache. Consequently, the same medication is often used to treat various types of GIT disorders. *P. nigrescens* is one of the herbs commonly used for this purpose.

The human body is home to more than 1 trillion microbes, with the gastrointestinal (GI) tract alone harbouring a diverse array of commensal microbes that are believed to contribute to host nutrition, developmental regulation of intestinal angiogenesis, protection from pathogens and the development of immune response (Johnson and Versalovic, 2012). Human-associated bacteria communities likely play a central role in host nutrition, development of immunity and protection from diverse pathogens (Hooper & Gordon, 2001; Maslowski & Mackay, 2011). The human body contains many different sites that are colonized by microbial communities during neonatal and childhood development and throughout the lifetime of individual in health and disease states (Johnson & Versalovic, 2012). Predominant bacteria phylla composed of hundreds of bacteria genera and species in the human body, regardless of body sites include Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (Spore *et al*., 2011). Bacteria species populations vary significantly between individuals and bacteria community composition appears to be driven primarily by body habitat (Castello *et al*., 2009).

Human microbiota have been found to be influenced by many factors such as mode of birth delivery, mode of first food in infants, hospitalization and gestational age, diets, antibiotics (Johnson & Versalovic, 2012). Moreover, profound alterations of microbial communities have been shown within days of treatment with Ciprofloxacin and Fluoroquilonone. The lack of recovery from this perturbation by several organisms emphasizes the potential impact of excessive antibiotics therapy (Dethlefsen & Ralman, 2011).
Many herbal extracts have been shown to be toxic and affect type and number of microorganisms inhabiting the gastrointestinal tract when consumed, therefore there is a need to control the amount of herbal product being consumed so as to safeguard the microbiota of the GI (Davis and Milner, 2010).

Although most of the acclaimed medicinal properties, have not been confirmed scientifically, the plant is extensively used in traditional medical practice in the Yoruba speaking part of Nigeria where its Yoruba name is (Ewe Ogbo), meaning the leaf that hears. Also, a part of the incantation that precedes its use says "ohun ti a ba wi fun ogbo ni ogbo ngbo" meaning that whatever we tell 'ogbo' it hears and does (Odetola *et al*., 2006) reflecting the importance and belief in its efficacy for cure of virtually all ailments.

In some Nigerian folklore, water extract of *P. nigrescens* is freely consumed because of people's belief that it increases the packed cell volume (PCV) of people with anaemia. However there is scarcity of information on its effect on beneficial microbes inhabiting the gastrointestinal tract of mammals. Hence the purpose of this research is to study the impact of the extract of the plant on some useful microorganisms present in human gastrointestinal tract using a mammal (*Rattus norvegicus*) as model.

MATERIALS AND METHODS

Plant Materials: Fresh aerial part of *Parquetina nigrescens* were collected in a farm in Abeokuta, Ogun state and certified in the Department of Pure and Applied Botany, Federal University of Agriculture Abeokuta, Nigeria.

Preparation of plant Extracts : Leaves were washed and dried in a cabinet air drier at room temperature for 3 weeks. The dried leaves were grinded to powder and weighed in two separate containers for ethanol and water extract. The extraction was allowed for 72h at room temperature (27 ± 2^0C). After extraction, filtration was done with a 3 fold sterile muslin cloth. Filterates were vapourized to dryness using rotary evaporator (Resona, Germany). Dried extracts were weighed as percentage yield and preserved in sterile bottles at 4°C before use.
The crude aqueous extracts were prepared by reconstituting with sterile distilled water at a concentration of 50-200mg/ml, while the ethanol extract was prepared in 10 % (v/v) ethanol at concentrations of 50-200mg/ml.

Animals: Twenty-one wistar strain rats were used in the study. The animals were housed in wire mesh cages and allowed to acclimatize for one week. They were fed with standard rat feed and water at libitum before commencement of study.

