



Bacteriological Quality, Antimicrobial Activity, and Public Health Implications of Selected Herbal Remedies Sold in Offa, Kwara State, Nigeria

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Abstract

The increasing use of locally produced herbal concoctions in Nigeria for medicinal purposes has raised concerns about their safety, as many are used without scientific validation. This study aims to investigate the bacteriological quality and histo-pathological effects of select herbal concoctions to assess their safety to human use. Thus, bacteriological quality of herbal remedies, the identity of isolates and histopathological effects of select herbal remedies using Wistar albino were determined using standard methods. Findings from means bacterial population of bacterial isolates of herbal remedies ranged between 5.0 and 10.0 ($\times 10^6$ cfu/ml), and no significant difference recorded among the values obtained at $p \leq 0.05$. Bacterial genera isolated from the herbal remedies included *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas sp.*, *Streptococcus sp.* and *Enterococcus sp.* The minimum inhibitory concentrations (MICs) of herbal remedies ranged between 60 and 90 (mg/ml) concentrations. Findings from toxicological studies confirmed that herbal remedies had adverse effects on the intestine, liver and kidney of the test Wister albino rats. This study suggests that extra care should be taken before and while taking herbal remedies despite its proven potency in curing some human ailments.

Keywords: Bacteriological, Herbal Remedies, Experimental Rats, Histopathology, Bacteria

Introduction

Traditional herbal medicine has been a reliable mean of healthcare in Nigeria for decades, with appreciable portion of the population relying on folk remedies for various ailments. Despite its wide use, the safety of many locally produced herbal concoctions remains largely unconfirmed. Concerns about potential toxicity, especially with prolonged use, have led to increased interest in the scientific evaluation of these treatments (Oreagba et al., 2011). Medicinal plants are widely known to exhibit different positive effects in animals, especially humans. Several species of plants are used for the management or treatment of some diseased conditions in herbal medicine, some of which include fruits of *Xylopiya aethiopica* (*X. aethiopica*) and seeds and leaves of *Piper guineense* (Uhegbu et al., 2015). Despite the global use of herbal medicines and their inherent health profits, they have their health hazard. The unethical and careless use of some herbs may endanger and put the health of their users at risk [(Nnorom et al., 2006; Bury & Fullinlaw (1987)]. More so, no convincing logical analysis has appraised the safety and effectiveness of traditional medicinal products (WHO Media Centre 2008). According to Oshikoya et al. (2007); Langlois-Klassen et al. (2007), herbal medicines used alone or concurrently with conventional or orthodox medicines had cases of adverse reactions. Despite the global multiplicity and acceptance of traditional medicines in diverse beliefs and areas, there are no corresponding global principles and approaches for its assessment (WHO, 2005). According to history, Jamaica is over dependent on herbal medicine (Asprey & Thornton, 1955), and this supported the claims of world health organization (WHO, 2002) estimate that, in developing countries up to 80% of the population depends on traditional herbal medicine for primary health care. Folk's medicines have long history of use in managing several ailments, based on the attributed efficacy and safety (Mansi et al., 2023).

In Nigeria, herbal medicines are widely used due to the country's diverse plant population, great socio-diversity, and belief originating from three ethnic backgrounds. Presently, African and European, use herbal medicines concurrently with synthetic medicines to lessen health care expenses owing to inadequate access to modern health care facility, and also because most individuals do not have health insurance and have low level of educations (Delgoda et al., 2010). Herbal medicines are cheap alternatives because they are easy to make or purchase in street markets in the West African regions. These herbal medicines are used not only because of

their therapeutic properties for the gastrointestinal tract but also because they are multifunctional herbs (Zank & Hanazaki, 2017). The increasing use of locally produced herbal remedies in Nigeria for medicinal purposes has raised concerns about their safety, as many are used without scientific validation. Traditional remedies are often perceived as safe due to their natural origins, but they may pose risks, including toxicity and adverse effects on vital organs like the liver and kidneys. This study is justified because it seeks to fill a critical gap in understanding the potential histopathological impacts of these widely consumed herbal medicines. By using Wistar albino rats as a model, the research will provide essential data that can guide the safe use and regulation of herbal concoctions, contributing to public health protection in Nigeria. This study aims to investigate the histopathological effects of selected herbal remedies on Wistar albino rats to assess their safety. The histopathological analysis will be limited to key organs such as the intestine, liver and kidneys.

