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ORIGINAL RESEARCH ARTICLE

Evaluation of Synergistic Antimicrobial and Toxicity Effect of *Anogeissus Leiocarpus* (DC.) Stem Bark and *Acacia Ataxacantha* (Linn) Leaves

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ABSTRACT

The purpose of this study was to assess the safety level of the aqueous and Hexane extracts of the stem bark of *Anogeissus leiocarpus* and the leaves of *Acacia ataxacantha* against *Staphylococcus aureus* and *Candida albicans* that cause vaginal discharge and vulvar itching (pruritis vulvae), as well as their synergistic antimicrobial activity. The aqueous extract of the extracts contained carbohydrates, flavonoids, alkaloids, tannins, anthraquinones, steroids, and terpenes, according to phytochemical screening; however, the hexane extract only contained carbs and phenols. The crude extracts' antimicrobial activity against the clinical isolates was determined to be active (18.00±0.00 for the aqueous extract and 13.00±0.00 for hexane extract against *Candida albicans* and 15.30±0.57 and 13.00±0.50 for the aqueous and Hexane extracts against *Staphylococcus aureus* respectively). The Fourier Transformed Infrared Spectroscopy (FT-IR) spectra revealed many peak positions, demonstrating the presence of phenol, alkyl, carboxylic acid, alcohol, aldehyde, alkenes, amines, ketones, and esters. Because no behavioural abnormalities or deaths were noted during the study's stages when the test animals were administered with up to 1000 mg/kg of the extracts, the data demonstrated that the plant extracts were comparatively safe. As a result, synergistic extracts of *Anogeissus leiocarpus* stem bark and *Acacia ataxacantha* leaves are considered safe for use when their oral median lethal dose (LD_{50}) is larger than 1000 mg/kg.

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INTRODUCTION

Pruritus vulvae, or abnormal vaginal discharge and vulval itching, is an infection brought on by a variety of organisms. When it persists, this creates distress for women of all ages, particularly sexually active women of reproductive age (Roqaiya *et al.*, 2016). Women who have untreated gonorrhoea infections may experience severe long-term issues, among other consequences (Grant and Nunns, 2012). According to Roqaiya *et al.* (2016), this infection has been linked to bacterial vaginosis, candidiasis, and other STDs.

Throughout history, people have used plants to prepare medications to treat ailments. Because they contain a variety of bioactive substances that aid in the healing of illnesses, innumerable plant species are consumed or used in one way or another throughout the world (Victor and Grace, 2013). Numerous microorganisms worldwide have developed antibiotic resistance, making infectious diseases one of the conditions that continue to threaten human health despite global advancements in antibiotic discovery. For this reason, the majority of people from low-income families are still forced to use traditional medicines to treat everyday illnesses (Keta, 2016). Plant extracts or herbal medicines are an easy way to treat these germs and the sickness they produce (Izah *et al.*, 2023). Among the microbes that are resistant to these antibiotics include *Staphylococcus aureus* and Candida albicans (Izah *et al.*, 2023). Because they contain a variety of phytochemicals, plants have been demonstrated to have a broad range of antibacterial activity (Ugboko *et al.*, 2020) against various microbes that are either superior to or equivalent to those of the conventional antibiotic drug (Njeru *et al.*, 2013).

The goal of herbal practitioners' medicinal plant mixtures is to treat multiple ailments and achieve better results than single herbs (Odhiambo *et al.*, 2011). Plants' varied antibacterial activity may be explained by the antagonistic

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or synergistic effects of different bioactive components in different concentrations (Abuto and Morono, 2018). Njeru *et al.* (2013) state that the growing drug resistance of the majority of bacteria has necessitated ongoing research into beneficial medicinal plants for safer and better medications that could provide a greater effect in overcoming the organisms' resistance to traditional antibiotics. According to this research, medicinal plants may be able to treat certain drug-resistant microbial diseases, which will support their usage in traditional medicine. This research investigated the potential synergistic effects of combining these plant extracts, which could increase antimicrobial activity when compared to the single plant extracts as earlier reported, as well as demonstrating clinical evidence on the efficacy and safety of using the plant extracts combination to treat infections and the potential modulation of microbial resistance mechanisms by multi-plant treatments in comparison to single-agent therapies.

MATERIALS AND METHODS

Plants Collection and Identification.

Selected plants parts *Anogeissus leiocarpus* stem bark and *Acacia ataxacantha* leaves for the research were collected from Darki forest, Wudil Local Government Area in Kano state and taken to herbarium section, Bayero University Kano for identification.

Plant material Extraction and Preparation

Parts of the chosen plants were mashed with a mortar and pestle and allowed to air dry at room temperature. To create 1000 g of powder, 500 g of each powdered plant material was weighed and well mixed from the samples of powdered plants. Using the maceration procedure, 500 g of each powder were extracted completely over two (2) weeks using 2.5 litres of Hexane and distilled water. The solvents were drained off at the end of the procedure, and centrifugation was used to extract the leftover miscellaneous material from the plant materials (Tiwari *et al.*, 2011). After being weighed, the extracted materials were placed in sterile, airtight containers and stored in desiccators until needed (Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002).

Phytochemical Tests

Phytochemical screening of the crude extracts was carried out using standard methods described by Sofowora (1993), Trease and Evans (2002), and Yadav and Munin(2011).

Test Isolates

The microbiology department of Murtala Muhammad Specialist Hospital in Kano provided the clinical isolates that were utilised to evaluate the antibacterial qualities of the chosen plants' aqueous crude extract. Prior to usage, the organisms were subcultured on nutritional agar, sloped at 4ºC, and maintained (Parekh and Chanda, 2007).

Bioassay studies

Antimicrobial activity of the crude aqueous extract of the selected plants was carried out according to standard method.

Culture Media

Mueller Hinton agar media was used to grow *Staphylococcus aureus* while Potato Dextrose Agar medium was used to grow *Candida albicans*.

Standardization of Inoculum was conducted as described by Perez *et al.* (1990) and Kirby (1996).