Experimental Design: The experiment lasted for twenty one (21) days and the rats were divided into seven groups of three rats each. Group A, the control, were fed standard chow and water at libitum, Group B, C and D were fed standard chow and water and administerd 50mg/kg, 100mg/kg and 200mg/kg of aqueous extract respectively, Group E, F and G were fed standard chow and water and administered 50mg/kg, 100mg/kg and 200mg/kg of ethanol extract respectively. All extracts were administered in respect to the body weight of each rat.
At the end of the experiment, all rats were sacrificed and caecum of each rat was dissected and samples taken for culturing and identification of microbial isolates.

Collection of samples: The Caecum of each rat was aseptically dissected and a gram was placed and rinsed in 9 ml sterile peptone water after which dilutions were made to 10^{-7} dilutions before plating on agar (Nutrient Agar, MacConkey Agar and de Man, Rogosa and Sharpe Agar). The total number of colony forming units (CFU) of bacteria was enumerated.

In-vitro analysis of *Parquetina nigrescens* extract on isolated microorganisms*:* The inocula of bacteria were prepared from 24 h old broth cultures. The absorbance was read at 530 nm and adjusted with sterile distilled water to match that of a 0.5 Mac farland standard solution. From the prepared bacterial solutions, other dilutions with sterile distilled water were prepared to give a final concentration of 10^6 colony forming unit (Cfu) per milliliter. Bacterial suspension (0.5 ml) was separately plated using spread plating technique. Plates were allowed to stand for 1.5 h for the inoculated bacteria organisms to be established in the medium.

Wells of 7 mm width were made on the seeded plates. Various concentrations of the extracts were introduced into each well using sterile syringe. Plates were labelled and incubated at 37^0C for 24-48 h. After incubation, clear zones of inhibition around the wells indicates the sensitivity rate of the test bacteria to each extract and diameter of the clear zones was recorded as index of the degree of sensitivity by measuring with calliper. Tests were carried out in triplicates. Ofloxacin (10 µg) was used as positive control.

Phytochemical screening

Saponin: 2.5gm of the powdered sample were boiled in 25ml of distilled water in a water bath and filtered. 10ml of the filtrate were mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. 3 drops of olive oil was added to the frothing and shaken vigorously, then observed for the formation of emulsion which indicates the presence of saponin.

Flavonoids: A portion of the powdered leaves sample were separately heated with 10ml of ethyl acetate in a water bath for 3 min, mixtures were filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids.

Tannins: 0.5g of powdered sample was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour.

Phlobatannins: Deposition of a red precipitate when an aqueous extract of powdered leaves sample is boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Anthraquinones: A half grams (0.5g) of the extract was boiled with 10 ml of 5% sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia solution was added. The resulting solution was observed for colour changes.

Terpenoids (Salkowski test): Two millimeter (2ml) of chloroform was added to 0.5g each of the extract. Concentrated Sulphuric acid (H_2S0_4) (3ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Ascorbic acid: A tablespoon of cornstarch was mixed into 20 millmetres of water to make paste, 250 millimetres of water was added to the paste and boiled for 5 minutes. Ten drops of the starch solution was added to 75 millimetres of water (use an eyedropper) then iodine solution was added to produce a dark purple-blue colour. This serves as an indicator. Five millimetres of indicator solution was put in a 15 millimetres test tubes, a clean eyedropper was used to add 10 drops of 0.5g of the extract diluted in 5 ml of distilled water. The test tubes were held against a white background. The test tubes were lined up from lightest to darkest purple. The lighter the solution, the higher the vitamin C content. This is because vitamin C causes the purple indicator solution to lose its colour.

Cardiac glycosides (Keller-Killianitest): A half grams (0.5g) of extract were diluted in 5ml of distilled water, 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer, boiled and filtered.