Materials and Methods

MATERIALS

Sample collection

Four (4) different herbal remedies (concoctions) were collected from two different locations in Offa, Kwara State, Nigeria. The samples were collected into sterile universal containers and brought to the laboratory for analyses. The GPS of the locations where the concoctions were obtained were accurately taken and recorded.

METHODS

Sterilization of materials and culture media

This was carried out to ensure that materials and media used were free from microorganisms which could be a source of contamination in this work. All the materials used were washed with soap and water and then sterilized in an oven at 160 °C for 60 minutes. Seventy percent (70%) alcohol was used to disinfect (swabbing) the bench work surface. All culture media were prepared according to the manufacturers guidelines, sterilized in an autoclave at 121 °C for 15 minutes at 15 Pascal.

Determination of microbial load of herbal concoction samples obtained from Offa Kwara State.

Six – fold serial dilution was carried out on the samples. An aliquot (1 ml) of the diluent was pipetted into Petri-dished. Then pour plated with 20 ml of molten nutrient agar, incubated at 37°C. The emerged colonies were counted using colony counter, and values recorded after 24 hours of incubation.

Preservation of culture

The bacteria isolates were inoculated into nutrient agar and incubated at 37 °C. The bacteria culture on nutrient agar stored and kept in the refrigerator at 4 °C until needed for analyses.

Identification of bacterial isolates from herbal concoction

The identity of all isolates was determined using standard methods of Cheesbrough (2010) Biochemical tests were also carried out on distinct colonies to ascertain the identity of the isolates. The isolates were tentatively identified by means of morphological characteristics, cellular and biochemical test. Morphological characteristics were observed for each bacterial colony after 24 hours of incubation. The appearance of the colony of each isolate on the media was studied and the characteristics observed included; cell shape, elevation, edge, optical characteristics, consistency colony surface and pigmentation. Biochemical tests carried out included; Catalase test, Production of hydrogen sulphide (H₂S), indole, urease, methyl red, oxidase, coagulase, motility, methyl red, vogesproskauer, starch hydrolysis and sugar fermentation. The results were compared with Bergey's Manual of Determinative Bacteriology (Fawole & Oso, 2007).

Gram's staining

This was carried out to know the Gram reaction of the isolates. A smear of each isolate was made on grease free glass slide, crystal violet stain was added to the smear for 30-60 seconds after which the stain was washed off with sterile water, and then Gram's iodine was added to the smear for another 30-40 and washed off with sterile water. Alcohol was added to the smear for 60 seconds to decolorize the stain. Safranin was finally added to the smear as a counter stain, rinsed with sterile water and dried. The glass slide was mounted on microscope and observed under oil immersion objectives lens (x100) A purple indicated the presence of Gram-positive microorganism while pink colour indicated the presence of a Gram-negative microorganism (Fawole & Oso, 2007).

Catalase test

The isolates were tested for the presence of enzyme catalase by placing a small amount of 24 hours of bacterial culture on a clean glass slide using a sterile inoculating loop, few drops of 3% hydrogen peroxide was added to

the smear, presence of bubbles indicates liberation of oxygen i.e. The presence of enzyme catalase which breaks down H_2O_2 to H_2O and O_2 (Fawole & Oso, 2007)

Citrate test

The isolates were inoculated into citrate agar slants and incubated at room temperature for 24 hours. Organisms that is able to utilize citrate as their carbon source will be indicated by a colour change of the agar from green to deep blue while microorganisms that cannot utilize citrate will be indicated by no colour change (Cheesbrough, 2010; Fawole & Oso, 2007),

Oxidase test

This was carried out to know the ability of isolates to produce the enzyme cytochrome oxidase. A piece of filter paper was soaked with few drops of oxidase reagent. Inoculum of each isolate was then smeared on the filter paper and observed for 10 seconds. The appearance of purple coloration on the filter paper implies that the microorganism is oxidase positive (Fawole & Oso, 2007),

Motility test

This test was carried out to know the motility of the isolates due to the presence of a structure called flagella. It was carried out by inoculating nutrient agar medium (with reduced percentage of agar component of the medium) with a straight wire, making a single stab down the centre of the tube to about half the depth of the medium. The tubes were then incubated at 37 °C. Non motile bacteria will give growths that are confined to the stab line while motile bacteria give diffuse growth that spread throughout the medium (Fawole & Oso, 2007).