Preparation of the Test Concentrations for Sensitivity

Two grammes (2g) of each extract were dissolved in four millilitres of Dimethyl Sulfoxide (DMSO) in separate Bijou bottles to create 1000 mg/ml solutions, which were then labelled as the stock solutions for the aqueous and Hexane extracts. Using the serial doubling dilution procedure, the working solutions were made from the stock solution of each aqueous and Hexane extract. 500 mg of flucoxacillin for bacteria and 500 mg of fluconazole for fungi were used as controls. To achieve a 500 mg/ml concentration, each tablet was dissolved in 1 ml of DMSO (Esimone *et al.*, 2012).

Antibacterial assay

The agar well diffusion method was used for this assay using Mueller Hinton Agar, and after the plates were incubated, the diameters of the zones of inhibition surrounding the wells were measured in millimetres using a transparent ruler. These tests were performed in duplicate.

Assay for antifungals

This assay was carried out using the agar well diffusion method with Potato Dextrose Agar (PDA). Following incubation, the plates were examined for zones of inhibition surrounding the wells, and the diameters of the zones were measured in millimetres using a transparent ruler (Mukhtar and Okafor, 2002; Esimone *et al.*, 2012; Singh and Tafida, 2013). These tests were performed in duplicate.

Minimum Inhibitory Concentration (MIC) calculation

Using the tube doubling dilution procedure with Dimethyl-sulfoxide (DMSO), the extracts (5 mg/ml) were subjected to the minimum inhibitory concentration (MIC) experiment in order to reach four distinct concentrations. In separate test tubes, one millilitre (1 ml) of the plant extract was added to each of the two media: Mueller Hinton agar (*Staphyloccocus aureus*) and Potato Dextrose broth (*Candida albicans*). Additionally, 0.1 ml of the standardised inocula were added and thoroughly mixed. For 48 hours, the test tubes were kept in an aerobic environment at 35 °C. The extracts without inocula and test tubes with broth were used as the positive control,

while test tubes with inocula and broth were used as the negative control for comparison. Following a 24-hour incubation period, the test tubes were examined, and the presence of growth (turbidity) and absence of growth (clear solution) were identified and noted. Minimum inhibitory concentrations (MIC) were defined as the lowest doses that did not exhibit any signs of growth or turbidity, (Baker *et al.*, 1993, Vallekobia *et al.*, 2001).

Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

Sub-culturing was used to measure the Minimum Bactericidal Concentration (MBC) and Fungicidal Concentration (MFC) of aqueous and Hexane extracts from each MIC test tube that did not exhibit any signs of growth (turbidity). To ascertain the MBC and MFC, the plates were incubated for an additional 24 hours at 37°C. The lowest bactericidal and fungicidal concentrations were determined to be the maximum dilutions that produced no single microbial colony on the solid media (Baker *et al.*, 1993; Vallekobia *et al.*, 2001).

Studies of Toxicity

Origin and Care of Experimental Animals

The Department of Pharmacology and Therapeutics at Bayero University Kano provided sixteen (16) albino rats of both sexes, weighing approximately 100–180 g for each extract. In the animal home, they were kept in cages with adequate ventilation, given their regular diet and unlimited water, allowed to acclimatise for three (3) days, and kept in typical laboratory settings.

Experimental Design

The rats were divided into four groups with three animals per group, sex and weight not considered (n = 3):

Group I: Infected +1000 mg/kg of the plant extract.

Group II: Infected + 500 mg/kg of the plant extract.

Group III: Infected + 250 mg/kg of the plant extract.

Group IV: Normal Negative control (un-infected + distilled water).

Acute Toxicity Studies (LD_{50} Calculation).

Rats were given the modified Lorke (1983) method to determine the acute toxicity of the aqueous and Hexane crude extracts. The study was broken up into two phases. In phase I, three groups of nine (9) randomly chosen adult rats were created, with three rats in each group (n=3). The groups were given aqueous and Hexane extracts at 50, 100, and 200 mg/kg body weight doses. Over the course of 24 hours, the number of deaths and indications of toxicity were noted. A new group of animals was employed, using the same protocol as in phase I, and given higher dosages (250, 500, and 1000 mg/kg body weight) of the extracts after 24 hours, when there were no deaths. For a further 24 hours, the animals were monitored for any indications of toxicity and potential fatalities. The lowest lethal dose and the greatest non-fatal dose were geometrically mean to determine the LD_{50} (Lorke, 1983).

Studies on Sub-chronic toxicity

The sub-acute toxicity study was conducted using the standard operating method for toxicity testing. Three groups of one rat each were created by randomly selecting three rats. Rats in the treatment groups received oral extract at doses of 250, 500, and 1000 mg/kg body weight for 24 hours, whereas the control group was given normal saline. Following administration for six hours, the rats were closely observed for twenty-four hours. Rats' body weights were recorded until the study's conclusion, and their water and feed consumption was also tracked. Following a 24-hour period, the rats were put to sleep in a glass chamber that was sealed and saturated with chloroform. Following the rats' surgical dissection, three millilitres of blood were extracted from each of them via cardiac puncture and placed into bottles of ethylenediaminetetraacetic acid (EDTA) for measuring haematological parameters. A histological investigation was performed after the liver and kidneys were removed, weighed, and preserved in formaline. After that, each rat's relative organ body weight ratio (ROW) was computed (Salawu *et al.*, 2009; WHO, 2000; OECD 407, 2008; Tauheed *et al.,* 2021).

Hematological investigations

Packed cell volume (PCV), haemoglobin concentration (HB), platelets (PLT), white blood cell count (WBC) and differentials, and mean corpuscular haemoglobin concentration (MCHC) were all estimated using an automated haematology analyser, Sysmex KX21N (Sysmex Japan) (Ekakitie *et al.,* 2021), after blood samples were collected into EDTA bottles.