Alkaloids: A half gram (0.5g) of extract was diluted to 10ml with acid alcohol, boiled and filtered Two millimeters (2 ml) of dilute ammonia was added to 5ml of the filtrate. Five millimetres (5ml) of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Statistical analysis: Data was recorded as Mean ± standard error of the mean. Statistical difference between the means was determined by ANOVA. Any significant difference between means was assessed by the Student's t-test

RESULTS

Evaluation of microbial load (CFU/g) of Caecum samples: Group A rats which is the control, has the highest bacteria count of 98.33±9.528 followed by Group B (50mg/kg water extract administration) with 95.0±8.145. The lowest count (37.42±2.08) was observed in Group G rats that were administered 200mg/kg ethanol extract (Table 1). For the Enterobacteriaceae counts, the trend changes, it was observed that the Group A (control), has the highest bacteria count with 57.33±1.764 followed by Group E (50mg/kg ethanol extract administration) with 56.67±2.333. The lowest Enterobacteriaceae count was observed in Group G (200mg/kg ethanol extract administration) with 25.33±1.20.

For the Lactobacilli count, the same trend was followed as the Enterobacteriaceae counts with the control having the highest count with 71.33±1.886 followed by Group E (50mg/kg dilution of ethanol extract) with 70.00±8.622. The lowest count was observed in Group G (200mg/kg dilution of ethanol extract) with 19.33±0.88 (Table 1).

Furthermore, Table 1 (mean total bacteria counts, Enterobacteriaceae and Lactobacilli counts) depicts a significant ($p \leq 0.05$) reduction in the microbial load of group C, group D, group F and group G (100mg/kg water extract administration, 200mg/kg water extract administration, 100mg/kg ethanol extract administration and 200mg/kg ethanol extract administration) respectively.

Bacteria species isolated from specific sample group: Ten (10) distinct Bacteria species were isolated across all the groups studied and these include; *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter aerogenes*, *Pseudomonas fluorescens*, *Klebsiella sp*, *Lactobacillus fermentum*, *Lactobacillus acidophilus* *Lactobacillus plantarum* and *Lactobacillus alimentarius* (Table 2).

Table 1:
Microbial load (CFU/g) of Caecum samples collected from rats in different groups

Group	Treatment	Total bacteria count x10^5 CFU/g	Total Enterobacteriaceae count x10^6CFU/g	Total Lactobacilli count x10^6CFU/g
Group A	Control	98.33±9.528^b	57.33±1.764^c	71.33±1.886^b
Group B	50mg/kg of water extract	95.00±8.145^b	52.00±1.732^c	63.33±5.207^b
Group C	100mg/kg of water extract	51.67±3.756^a	41.67±1.764^b	40.67±3.528^a
Group D	200mg/kg of water extract	37.33±2.848^a	34.33±2.333^a	35.00±2.646^a
Group E	50mg/kg of ethanolic extract	94.33±2.906^b	56.67±2.333^c	70.00±8.622^b
Group F	100mg/kg of ethanolic extract	38.00±2.309^a	34.00±1.155^a	37.33±2.728^a
Group G	200mg/kg of ethanolic extract	37.42±2.08^a	25.33±1.200^a	19.33±0.88^a

Values are mean ± standard error of mean. Values followed by different letters within a column indicates significant differences according to the Duncan Multiple Range Test (DMRT), where $p \leq 0.05$.

Table 2:
Bacteria species isolated from the gut of each of the sample group

Bacteria species	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Escherichia coli	+	+	+	+	+	+	+
Proteus mirabilis	+	+	+	-	+	-	-
Proteus vulgaris	+	+	-	-	+	-	-
Citrobacter aerogenes	+	+	+	-	+	-	-
Pseudomonas fluorescens	+	+	+	+	-	-	-
Klebsiella oxytoca	+	+	-	-	+	-	-
Lactobacillus plantarum	+	+	-	-	-	-	-
Lactobacillus fermentum	+	+	+	+	+	-	-
Lactobacillus acidophilus	-	-	-	-	-	+	+
Lactobacillus alimentarius	+	-	-	+	+	+	-

KEY: + = Present; - = Absent
Group treatments: A- Control; B, C, D (50, 100 and 200mg/kg water extract respectively); E,F, G ((50, 100 and 200mg/kg ethanolic extract respectively)

As seen in the Table 2, *Escherichia coli* is the most prevalent specie as they are been isolated from rats in all the study groups, followed by *Lactobacillus fermentum* which was isolated from five of the groups. The least isolated of the bacteria species are *Lactobacillus plantarum* and *L. acidophilus* as they were isolated from only two groups respectively followed by *Proteus vulgaris* isolated from groups A,B and E.