Indole test

This was carried out to know the ability of the isolates to hydrolyze amino acid tryptophan with the release of indole. It was carried out by inoculating the test organism in a Biotin bottle containing 3 ml of sterile tryptone water and incubated at 37 °C for 48 hours. The release of indole was tested by adding 0.5 ml of Kovac's reagent and shaken gently. Negative indoles test was confirmed by absence of red colour in the surface layer within 10 minutes (Cheesbrough, 2010).

Coagulase test

A drop of distilled water was placed on clean slide, a sterile loop was used to pick colonies and a thick suspension was made. A loopful of plasma was added to the suspension agglutination within 10 seconds indicates positive reaction and absence of agglutination indicates negative reaction (Fawole & Oso, 2007)

Spore staining

A smear of the isolate was placed on a clean glass slide, which was flooded with malachite green stain and heated over beaker of boiling water bath for 10 minutes. The slide afterward flooded safranin for 60 seconds, washed with water and allowed to dry. The stained slide was examined under and oil immersion microscope with the spore appearing green while the negative cells appear red (Fawole & Oso, 2007)

Starch hydrolysis

A 1.4 gram of nutrient agar and 1.0 gram soluble starch were prepared in 100 ml distilled water and sterilized at 121 °C for 15 minutes, allowed to cool, poured on sterile plates and inoculated by single streak of the isolate across the surface of the media. Un-inoculated plates serve as control for the test. The control and experimented plates were incubated at 37 °C for 5 days. Hydrolysis of starch was determined by flooding the plates with Lugol's iodine. The blue-black colour showed negative, clear zone around the colony shows positive, while reddish brown zones around the colony showed partial result (Fawole & Oso, 2007).

Methyl red test

Nine milliliter (9 ml) buffered (phosphate) glucose broth (1g glucose, 0.5% KH_2PO_4 , 0.5% peptone and 100 mg stiller water) were dispensed aseptically into clean test tubes and covered with absorbent cotton wool and aluminum foil, sterilized at 121°C for 15minutes. After cooling, it was inoculated with bacterial isolate and incubated at 37°C for 48hours. Five (5) drops of methyl red reagent was added to each test tubes, development of bright red colour indicate negative result (Fawole & Oso, 2007).

Sugar fermentation test

Five milliliter (5 ml) each of the sugar solution was dispensed into different tubes with Durham's tube inverted into each test tube. The mouths of the tubes were plugged with cotton wool and labeled appropriately; it was then sterilized in an autoclave for 15 minutes at 121 °C. The tubes were then allowed to cool down. The bacterial isolates were inoculated aseptically into the sugar solutions in the tubes and incubated at 37 °C for 72 hours. The change of colour from red to yellow indicates acid production which implies the utilization of the

sugar by the microorganism and appearance of bubble in the Durham tube indicates gas production but if otherwise, acid or gas is not produced (Fawole & Oso, 2007).

Test organisms for antimicrobial assay

The bacterial isolates used for the experiments were *Bacillus subtilis*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Micrococcus* spp., *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp., *Enterococcus* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis* obtained from the Microbiological Unit of Department of Biological Science, Federal Polytechnic, Offa, Kwara State, Nigeria.

Standardization of inoculums for antimicrobial assay (0.5 McFarland standard)

A 0.1 ml of 1% Barium chloride added to 9.9 ml of 1% sulphuric acid which was later reconstituted into 10 ml of sterile distilled water to make 0.5 ml McFarland standard solution. The broth culture of 24 hours test organism was then compared in terms of turbidity to 0.5% McFarland which is equivalent to 10^5 cfu/ml. A loopful of the standardized (0.5 McFarland standard solution equivalent) culture was used for antibiotic susceptibility assay.