Histology of liver and kidney

The liver and kidney were carefully removed, exteriorised, and preserved in 10% formal saline for a minimum of 48 hours. After being dried using a graded series of ethanol, each sample was embedded in paraffin. The samples were cut into 5–6 µm thick paraffin slices, which were then placed on coated slides. Sections were rehydrated using a decreasing series of ethanol after being dewaxed in xylene prior to staining. Eosin and haematoxylin were used to stain the sections. A consulting histopathologist reviewed the histological sections. Representative lesions were photographed under a variety of magnifications (Olorunsogbon, 2017; Tauheed *et al.,* 2021).

Fourier Transformed Infrared Spectroscopy Analysis (FT-IR)

Fourier Transformed Infrared Spectroscopy Analysis (FT-IR) was conducted using FTIR Carry 630 Agilent technology. The extracts FTIR spectra were captured in the mid-IR range (650–4000 cm–1) at a resolution of 8 cm–1 with different scans. FTIR spectra were recorded and analysed using software that was attached to the equipment. The mean data were utilised for the analysis, and each experiment was carried out in triplicate.

Following interpretation of the spectra, the different spectra were superimposed to evaluate sample similarity (Mansoori *et al*., 2020; Shehu *et al*., 2022).

RESULTS AND DISCUSSION

Phytochemical Constituents of the Extracts

Several phytochemicals were found in *Anogeissus leiocarpus* stem bark extract and *Acacia ataxacantha* leaves after preliminary phytochemical screening, as indicated in Table 1. Similar studies have also identified numerous phytochemical elements in the stem bark of *A. leoicarpus* (Hussaini, *et al.,* 2022). Additionally, the results of this investigation are consistent with those of Mann *et al.* (2008) and Mann *et al.* (2014), who studied the methanol extract of *Anogeissus leiocarpus*. According to Ochola *et al.* (2024), *Acacia ataxacantha* leaves' methanol extract contains a variety of phytochemicals. According to Madubuike *et al.* (2018), *A. ataxacantha* leaves contain a number of phytochemicals. According to Malgwui *et al.* (2024), the aqueous root extract of *A. leiocarpus* contain saponins, cardiac glycosides, and other phytochemicals. Namadina *et al.* (2019) also reported several phytochemicals, including cardiac glycosides and saponins in the aqueous stem bark extract of *A. leiocarpus*.

Table 1: Phytochemical Constituents of *Anogeissus leiocarpus* Stem bark and *Acacia ataxacantha* Leaves Extracts.

Phytochemical	Solvent	
	Aqueous	Hexane
Caebohydrates	+	+
Saponins	-	-
Flavonoids	+	-
Alkaloids	+	-
Steroids	+	-
Triterpenes	+	-
Cardiac glycosides	-	-
Tannins	+	-
Anthraquinones	+	-
Phenols	-	+
Resins	-	-

Key: + = present - = absent

Antimicrobial Activity

The Synergistic effect of aqueous and Hexane extracts of *Anogeissus leiocarpus* stem bark and *Acacia ataxacantha* leaves evaluated for in vitro antibacterial activity against, *C. albicans* and *S. aureus* indicated different zones of inhibition (Table 2). The results showed that the aqueous extract had a significantly higher inhibitory activity (16-18 mm) against *Candida albicans* and *S. aureus*, while the hexane extract had a lower activity (12-13 mm) against the same organisms. This demonstrated that the extracts had good antibacterial activity against the tested organisms due to the extracts' strong presence of various phytochemical compounds such as flavonoids, tannins, alkaloids, steroids, and saponins. The results also showed that even though the plant extracts had demonstrated impressive activities, the positive control inhibited the growth of the organisms higher than the plant extracts. In a related investigation, Mann *et al.* (2008) found that *Anogeissus leiocarpus* ethanol stem bark extract was effective against *Staphylococcus aureus*. *A. leiocarpus's* roots, stem bark, and leaves have been found to contain antibacterial compounds that can be used to treat a variety of microbial infections in other studies (Ali *et al*., 2017; Magashi and Nuhu, 2017; Dayok *et al.,* 2018; Agada *et al.,* 2019; Muhammad *et al*., 2022). Antifungal properties of the herb have also been reported (Adedotun *et al.,* 2023). A study by Mann *et al.* (2014) and Elsiddig *et al.* (2015) demonstrated the potent antibacterial qualities of the leaf, bark, and root extracts of *A. leiocarpus* by demonstrating the in vitro susceptibility of five bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella aerogens, Pseudomonas aeruginosa*, and *Salmonella typhi*). It has also been observed that *A. leiocarpus* has antifungal and antibacterial properties (Elsiddig *et al*., 2015; Muhammad *et al*., 2019; Usman *et al.,* 2020).

Likewise, it was observed that *A. ataxacantha* exhibited antibacterial action against both Gram-positive and Gram-negative yeast and bacteria (Amoussa *et al*., 2016). According to a different study, *Staphylococcus aureus* was among the bacteria that the methanol extract of *Acacia ataxacantha* had bacteriocidal activity against (Ochola *et al.,* 2024). Numerous studies have documented the antifungal (Tissouras *et al.,* 2014) and antibacterial (Saini *et al*., 2008; Okoro *et al.,* 2012; Olajuyigbe and Afolyan, 2012; Elmi *et al*., 2020) properties of various Acacia species. It was discovered that the minimum inhibitory concentration (MIC) for the plants extracts was 2.2 ug/ml for Hexane and 3.7 ug/ml for aqueous in *C. albicans*. The MIC values for Hexane and aqueous extracts of *S. aureus* were found to be 2.4 ug/ml and 2.8 ug/ml, respectively. Additionally, it was discovered that the minimum bactericidal concentration (MBC) for Hexane and aqueous extracts in *C. albicans* was 2.1 mg/ml and 3.3 mg/ml, respectively, while for *S. aureus*, it was 2.0 mg/ml and 2.4 mg/ml.

FT-IR Analysis of Aqueous and Hexane Extracts of *Anogeissus leiocarpus* Stem bark and *Acacia ataxacantha* Leaves.

Alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid, and phenol were all validated by the FTIR analysis results, which are displayed in Table 2 and Figure 1 (aqueous extract). In the bio-reduction process, the absorbance bands that were seen in the 400–4000 cm-1 range are 1123, 1246, 1465, 1648, 2925, and 3648 cm-1. Several secondary metabolites were present, including steroids, terpenoids, saponins, phenols, and carbohydrates. The main peak, which was 3648 cm-1, was attributed to the O-H stretching vibration of the alcohol and phenols functional group. Alcohol, aldehyde, alkenes, amines, ketones, esters, alkyl, carboxylic acid, and phenol were determined to be present in Table 4 and Figure 2 (hexane extract). Between 400 and 4000 cm-1, the absorbance band analysis in the bioreduction process showed values between 1640 and 3324 cm-1, with 3324 cm-1 potentially belonging to O-H. Alcohol and phenol functional groups' stretching vibrations revealed the presence of many secondary metabolites, including steroids, terpenoids, saponins, phenols, and carbohydrates. 1640 cm-1 revealed the existence of

terpenoids, streroids, saponins, fatty acids, and C=C stretching and N-H bending of primary amines. In addition to agreeing with Adigun *et al.* (2000) and Rao *et al.* (2016), this validates the findings on Table 1. The results showed the availability of flavonoids, terpenoids, streroids, and saponins, supporting prior results (Adigun *et al.*, 2000; Kawo *et al.*, 2009; Aliyu and Sani 2011; Rao *et al.*, 2016). C-H (Stretching) and C=O Stretching of Carboxylic acid and ketone were detected at 2925 cm-1. C=C stretching and N-H bending of primary amines were detected at 1648 cm-1. This is consistent with the results of Aliyu and Sani (2011) and Mann *et al.* (2014), which demonstrated the presence of fatty acids, terpenoids, steroids, and saponins. C-N stretching of aromatic amines and C-H bending, which demonstrated the existence of alkaloids, were detected at 1465 cm-1 (Mann *et al.*, 2008, Rao *et al.*, 2016, Salih *et al.*, 2017). In accordance with the findings of Mann *et al.* (2008), Rao *et al.* (2016), and Salih *et al.* (2017), C-O stretching of aryl-ether and phenols (containing anthraquinones) and C-H stretching of aromatic hydrocarbons (containing terpenoids, steroids, saponins, glycosides, and carbohydrates) were observed at 1246 cm-1 and 1123 cm-1.

Table 2: Antimicrobial Activity of *Anogeissus leiocarpus* Stem bark and *Acacia ataxacantha* Leaves Extracts.

Test organism	Plant Extract	Zone of Inhibition (mm) After 24 hrs		Zone of Inhibition (mm) After 48 hrs		Mean±SE After 24 hrs	Mean±SE After 48 hrs
		R1	R2	R1	R2		
Candida albicans	Aqueous	17.90	18.00	18.00	18.00	17.95±0.07	18.00±0.00
	Hexane	13.00	13.10	12.90	13.00	12.50±0.07	13.00±0.00
	PC (Fluconazole)	31.23	31.07	31.27	31.22	31.15±0.47	31.24±0.05
S. aureus	Aqueous	18.61	15.30	18.50	15.34	18.60±1.15	15.30±0.57
	Hexane	13.00	12.00	13.10	13.20	13.00±0.57	13.00±0.50
	PC (Flucoxacillin)	21.30	22.04	20.14	20.78	21. ±0.97	20.46±0.02

Values are expressed as the mean ± standard deviation ($p < 0.05$). PC = Positive control

Table 3: Peak Position and Probable Inter-Atomic Bond of Aqueous Extract of *Anogeissus leiocarpus* Stem bark and *Acacia ataxacantha* Leaves.

IR-SA (CM^{-1})	FUNCTIONAL GROUP	REMARK
3648	O-H Stretching vibration of alcohol and phenols	Steroid, terpenoids, saponins, phenols, carbohydrates
2925	C-H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, terpenoids, steroid, saponins
1648	C=C Stretching, N-H bending of primary amines	Steroid, terpenoids, saponins, fatty acid
1465	C-N stretching of aromatic amines, C-H bending	Alkaloids
1246	C-O stretching of aryl-ether and phenols	Anthraquinones Terpenoid, steroid , saponins,
1123	C-H stretching of aromatic hydrocarbon	glycosides, carbohydrate

IR-SA = Infrared Spectra Absoption

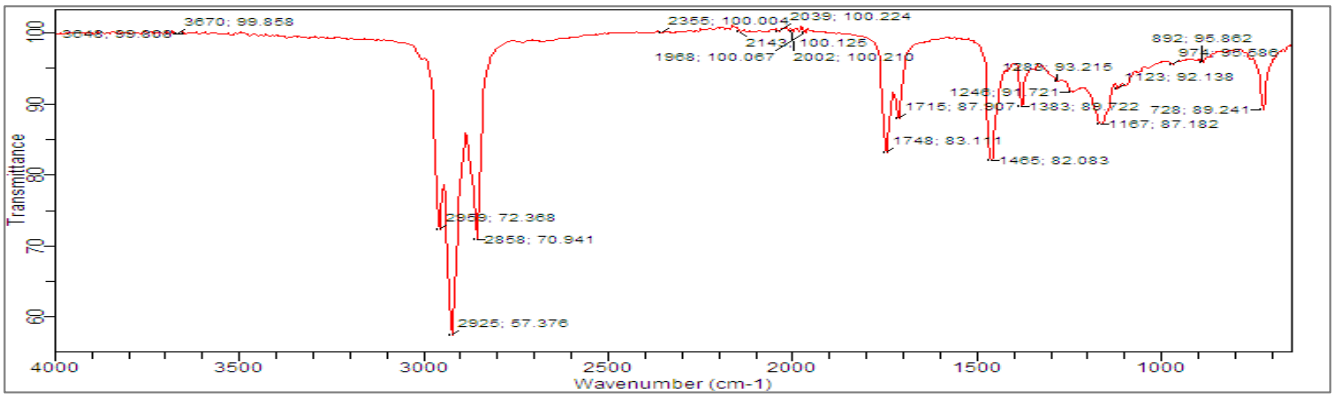

Figure I: FT-IR spectrum of Aqueous Extract

Table 4: Peak Position and Probable Inter-Atomic Bond of Hexane Extract of *Anogeissus leiocarpus* Stem bark and *Acacia ataxacantha* Leaves (IR-SA= Infrared Spectra Absoption)