The treatment received by rats in Group G (200mg/kg ethanol extract) had the most lethal effect because there were no growth of all the bacteria species except *Escherichia coli* and *Lactobacillus acidophilus* followed by treatment received by rats in group F (100 mg/kg ethanol extract). There was a lethal effect on seven (7) out of the ten (10) bacteria species inhabiting the gastrointestinal tracts of the rats. The bacteria species inhabiting guts of rats in group B least experienced a lethal effect of the extract as 8 bacteria species out of 10 were isolated from the ceacum of the rats as compared to control (Table 2)

Mean weight of rats observed during experiment
Weekly mean weight of the experimental rats, starting from the day extract was given to the day they were sacrificed were studied and results presented in Figure 1.

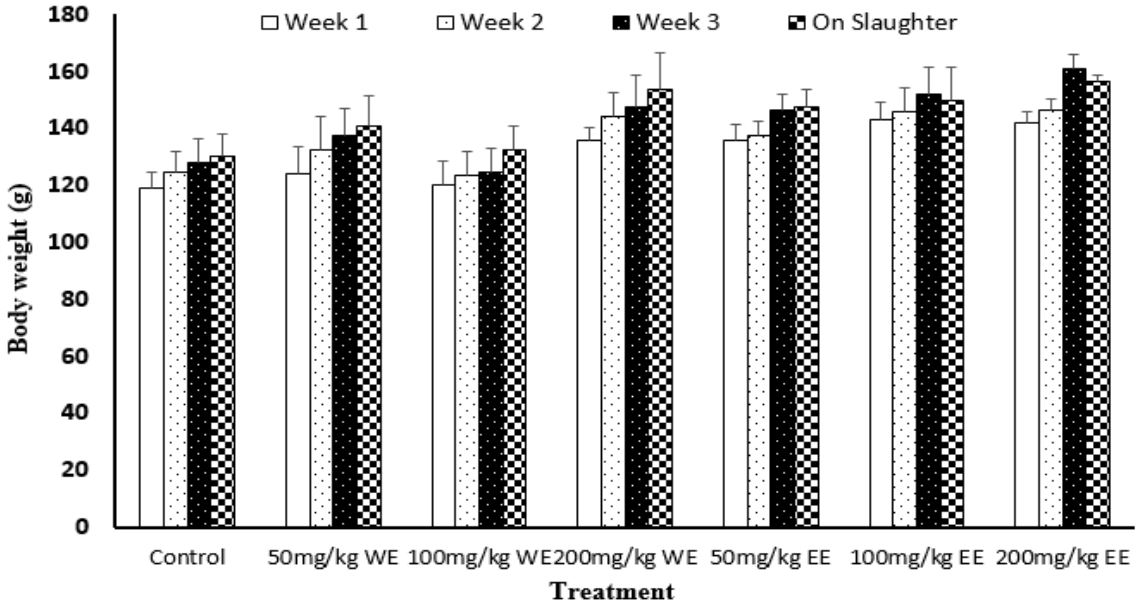

Figure 1:
Mean weight of rats during treatments with water extract (WE) and ethanolic extract (WE) of *Parquetina nigrescens*. Each bar represents mean ± standard error of mean

Table 3:
In-Vitro antimicrobial activity of *Parquetina nigrescens* with Ofloxacin as positive control