Screening of herbal concoctions for antimicrobial assay

Antimicrobial activities of herbal concoctions

Agar well diffusion method of Daoud et al. (2015) was used to assay the antibacterial activities of herbal concoctions. One ml aliquot of 24 hours bacterial culture was poured plated into freshly prepared tempered Muller Hinton agar (MHA) and allowed to gel. Upon solidification, wells were made using a sterile cork-borer (6 mm in diameter) into agar plates containing inoculums. A few drops of 100% concentration of herbal concoction were poured into agar well containing microbial inoculum. Then, the plates were incubated at 37 °C for 24 hours along with control plate that is organisms viability control (OVC). Antimicrobial activity was determined by measuring the zone of inhibition in millimeter.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined by broth dilution method of NCCLS (2002). Varying concentrations of medicinal plant extract were prepared by accurately measuring 0.9 ml, 0.8 ml, 0.7 ml, 0.6 ml and 0.5 ml into 1 ml of sterile distilled water to give final concentrations of 90, 80, 70, 60 and 50 percent respectively. Then 1 ml aliquot of 24 hours inoculum of 0.5 McFarland standard equivalent was transferred into each of extract concentration. The tubes containing the culture and the extract were incubated at 37 °C for 24 hours. The results of extract minimum inhibitory concentration (MIC) was checked by comparing the growth on agar culture with that of the control; organism viability control (OVC). Growth confirmed no activity and no growth confirmed activity of the extract. The least concentration of the extract with no growth confirmed minimum inhibitory concentration.

Determination of minimum bacteriocidal concentration (MBC)

The minimum bacteriocidal concentration (MBC) values were determined by agar dilution method of NCCLS (2002). Organisms from agar culture that showed activity under minimum inhibitory concentration were sub-cultured into freshly prepared agar culture containing no extract. The least concentration of extract where microorganism could no revive indicated minimum bacteriocidal concentration (MBC).

Phytochemical screening of herbal concoctions extracts

Phytochemical screening for the presence of alkaloids, cardiac glycosides, Terpenoids, triterpenes, flavonoids, saponins, and tannins was carried out using the methods described by Alhaithloul (2023).

Test for alkaloid

The filtered ethanol extract was diluted in a few milliliter of dilute Hydrochloric acid. A few drops of Hager's reagent (picric acid in a saturated aqueous solution) were added to 2 ml of filtrate. The presence of a bright yellow precipitate indicated the presence of alkaloids.

Test for saponins

Twenty milligram (20 mg) of the crude extract and fractions was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. Ten milliliter of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation.

Test for flavonoids

To 50 mg of the crude extract and fractions, 100 ml of distilled water was added, stirred and then filtered to get the filtrate. Five milliliter (5 ml) of dilute ammonia solution added to 10 ml of the filtrate followed by few drops of concentrated H_2SO_4 . Yellow coloration indicated the presence of flavonoid.

Test for tannins

To 0.5 g of the crude extract and fractions 10mls of distilled water was added, stirred and then filtered. Few drops of 1 % ferric chloride solution were then added to 2 ml of the filtrate. Tannins confirmed present by production of a blue –black color

Test for terpenoids

To test for terpenoids (0.5 g) of the crude extract and fractions was mixed with 2 ml chloroform and 3 ml H_2SO_4 was carefully added to form a layer. Terpenoids confirmed present by a reddish brown colouration of the interface

Test for Flavonoid

Two to three ml of (methanol extract) filtrate were treated with a piece of magnesium ribbon and one ml of strong HCl. Pink-red or crimson coloring of the solution confirmed presence of flavonoids.

Experimental animals

Ten (10) three (3) weeks old Wister albino rats of similar sexes weighing between 140-250g were obtained from Science Laboratory Department Biological garden of Federal Polytechnic Offa, Kwara State, Nigeria. They were allowed to acclimatize for 7 days, fed with grower's mash and sachet water before treatment with plant extracts.

Treatment of experimental animals

The acclimatized rats were randomly categorized into five groups of two per group. Groups 1 were fed grower's mash, water and 1 ml concoction A, group 2 fed with grower's mash, water and 1 ml concoction B, group 3 fed with grower's mash, water and 1 ml concoction C, group 4 fed with grower's mash, water and 1 ml concoction D and group 5 (control) were maintained on grower's mash and water only for 7 days after which the animals were sacrificed. The animal were taken good care of according to the "care and use of laboratory animals" at Federal Polytechnic Offa, Kwara State, Nigeria.