IR-SA (CM^{-1})	FUNCTIONAL GROUP	REMARK
3224	O-H Stretching vibration of alcohol and phenols	Steroids, terpenoidssaponins carbohydrates and phenols
1640	C=C Stretching, N-H bending of primary amines	Terpenoids, streroids, saponins and fatty acids

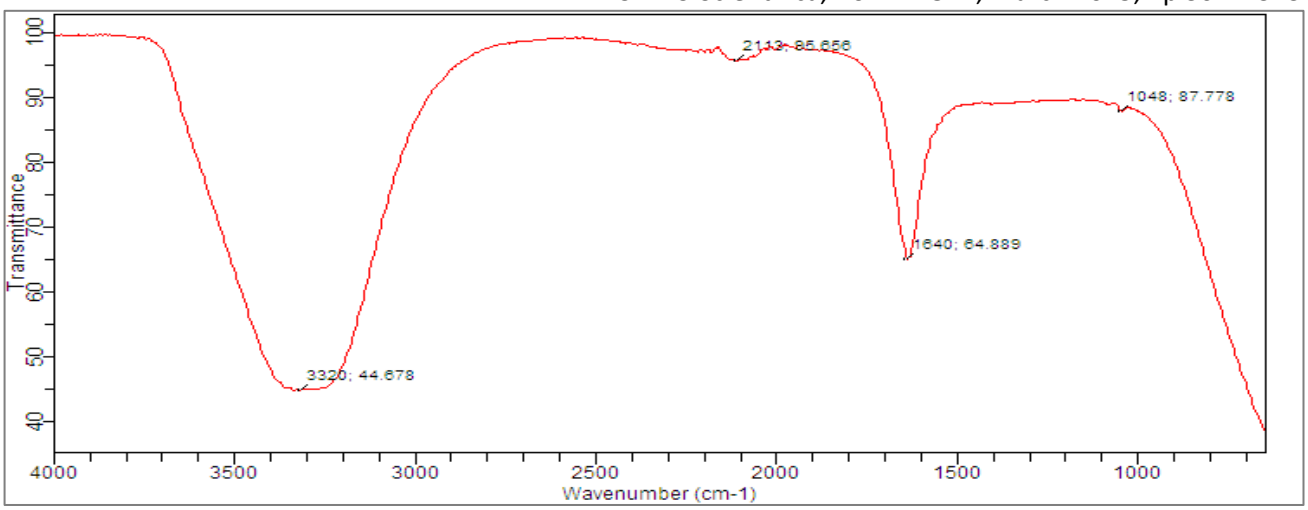

Figure II: FT-IR spectrum of Hexane Extract

Acute and Sub-acute Toxicities Examination of the Extracts

According to the findings of acute toxicity studies, no behavioural changes or deaths were noted in the first (50, 100, and 200 mg/kg) or second (250, 500, and 1000 mg/kg) phases of the study for the aqueous extract; however, one rat experienced appetite loss at 500 mg/kg of hexane extract. Therefore, the synergistic aqueous and Hexane extracts of *Acacia ataxacantha* and *Anogeissus leiocarpus* stem bark are calculated to have an oral median lethal dose (LD_{50}) of more than 1000 mg/kg. This supports the findings of Datok *et al.* (2022) and Namadina *et al.* (2019), who also observed that *Anogeissus leiocarpus* aqueous stem bark extract was non-toxic to experimental animals, even at higher doses of 5000 mg/kg (Tauheed *et al.*, 2021). It was also deemed safe for ingestion after rats at doses of up to 200 mg/kg showed no signs of liver toxicity (Ahmad and Wudil, 2013). The rats exhibited elevated hair, appetite loss, and respiratory difficulties at 5000 mg/kg of *Anogeissus leiocarpus* methanol extract, but at 1000 mg/kg there were no outward signs of toxicity, minor body weakness, and appetite loss (Adamu *et al.*, 2022). Tauheed *et al.* (2021), in contrast to the findings of this study, found that rats given *A. leiocarpus* at 5000 mg/kg within 24 hours did not exhibit any significant physical alterations. At 5000 mg/kg, the rats showed signs of sadness, lethargy, and a rough and hairy coat, but they soon recovered. In addition Sodipo *et al.* (2023) recorded the oral LD_{50} of *A. leiocarpus* as 3807 mg/kg. Furthermore, it was discovered that the methanol leaves extract of *Acacia ataxacantha* has an oral median lethal dose (LD_{50}) of above 5000 mg/kg body weight, according to reports from Abbas *et al.* (2017) and Abbas *et al.* (2018). Thus, the extract appears to be almost non-toxic when taken orally (Loomis and Hayes, 1996; Lorke, 1983).

All of the test animals' body (Table 5) and organ (Table 6) weights increased as a result of the extracts, with the exception of one, which showed a drop in body weight at 500 mg/kg in hexane extract, which may have been caused by a decrease in food and water intake (Teo *et al.*, 2002; Chindo *et al.*, 2012). The methanol extract of *Acacia ataxacantha* increased body weight in comparison to the negative control group (Abbas *et al.*, 2018). The relative organ weights of all the tested doses showed no discernible differences, leading them to infer that the extract is generally safe for the rats. The non-toxicity of *A. ataxacantha's* hydro-alcoholic bark extract up to 2000 mg/kg body weight was confirmed by Maroyi (2018).

