Trop J Nat Prod Res, May 2020; 4(6):237-242 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Screening, Liver Toxicity and Antibacterial Properties of Aqueous and Ethanol Extracts of a Local Herbal Mixture "Aju Mbaise"

Oprah Evbakhavbokun*, Taidi Ekrakene, Ebakota O. Daniel, Frances N. Olisaka

Department of Biological Sciences (Microbiology option), Benson Idahosa University, Ugbor, Edo- state, Nigeria.

ARTICLE INFO

Article history:
Received 03 June 2020
Revised 29 June 2020
Accepted 30 June 2020
Published online 02 July 2020

ABSTRACT

Aju Mbaise is a local herb with different combination of plant leaves and stem, which has several health benefits. The aim of this research was to study the phytochemical properties, liver toxicity and antibacterial activity of Aju Mbaise. The crude aqueous and ethanol extracts of Aju Mbaise were screened for their phytochemicals using standard methods. The antibacterial potential of Aju Mbaise was evaluated using agar well diffusion methods. The haematological, biochemical and the hepatotoxic effect on rats fed with Aju Mbaise decoction were also investigated. The results of the phytochemical screening of Aju Mbaise ethanol and aqueous extracts revealed the presence of alkaloids, flavonoids, tannins, cardiac glycoside, and coumarins. The ethanol extract of Aju Mbaise had antibacterial activity at 0.2 g/mL with 8 mm zone of inhibition against *Escherichia coli*, while at 2 g/mL, the ethanol extract had 25 mm inhibition zone against *Proteus* sp. The aqueous extract of Aju Mbaise had antibacterial activity at 0.2 g/mL with 8 mm zone of inhibition against *Klebsiella* sp, while at 2 g/mL the aqueous extract had 25 mm zone of inhibition against *Salmonella* sp. There was significant weight reducing effect on rats fed with Aju Mbaise decoction (loss of weight from 180 g in week one to 50 g in week six). Administration of the herbal decoction also showed signs of liver toxicity in the rats. In conclusion, the study has shown that Aju Mbaise extract has antibacterial activity and may potentially be toxic to the liver on chronic administration.

Keywords: Aju Mbaise**,** Antibacterial activity, phytochemicals, liver toxicity.

Introduction

Plants have been used worldwide for the treatment of various human ailments for centuries.[1] Their use is still quite prevalent, especially in developing countries in the form of traditional medicine.[2] Extensive chemical and antimicrobial studies on several medicinal plants during the last decades have led to the validation of traditional claims in many cases and have facilitated the identification of their active components.[3]

Medicinal plants are the richest source of natural antimicrobial agents.[4,5] Traditional healer's claim that some plants are more efficient to treat infectious diseases than synthetic antibiotics. From ancient times, different parts of plants have been used to cure specific ailments. The plants are widely used because of their availability and cost-effectiveness.[6,7]

"Aju Mbaise" is a very popular decoction native to the Igbo people of South-East Nigeria, which is used in treating a variety of conditions and diseases. Traditionally it is believed to be a strong remedy for weight loss, ovarian cyst, diabetes, irregular menstrual cycle, and eczema. It is a combination of leaves and stem of various plants, tied together with twine and administered after boiling as tea or soup.[8]

According to Ogueke *et al*,[8] the plants that makeup Aju Mbaise are *Jatropha curcas* (Barbados nut, purging nut), *Cnestis ferruginea*, *Combretum racemosum* (Bushwillow tree), *Chrysophyllum albidum* (African Star apple), *Heterotis rotundifolia* (rock rose, Spanish shawl), *Sphenocentrum jollyanum* (called Akerejupon in Yoruba language, Nigeria) and *Psidium guajava* (guava). Some of the plants that make up "Aju Mbaise" such as *Jatropha curcas*, *Censtis ferruginea*, and *Combretum recemosum* have been shown to improve the immune system and possess good antimicrobial and antioxidant activity.[9-11] However, most of the bioactive compounds contained in the plants are neither known nor quantified. The scientific literature on the beneficial health claims associated with the intake of "Aju Mbaise" is few. With the increase in the use of this decoction, there is a need for a thorough scientific evaluation to validate the supposedly therapeutic effects and toxicity. It was based on this background that the study seek to evaluate the phytochemical properties, liver toxicity and antibacterial activity of "Aju Mbaise" decoction.