Histopathology examination

The removed kidney, liver and large intestine were fixed in formal saline, after which they were dehydrated in different alcohol concentrations 50, 70, 90 and absolute alcohol. Thereafter the alcohol was removed in xylene and tissues were embedded in paraffin and mounted on chuck. Sectioning was done using rotary microtome. The sectioned tissues were floated in water and picked with glass slides and finally stained with haematoxylin and eosin as thus: the sectioned were de-waxed in xylene (5 minutes), hydrated through this following grades of alcohol, 100, 90, 70 and 50 spending 1 minute in each stage, rinsed in water and stained with haematoxylin for 5 minutes, differentiated in 1% acid alcohol for 10 seconds and then blued in running water for 5 minutes. It was counter stained with 1 %eosin for 5 minutes and finally, the sectioned were dehydrated in 50, 70, 90 and 100 alcohol impregnation, dropped in xylene and mounted on distrene (a polystyrene) a plasticizer and xylene

Results**Table 1: Means population of microbial load of local herbal concoctions**

S/N	Samples	Samples sites	Sites' coordinates	X 10^6cfu/ml
1	A	Ijagbo new site	Long. 4.73057, Lat.8.13054	5.667 $\pm$ 2.082 ^a
2	B	Ijagbo main town	Long. 4.72704, Lat. 8.13630	5.000 $\pm$ 1.732 ^a
3	C	Ijagbo new site	Long. 4.73057, Lat.8.13054	10.000 $\pm$ 1.732 ^a
4	D	Ijagbo main town	Long. 4.72704, Lat. 8.13630	10.000 $\pm$ 8.718 ^a

Values are mean $\pm$ SD three replicates (n=3) values in the same column with the same alphabet are not significantly different at $p \leq 0.05$

Key = S/N= serial number, A-D= samples, cfu= colony forming unit, Long. = Longitude, Lat. = Latitude, A= Pile-a, B= Fever-a, C= Pile-b, D= Fever- b

The mean count of bacteria isolated from herbal concoction in Offa, Kwara State, Nigeria is shown in Table 1. The findings revealed that sample obtained from site with coordinates Long. 8.13630, Lat. 4.72704 recorded the

highest means bacterial count of 10.000×10^6 CFU/ml, while sample from site with coordinates Long. 8.13630, Lat. 4.72704 recorded least means bacterial count (5.000×10^6 CFU/ml) and there were no significant differences among values obtained from herbal concoction samples at $p \leq 0.05$.

Table 2: Bacterial isolates from herbal concoction in Offa (Kwara State) Nigeria

S/N	Samples	Samples sites	Sites' coordinates	Bacterial isolates
1	A	Ijagbo new site	Long. 4.73057, Lat. 8.13054	<i>Proteus mirabilis</i> , <i>Bacillus subtilis</i> ,
2	B	Ijagbo main town	Long. 4.72704, Lat. 8.13630	<i>Pseudomonas sp.</i> , <i>Proteus mirabilis</i> , <i>Bacillus subtilis</i>
3	C	Ijagbo new site	Long. 4.73057, Lat. 8.13054	<i>Bacillus subtilis</i> <i>Pseudomonas sp.</i> , <i>Streptococcus sp.</i> ,
4	D	Ijagbo main town	Long. 4.72704, Lat. 8.13630	<i>Bacillus subtilis</i> , <i>Streptococcus sp.</i> , <i>Enterococcus sp.</i> , <i>Staphylococcus aureus</i> ,

Key: S/N= serial number, A-D= samples, Long. = Longitude, Lat. = Latitude, A= Pile-a, B= Fever-a, C= Pile-b, D= Fever- b

Using standard conventional methods, in herbal samples analyzed *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus sp.*, and *Micrococcus sp.* were presumptively confirmed present (Tables 2).

Table 3: Antimicrobial activities of local herbal concoction on selected microbes

S/N	TEST ISOLATES	Extracts concentrations (in mg)/ zones of inhibition (in mm)			
		Pile-A	Fever B	Fever A	Pile B
1	<i>Proteus mirabilis</i>	20	15	20	25
2	<i>Enterococcus sp</i>	13	10	-	-
3	<i>Pseudomonas earuginosa</i>	15	-	-	12
4	<i>Escherichia coli</i>	20	8	-	-
5	<i>Micrococcus sp</i>	12	10	7	-
6	<i>Streptococcus sp.</i>	25	10	-	-
7	<i>Bacillus subtilis</i>	23	12	-	13
8	<i>Staphylococcus epidermidis</i>	-	12	-	-
9	<i>Salmonella sp</i>	15	24	-	-
10	<i>Staphylococcus aureus</i>	-	13	-	-

Key: S/N= serial number, - = no zone of inhibition

Findings from *in vitro* antimicrobial activities of herbal concoction on the test isolates revealed that herbal concoction displayed activities against *Proteus mirabilis*, *Enterococcus sp.*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Streptococcus sp.* at 100 mg/ml concentrations (Table 3).