Table 5: Effect of Oral Administration on Body Weight of Rats

	Means of Body Weight± SD		
Concentration (mg/kg)	1	2	**3**
1000	131.30 ± 25.60[a]	153.00 ± 66.20[a]	153.40 ± 20.50[a]
500	120.60± 23.10[a]	98.60 ± 57.02[a]	113.8 ± 40.10[a]
250	145.20 ± 19.20[a]	159.40 ± 13.09[a]	136.80 ± 27.60[a]
DW1.0(ml/kg)	125.00 ± 5.90[a]	134.40 ± 10.01[a]	128.00 ± 50.22[a]
LSD	25.21	35.87	65.24

Key for both Table 5 & 6: Means ± SD= Standard deviation; Conc.= concentrations(mg/ml); DW=distilled water; LSD= Means with superscript shows no significant difference (p>0.05) ANOVA

Table 6: Effect of Oral Administration on Relative Organ Weight of Rats

	Means of Relative Organ Weight± SD	
Concentration (mg/kg)	**Liver**	**Kidney**
1000	4.55 ± 0.17[a]	1.16 ± 0.13[a]
500	5.24 ± 0.25[a]	1.22 ± 0.08[a]
250	3.26 ± 0.14[a]	1.23 ± 0.09[a]
DW 1(ml/kg)	5.43± 0.19[a]	0.87 ± 0.03[a]
LSD	2.46	0.26

Table 7a: The Effect of Oral Administration of the Extracts on some Hematological Parameters.

	Means ± SD of Hematological Parameters						
Extract used	Wbc (10^3/uL)	Lymp(10^3/uL)	Mid (10^3/uL)	Gran 1(0^3/ul)	Lymp(%)	Mid (%)	Gran (%)
Normal Range	4.0-11.0	1.2-4.0	0.1-1.5	1.5-7.5	20.0-45.0	3.0-15.0	40.0-45.0
Aqueous	3.90 ± 0.10a	6.10 ± 1.00a	1.30 ± 0.03a	2.90 ± 0.46a	66.00± 2.10a	3.30 ± 0.04a	30.80 ± 0.76a
Hexane	4.90 ± 0.04a	5.60 ± 0.67a	0.30 ± 0.54a	2.60 ± 0.30a	63.50 ± 1.60a	4.80 ± 1.14a	31.60 ± 0.03a
DW 1.0(ml/kg)	4.83 ± 0.29a	5.63 ± 0.40a	0.30 ± 0.10a	3.50 ±0.34a	57.56 ± 1.93a	5.23 ± 0.13a	35.36 ± 1.80a
LSD	0.88	1.61	0.99	0.81	10.98	1.98	10.28

Means ± SD= Standard deviation; DW=distilled water; LSD= Means with superscripts show no significant difference (p>0.05) ANOVA, WBC (white blood cell), LYMP (lymphocyte), MID (monocyte, eosiniphil and basophil combined), GRAN(neutrophil)

The Impact of Extracts Taken Orally on Haematological Parameters.

Table 7a displays the impact on several haematological parameters of oral administration of aqueous and Hexane extracts of the stem bark of *Anogeissus leiocarpus* and the leaves of *Acacia ataxacantha*. LYMP (%) (Lymphocytes percentage), MID (%) (Monocyte, Eosiniphil, and Basophil Percentage), GRAN (10*3/µL) (Neutrophil count), WBC (white blood cell), and MID. (10*3/µL) (monocyte, eosiniphil, and basophil count) of the animals treated in any of the groups do not differ significantly from the control group. Abbas *et al.* (2018) found no discernible variation in the haematological parameter levels of *Acacia ataxacantha* leaf extract in methanol in Wistar rats, which is consistent with the results of this study. Amoussa *et al.* (2015) reported that white blood cell counts showed a modest increase.

Table 7b: The Effect of Oral Administration of the Extracts on some Hematological Parameters.

	Means ± SD of Hematological Parameters						
Extract used	Rbc (10^6/ul)	Hgb (g/dl)	Hct (%)	Mcv(fl)	Mch (pg)	Mchc (g/dl)	Rdw-cv(%)
Normal Range	3.5-5.5	11.0-16.0	37.0-54.0	80.0-100.0	27.0-34.0	32.0-36.0	11.0-16.0
Aqueous	5.10 ± 0.13a	11.6 ± 0.30a	37.00 ± 1.34a	97.1 ± 2.20a	38.60 ± 0.00a	33.5 ± 1.20a	167.60 ± 3.00a
Hexane	6.00 ± 0.57a	14.0 ± 0.27a	41.00 ± 2.00a	85.5 ± 0.20a	30.10 ± 3.56a	33.50 ± 1.66a	11.90± 0.77a
DW 1(ml/kg)	6.10 ± 0.06a	13.50 ± 0.36a	36.56 ± 1.67a	87.13 ± 0.63a	28.76 ± 2.84a	35.10 ± 2.40a	17.80 ± 1.30a
LSD	0.53	2.04	6.77	7.98	8.04	6.3	10.9

Means ± SD= Standard deviation; DW=distilled water; LSD= Means withsuperscripts show no significant difference (p>0.05) ANOVA, RBC (red blood cell count), HGB (haemoglobin count), HCT (hermatocrit percentage by volume), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin count), RDW-CV (red blood cell distribution width curve)

Table 7c: The Effect of Oral Administration of the Extracts on some Hematological Parameters

	Means ± SD of Hematological Parameters						
Extract used	Rdw-sd (fl)	Plt(10^3/ul)	Mpv (fl)	Pdw	Pct (%)	P-lcc (10^9/l)	Plcr(%)
Normal Range	35.0-56.0	15.0-45.0	6.5-12.0	7.0-9.0	0.1-.02	30-90	11.0-45.0
Aqueous	40.90 ± 1.23a	167.0 ± 2.27a	7.40 ± 1.10a	25.60 ± 3.44a	0.19 ± 0.02a	36.0 ± 4.97a	38.80 ± 0.30a
Hexane	37.20 ± 0.46a	397.3 ± 58.9a	7.10 ± 0.74a	7.90 ± 0.04a	0.10 ± 0.03a	44.0 ± 6.67a	29.60 ± 1.00a
DW 1.0(ml/kg)	38.53 ± 3.40a	177.33 ± 14.00a	7.56 ± 0.43a	14.26 ± 5.90a	0.25 ± 0.01a	45.00 ± 13.70a	34.73 ± 2.30a
LSD	7.43	90.34	2.24	16.52	0.17	27.49	8.01