	Water Extract (mg/kg)			Ethanol Extract (mg/kg)			
Isolates	50	100	200	50	100	200	Ofloxacin
E. coli	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)	21.00±0.58 (S)
Proteus vulgaris	12±0.56 (S)	13.07±0.62 (S)	13.57±0.37 (S)	11.20±0.40 (S)	15.30±0.55 (S)	19.45±0.50 (S)	23.33±0.88 (S)
Cirobacter aerogenes	0.00±0.00 (R)	6.70±0.40 (S)	7.00±0.00 (S)	11.50±0.52 (S)	11.90±0.30 (S)	15.30±0.67 (S)	20.33±0.88 (S)
Pseudomonas fluorescens	0.00±0.00 (R)	0.00±0.00 (R)	7.96±0.58 (S)	6.00±0.00 (S)	10.67±1.30 (S)	14.60±0.87 (S)	20.67±0.67 (S)
Klebsiella oxytoca	0.00±0.00 (R)	0.00±0.00 (R)	6.10±0.20 (S)	6.30±0.25 (S)	8.50±0.52 (S)	10.20±0.25 (S)	10.67±0.67 (R)
Lactobacillus acidophilus	13.20±0.58 (S)	13.90±0.87 (S)	14.20±1.37 (S)	12.30±0.36 (S)	13.70±0.77 (S)	16.00±0.50 (S)	22.33±0.33 (S)
Lactobacillus alimentarus	0.00±0.00 (R)	0.00±0.00 (R)	8.40±0.73 (S)	6.33±0.66 (S)	8.15±0.00 (S)	8.58±1.34 (S)	13.67±0.67 (I)
Lactobacillus plantarum	7.63±0.33 (S)	7.89±0.16 (S)	9.56±0.87 (S)	10.53±0.58 (S)	12.14±0.78 (S)	13.43±1.55 (S)	26.00±1.30 (S)
Lactobacillus fermentum	0.00±0.00 (R)	6.90±0.35 (S)	8.44±0.37 (S)	0.00±0.00 (R)	8.16±0.50 (S)	9.63±1.67 (S)	24.00±0.58 (S)

KEY: Resistance (R), Susceptible (S)

It was observed that similar trend was followed in all the groups with the weight of rats increasing through the first week to the day they were been sacrificed, except in group F and group G, in which a drop in weight was observed between the third week and the day they were been sacrificed from 152±9.54 to 149.67±12.14 and 161±5.03 to 156.33±2.60 respectively (Figure 1).

In Vitro antimicrobial activity of *Parquetina nigrescens* with Ofloxacin as positive control

Table 3 showed the positive result that was observed with both the aqueous and ethanol extracts of *Parquetina nigrescens* on

the tested microorganisms. Though, both extracts acted on the test bacteria at different concentrations, with various degree of inhibition, which were varied based on the susceptibility of the organisms to the extracts concentrations. Only three (3) of the test organisms (*Proteus vulgaris, Lactobacillus acidophilus* and *Lactobacillus plantarum*) were susceptible to 50mg/kg of the water extract. The ethanol concentration exhibited more inhibitory potentials on the tested organisms except *E. coli* which could not be inhibited as it proved its resistance ability even to the highest concentration (200mg/kg) of *P. nigrescens* employed in this study.

However, *E. coli* was much more inhibited by the positive control drug (Ofloxacin) than some of the tested bacterial organisms inhibited by both the aqueous and ethanol extracts as shown in the table

Table 4:
Some phytochemicals tested in *Parquetina nigrescens.*

Phytochemicals	***Parquetina nigrescens.***
Anthraquinones	+
Saponins	+
Terpenoids	+
Cardiac glycosides	-
Flavonoids	+
Tannins	-
Alkaloids	+
Phlobatannins	+
Ascorbic acid	+

KEY: +=Present, - =Absent

Phytochemical screening of *Parquetina nigrescens*
The phytochemical screening carried out on the leaf samples of *P. nigrescens* showed the presence of various phytochemicals such as anthraquinones, saponins, flavonoids, phlobatannins, alkaloids, Ascorbic acid and terpenoids, while cardiac glycosides and tannins were absent (Table 4).

DISCUSSION

From this study, it was observed in the in-vivo analysis that the intestinal microbial count in the groups administered with water and ethanol extracts of *P. nigrescens* at 100-200mg/kg aqueous and 100-200 mg/kg ethanolic extract was reduced respectively compared to the control group, which agrees essentially with several investigations and reports that *P. nigrescens* contains antimicrobial substances (Akujobi *et al*., 2006; Imaga *et al*., 2010). However at lower concentration of 50 mg/kg of both water and ethanolic extracts (Group B and E), there were no significant reduction in the total bacteria count and total lactobacilli count.