Table 4: Minimum inhibitory concentration of Fever-A concoction on selected isolates

S/N	TEST ISOLATES	Extracts concentrations (in mg)			
		90	80	70	60
1	<i>Proteus mirabilis</i>	14	12	9	6
2	<i>Micrococcus sp</i>	-	-	-	-

Key: S/N= serial number, - = no activity (turbidity),

Table 5: Minimum inhibitory concentration of Fever- B concoction on selected isolates

S/N	TEST ISOLATES	Extracts concentrations (in mg)			
		90	80	70	60
1	<i>Proteus mirabilis</i>	-	-	-	-
2	<i>Pseudomonas sp.</i>	-	-	-	-
3	<i>Bacillus sp</i>	-	-	-	-

Key: S/N= serial number, - = no activity (turbidity)

Table 6: Minimum inhibitory concentration of Pile A concoction on selected isolates

S/N	TEST ISOLATES	Extracts concentrations (in mg)			
		90	80	70	60
1	<i>Proteus mirabilis</i>	15	13	10	7
2	<i>Enterococcus sp</i>	20	18	16	-
3	<i>Pseudomonas sp.</i>	-	-	-	-
4	<i>Escherichia coli</i>	-	-	-	-
5	<i>Micrococcus sp</i>	-	-	-	-
6	<i>Bacillus sp</i>	-	-	-	-
7	<i>Salmonella sp</i>	16	15	14	13

Key: S/N= serial number, - = no activity (turbidity).

Table 7: Minimum inhibitory concentration of Pile B concoction on selected isolates

S/N	TEST ISOLATES	Extracts concentrations (in mg)			
		90	80	70	60
1	<i>Proteus mirabilis</i>	12	10	8	-
2	<i>Enterococcus sp</i>	13	11	9	6
3	<i>Staphylococcus epidermidis</i>	-	-	-	-
4	<i>Escherichia coli</i>	-	-	-	-
5	<i>Streptococcus sp.</i>	-	-	-	-
6	<i>Micrococcus sp</i>	-	-	-	-
7	<i>Bacillus sp</i>	-	-	-	-
8	<i>Staphylococcus aureus</i>	-	-	-	-
9	<i>Salmonella sp</i>	-	-	-	-

Key: S/N= serial number, - = no activity (turbidity),

The findings of minimum inhibitory concentration of ethanolic and aqueous extracts of medicinal plants were shown on (Tables 4, 5, 6 & 7). The results showed that Fever A displayed minimum inhibitory concentration on *Proteus mirabilis* spp. at 90, 80, 70 and 60 (mg/ml) concentrations respectively. Pile A displayed minimum inhibitory concentration on *Salmonella sp.*, *Enterococcus sp.* and *Proteus mirabilis* at 90, 80, 70, and 60 (mg/ml) concentrations respectively. More so, Pile B displayed minimum inhibitory concentration on *Proteus mirabilis* and *Enterococcus sp.*, at 90, 80, 70 and 60 mg/ml concentration.

Table 8: Phytochemical components of medicinal plant extracts

S/N	Components	Concoctions			
		JEDI A	JEDI B	IBA A	IBA B
1	Saponins	+	+	+	+
2	Terpernoid	+	+	+	-
3	Flavonoids	+	+	+	+
4	Tannin	+	+	+	+
5	Alkaloids	+	+	+	-

Key: S/N= serial number, + = present, - = absent

The phytochemical screening of herbal concoctions confirmed presence of saponins, terpernoid, flavonoids tannin and alkaloid in herbal remedies Pile A, Pile B and Fever A respectively (Table 8.)