Means ± SD= Standard deviation; DW=distilled water; LSD= Means with the superscripts show no significant difference (p>0.05) ANOVA, RDW-SD (red cell distribution width), PLT (platelets count), MPV (mean platelets volume), PDW (platelet distribution width), PCT (platelets volume), P-LCC (platelets large cell count), PLCR (platelets large cell ratio)

Other haematological parameters, as shown in Table 7b, indicated that the animals treated in any of the groups did not significantly differ from the control group in terms of their haemoglobin count (HGB), haematocrit percentage by volume (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin count (MCHC), or red blood cell distribution width curve (RDW-CV). One animal's red blood cell count (RBC) changed significantly when exposed to 500 mg/kg of the hexane extract in comparison to all other groups. When comparing the animals treated in each group to the control group, Table 7c demonstrated that there was no significant difference in the red cell distribution width (RDW-SD), platelets count (PLT), mean platelets volume (MPV), platelet distribution width (PDW), platelets volume (PCT), platelets large cell count (P-LCC), or platelets large cell ratio (PLCR).

The results of this study showed that there was no discernible change in the level of haematological parameters between the aqueous and Hexane extracts of the stem bark of *Anogeissus leiocarpus* and the leaves of *Acacia ataxacantha*, as indicated by Tables 7a, 7b, and 7c. This outcome is consistent with that of Abbas *et al.* (2018), who similarly discovered that there was no discernible variation in the haematological parameter examined by the methanol extract of *Acacia ataxacantha* leaves. According to the findings of Agaie *et al.* (2007), *Anogeissus leiocarpus* extract had no discernible impact (p>0.05) on any of the haematological parameters other than lymphocytes and packed cell volume. Cyril-Olutayo *et al.* (2013) also demonstrated that in mice infected with P. berghei, the methanol bark extract of A. leiocarpus changed haematological indicators, including haemoglobin, red blood cells, packed cell volume, lymphocytes, and neutrophils.

Histology of Test Animals' Liver and Kidney After Treatment with Hexane and Aqueous Extracts.

Plate I: Photomicrograph of Liver of animal administered Aqueous extract (at 500 mg/kg) showing slight Hepatic necrosis (HN)

The liver and kidney histopathology results for the control group had typical characteristics. In rats given 500 mg/kg body weight of the aqueous extract, the kidney displayed mild lymphocyte hyperplasia and the liver displayed mild hepatic necrosis. When exposed to 500 mg//kg of hexane extract, the liver showed mild lymphocyte hyperplasia and the kidney showed granular necrosis. Long-term administration of the plants resulted in focal necrosis, hepatic congestion, and necrosis, whereas extracts of *A. leiocarpus* and *K. senegalensis* at 5000 mg/kg caused

degenerative alterations in the hepatocytes. When rats were given extract of *A. leiocarpus*, their nephrons displayed necrosis and clogged blood vessels (Tauheed *et al.*, 2021). In a different study by Baba *et al.* (2022), the livers of experimental rats given 500 mg/kg and 1000 mg/kg of *Acacia nilotica*, respectively, displayed mild hepatocellular necrosis and kuffer cell hyperplasia. At 1000 mg/kg, the kidney of rats given 500 mg/kg of the same extract displayed tubular deformities and significant lymphocyte hyperplasia.

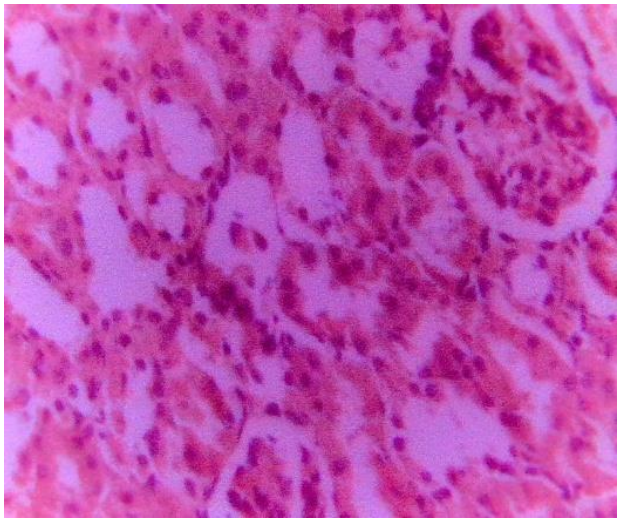

Plate II: Photomicrograph of Kidney of animal administered Aqueous extract (at 500 mg/kg) showing slight lymphocytes hyperplasia (LH)

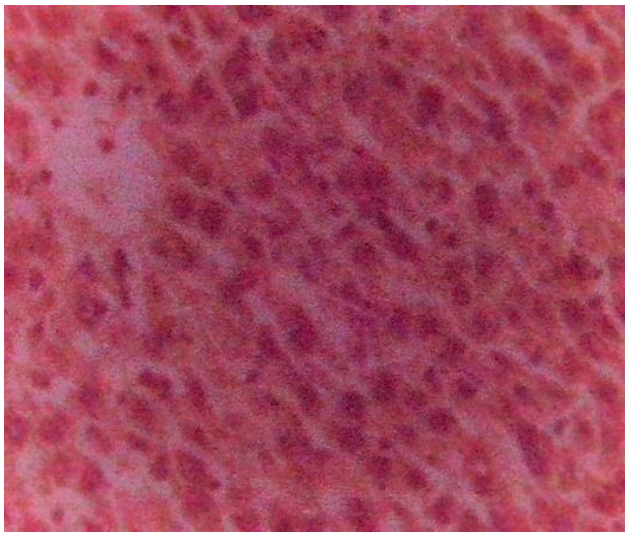

Plate III: Photomicrograph of Liver of animal administered Hexane extract (at 500 mg/kg) showing slight lymphocytes Hyperplasia