It was also shown in the in-vivo study that the groups administered with ethanol extracts of *Parquetina nigrescens* exhibit a lower microbial count compared to those administered with water extract. Differences in microbial count observed in the varied concentrations of the aqueous and ethanol extracts can be linked to the deleterious effect of the extract against the microbial flora at higher concentration. It is therefore imperative that the consumption of the water and ethanolic extracts of *P nigrescens* at higher concentration such as 100 mg/kg and 200 mg/kg should be with caution as its reduction effect on beneficial microbes in the gastrointestinal tract might lead to imbalances (Dethlefsen *et al*., 2008). Some of these beneficial microbes are the Lactic acid bacteria and their roles within the gastrointestinal tract have been one of the most controversial subjects area of intestinal microbial ecology (Hoves *et al.,* 1999). Lactic acid bacteria are described to be of nutritional and therapeutic benefit to the host in several clinical conditions which include the improvement of lactose absorption when compared to that of milk, the reduction of incidence of diarrhoea among infants and antibiotic associated diarrhoea (Hove *et al.*, 1999).

It was observed that *Escherichia coli* was highly resistant to all the doses of both the aqueous and ethanol extract of the plant. This is in consonance with the report of Makanjuola *et al*.(2010) who also reported the resistant nature of *E. coli* to both the aqueous and ethanolic extract of *P. nigrescens.*

The antimicrobial properties exhibited by the extracts may be attributed to the presence of saponins, flavonoids, anthraquinone, terpenoids and alkaloids found in the plant extracts. A large number of flavonoids have been reported to possess antimicrobial properties (Olowusulu and Ibrahim, 2006). Oluwafemi and Debiri, (2008) 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.

From the in-vitro study, it was observed that the growth of the microorganisms were maximally inhibited, as indicated by the zones of inhibition in both the water and ethanol extracts, which agrees essentially with several investigations and reports that *Parquetina nigrescens* contains antimicrobial substances (Oluwafemi and Debiri, 2008; Imaga *et al*., 2010). From this study, the highest concentration of ethanol extract was observed to be the most deleterious, exhibiting a lethal effect on the growth of most of the tested gastrointestinal microflora except *E. coli.* Furthermore, the ethanol extract is observed to be more effective than the water extract, showing wider zones of inhibition. This is corresponding to the work of Makanjuola *et al.* (2010).

The increase in weight of the rats must have come from their regular feeds as there was no detection of fat in the ethanol extract of *P. nigrescens.* However, a report from Omoboyowa *et al*., (2016) showed the presence of fat in *P. nigrescens* at the chloroform layer but not the ethanol layer. Furthermore, it also shows that some fats are present in the plant though not detectable with ethanol.

Known for their resistance to various synthetic antibiotics, *Pseudomonas spp.*was susceptible to all the concentration of the ethanol extract and the highest concentration of the aqueous extract. It is therefore important that the plant be employed in screening for antibiotic sensitivity ability of Pseudomonas species which have resisted many antibiotics over the years.

It is interesting to note that high aqueous and ethanol extracts concentrations in this study demonstrated an inhibitory potency to valuable standards that approves any plant extract as being antimicrobial (>2.5mm). The

information presented in this study proves the antibacterial properties of the leaf extracts of *Parquetina nigrescens*, and it proves quite promising for its therapeutic and pharmacological use. Variations existed in inhibitory potency of aqueous and ethanol extracts of *P. nigrescens* is as a result of the inhibitory compounds which was more extracted by ethanol from the plant leaves.

The detection of flavonoids in *P. nigrescens* is suggestive of the plant's antioxidant properties in accordance to Agbor and Odetola (2001). Therefore, the plant has been implicated in the treatment of diarrhea.

In conclusion, extract of *Parquetina nigrescens* was found to reduce the microbial count of some beneficial microbes inhabiting the gastrointestinal region of wistar rats. In vitro activities of this plant extract against the isolated microbes from the gut further confirmed the inhibitory properties of this important plant. Therefore, it is very imperative that the concentration at which the plant extract will not be eliminating the beneficial microbes inhabiting the gut be determined and recommended.

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