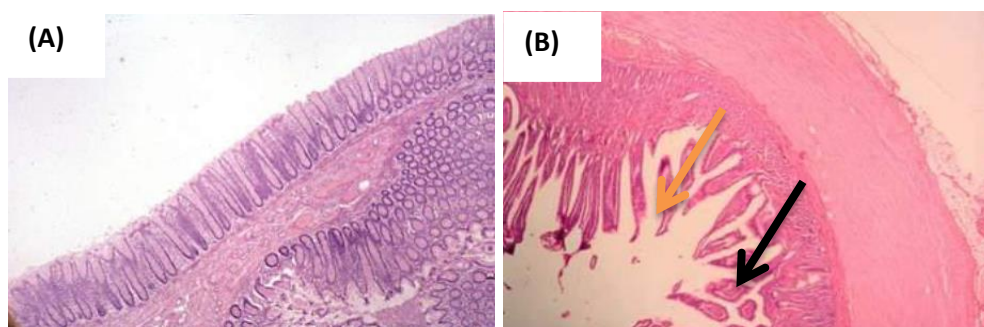


PLATE 1: Photomicrography of the Experimental Rats

(A) Photomicrograph of an intestine section of adult albino rats in control group, showing intact villi and empty lumen. All the intestinal layers were intact and the muscular components were normal.

(B) Photomicrograph of the intestine of treated rat showed necrosis and collapse of the villi into the intestinal lumen. Villi collapsed (yellow arrow) into the intestinal lumen and distorted intestinal epithelium (black arrow) was evident (H & E X100)

The histopathological examination of this study revealed the Photomicrograph of a Kidney section of a treated albino rats, showing degeneration of Glomerulus (G), Distal convoluted tubule (DT), Bowman space (BS), Proximal convoluted tubule (PCT) (H & E X400) (Plate 1)

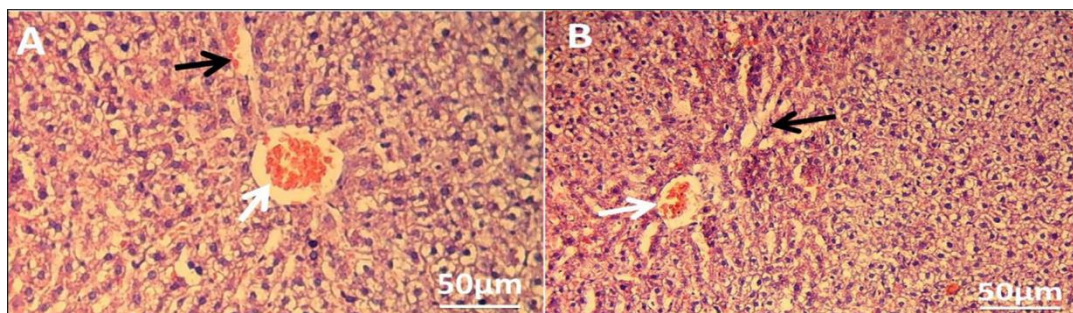


PLATE 2: Photomicrography of the Experimental Rats

(A) Photomicrograph of a Liver section of adult albino rats in control group (Group A), showing normal central vein and devoid of any occlusion (CV). The hepatocytes appear normal with nucleus. The profile was devoid of any cellular alterations, normal radial arrangement of hepatocytes around the central vein and hepatic sinusoids (white arrow). **(B)** Photomicrograph of the Liver of treated rat (Group B) showed degeneration of hepatocytes (black arrow) and dilation and elongation of central vein congestion (white arrow) and congestion and dilation in blood sinusoids (black arrow) (H & E X100). Photomicrograph of the Liver of treated rat (Group B) showed degeneration of hepatocytes (black arrow) and dilation and elongation of central vein congestion (white arrow) and congestion and dilation in blood sinusoids (black arrow) (H & E X100) (Plate 2).