Anogeissus leiocarpus methanol extract photomicrographs revealed normal liver and kidney functions with moderate histological abnormalities at 5000 mg/kg, according to Adamu *et al.* (2022). According to other research findings, the liver displayed hepatocellular necrosis with hyperplasia of kupffer cells and moderate glomerular necrosis on the kidney (50, 200, and 400 mg/kg) when the methanol extract of *Acacia ataxacantha* leaves was examined histologically (Abbas *et al.,* 2018). However, prolonged use may have negative effects on the liver and kidney.

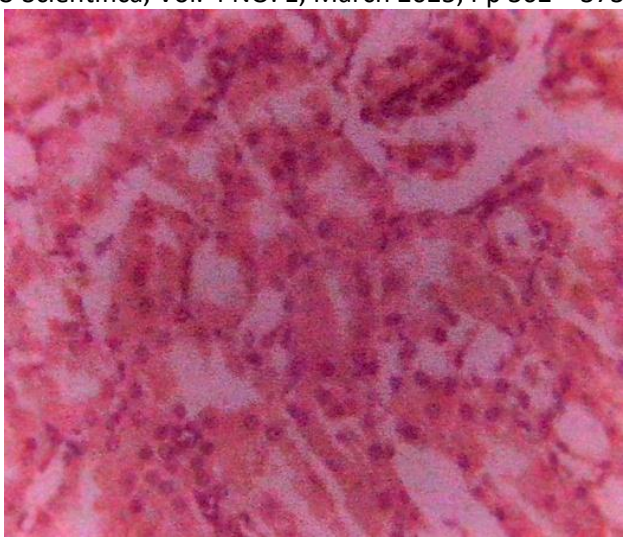

Plate IV: Photomicrograph of Liver of animal administered Hexane extract (at 500 mg/kg) showing slight Granular Necrosis (GN)

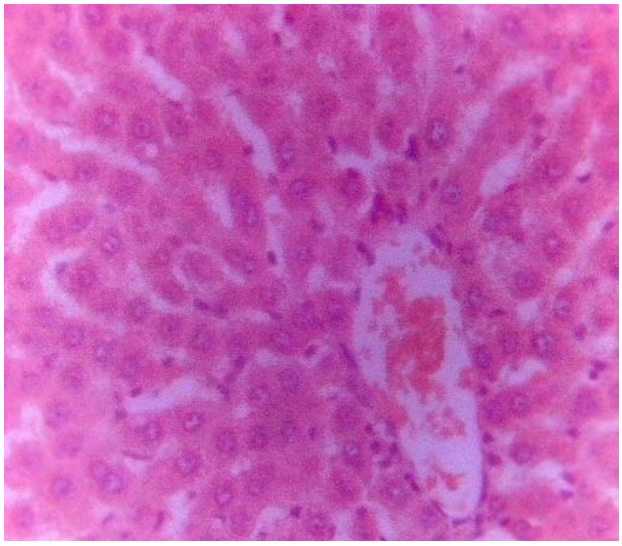

Plate V: Photomicrograph of Control group Liver Normal Future

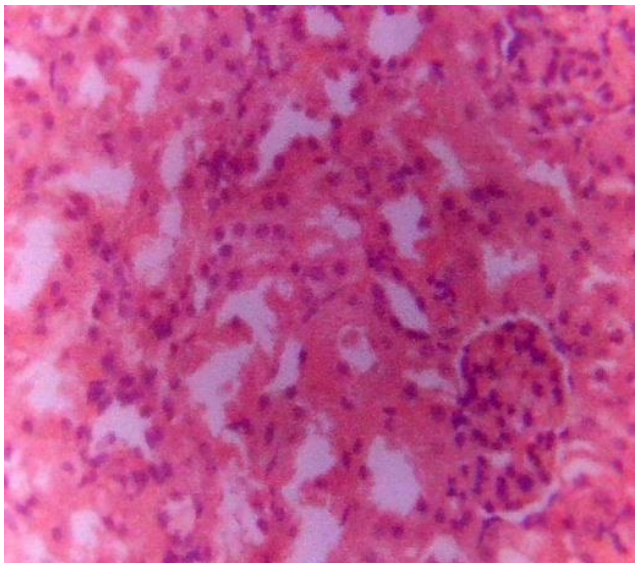

Plate VI: Photomicrograph of Control group kidney Showing Showing Normal Future

When compared, the haematological parameters and body weight of animals treated with *A. ataxacantha* to the controls, Amoussa *et al.* (2015) found no appreciable alterations. Likewise, normal architecture with no morphological abnormalities was found in the kidney and liver histopatological analyses. The frequent use of mixtures made from different sections of these plants in traditional medicine may be due to their capacity to maintain appropriate haematological parameters, bodyweight, and the absence of mortality, according to Tauheed *et al.* (2021).

CONCLUSION

The study's findings unequivocally supported the traditional use of these plants as medicines to treat microbial illnesses because the extracts demonstrated strong antibacterial and antifungal action against the organisms under investigation. The many phytochemicals found in the extracts are what give the synergistic extracts their wide range of antimicrobial activity. The plants' aqueous and Hexane extracts have LD_{50}s above 1000 mg/kg because no mortality was noted, and the recorded body weight, haematological parameters, and histological analysis all stayed largely normal. In order to combat the microorganisms that cause vaginal discharge and vulval itching, this study demonstrated the significant antimicrobial synergistic effect and safety of *Anogeissus leiocarpus* stem bark and *Acacia ataxacantha* leaves. It is advised that more research be done to extract the plants using different solvents and identify the active compound responsible for the plants' activity.

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