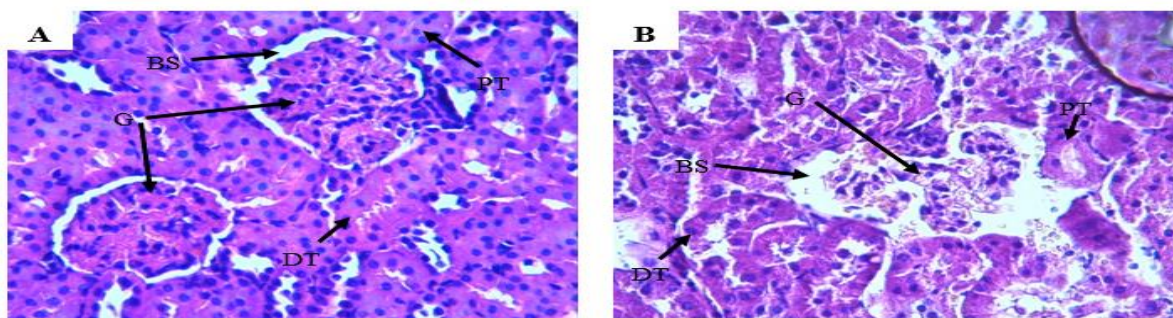


PLATE 3: Photomicrography of the Experimental Rats

(A) Photomicrograph of a Kidney section of adult albino rats in control group, showing normal Glomerulus (G), Distal convoluted tubule (DT), Bowman space (BS), Proximal convoluted tubule (PCT). The kidney section was devoid of any cellular alterations. (B) Photomicrograph of a Kidney section of a treated albino rat, showing degeneration of Glomerulus (G), Distal convoluted tubule (DT), Bowman space (BS), Proximal convoluted tubule (PCT) (H & E X400). Photomicrograph of the intestine of treated rat showed necrosis and collapse of the villi into the intestinal lumen. Villi collapsed (yellow arrow) into the intestinal lumen and distorted intestinal epithelium (black arrow) was evident (H & E X100) (Plate 3).

Discussion

Findings of the mean population of this study disagreed with the findings of work of Onyewenjo et al. (2022) whose total aerobic bacteria load is $1.0 \times 10^5 - 1.4 \times 10^5$ cfu/ml. The present study showed that the herbal remedies under investigation were contaminated by not following good manufacturing practices and this accounted for high bacterial load that renders the remedies completely abysmal especially when consumed indiscriminately. Also, the work of Onyewenjo et al. (2022) was somewhat in line with the findings of this study where the presence of *Bacillus* sp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were confirmed. Similar microorganisms were also isolated from the herbal remedies in previous studies of Chinakwe et al. (2023). However, *Proteus mirabilis* was found to be absent. Findings from the antimicrobial screenings of this study disagreed with the findings from work of Chinakwe et al. (2023), this is because the methods used differ and the concentration varies considerably. Also, the antimicrobial activity reported in the work of Chinakwe et al. (2023), does not correspond with the results of this study due to the difference in the solvent used in extracting the constituent of the herbal concoction. The solvent used were acetone and hexane in which aqueous extract were used in this finding. More so, findings of Onyewenjo et al. (2022) in the minimum inhibitory concentration do not correspond with the results of this due to the differences in solvent extraction. The histopathological examination of this study revealed the Photomicrograph of a Kidney section of a treated albino rat, showing degeneration of Glomerulus (G), Distal convoluted tubule (DT), Bowman space (BS), Proximal convoluted tubule (PCT) (H & E X400) (Plate 1). Photomicrograph of the Liver of treated rat (Group B) showed degeneration of hepatocytes (black arrow) and dilation and elongation of central vein congestion (white arrow) and congestion and dilation in blood sinusoids (black arrow) (H & E X100) (Plate 2). Photomicrograph of the intestine of treated rat showed necrosis and collapse of the villi into the intestinal lumen. Villi collapsed (yellow arrow) into the intestinal lumen and distorted intestinal epithelium (black arrow) was evident (H & E X100) (Plate 3), indicating the risk in high consumption of herbal concoction to human health.

Conclusion

The study investigating the bacteriological quality, antimicrobial properties, and histopathological effects of various herbal remedies on apparently healthy Wistar albino rats has provided invaluable acumens into the potential therapeutic uses and safety potentials of these herbal remedies. The results indicate that certain herbal concoctions exhibit significant antimicrobial properties, with the ability to inhibit the growth of pathogenic bacteria. In the aspect of histopathological effects, the study revealed detrimental outcomes, which depends on the composition and dosage of the herbal concoctions. Some herbs demonstrated minimal or no adverse effects on the histological structures of the organs, indicating their relative safety for use in vivo. However, others caused varying degrees of tissue damage, highlighting the importance of dosage optimization and careful monitoring of potential toxicity.

Recommendations

It is recommended that additional toxicological studies, including chronic toxicity and dose-response assessments be conducted to determine the safety limits of these herbal remedies for long-term use.

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