Materials and Methods

Collection of samples

Commercially sold Aju Mbaise" herbs were purchased from Mbaise Local Government Area of Imo State, Nigeria.

Extraction of herbal materials

Three hundred grams each of the grounded Aju Mbaise leaves and stem were air-dried, pulverized and then extracted using 500 mL distilled water and ethanol, respectively. The Aju Mbaise leaves and stem were soaked in different solvents (aqueous and ethanol) for 72 hours after which the mixture was filtered with muslin cloth and the filtrate concentrated with the aid of a hot box oven till all the solvent was evaporated. The extract was collected and stored in air-tight containers at 4°C till further analysis.

*Corresponding author. E mail: oprahevba@gmail.com
Tel: +2347031371748

Citation: Evbakhavbokun O, Ekrakene T, Daniel EO, Olisaka FN. Phytochemical Screening, Liver Toxicity and Antibacterial Properties of Aqueous and Ethanol Extracts of a Local Herbal Product "Aju Mbaise". Trop J Nat Prod Res. 2020; 4(6):237-242. doi.org/10.26538/tjnpr/v4i6.5

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Phytochemical screening
Tests for Flavonoids, Tannins, Cardiac glycosides, Saponins, Steroids, Terpenoids, Coumarins, Reducing Sugars, and Alkaloids, were carried out in both the aqueous and ethanol extracts as described by Kamba and Hassan, 2010.[12]

Source of bacterial isolates
The clinical isolates; *Proteus sp, Escherichia coli, Salmonella sp,* and *Klebsiella sp.* were obtained from the microbiology laboratory of Department of Biological Sciences Benson Idahosa University. Benin City, Nigeria. The isolates were identified and stored on nutrient agar slants at 4ºC until required.

Standardization of the microbial inoculum
All the test bacterial isolates were sub-cultured into the sterile nutrient broth and incubated overnight. The resultant turbidity of the bacterial suspension obtained was compared and matched to 0.5 McFarland standard representing approximately 10^7cfu/mL.[13]

Antimicrobial susceptibility test
The agar well diffusion method as described by Vandepitte *et al*.[13] was used to determine the antibiotic resistance of *Proteus sp, Escherichia coli, Salmonella sp, and Klebsiella sp.* against the following standard antibiotics. Ceftazidime (30 µg/mL), Cefuroxime (30 µg/mL), Gentamicin (10 µg/mL), Ceftriaxone (30 µg/mL), Erythromycin (5 µg/mL), Cloxacillin (5 µg/mL), Ofloxacin (5 µg/mL), Amoxycillin/Clavulanate (30 µg/mL). Different concentrations of the Aju Mbaise herb extracts were prepared to give a final concentration of 0.2 g/mL, 0.4 g/mL, 0.6 g/mL, 0.8 g/mL, 1 g/mL and 2 g/mL. Three wells of about 6 mm diameter were bored on already seeded nutrient agar plates using a sterile cork borer after which 0.5 mL of the different concentrations of the extracts were added to the wells and left to stand for prediffusion before incubation at 37ºC for 24 h. All antibiotic disc used were manufactured by Oxoid Ltd. (Basingstoke, Hampshire, England). Resultant zones of inhibition were measured in mm.

Determination of the minimum inhibitory concentration (MIC)
The MIC of the extracts was determined by the tube dilution method according to the methods described by Kamba and Hassan.[12] Freshly standardized overnight microbial cultures in nutrient broth were introduced into the freshly prepared nutrient broth in tubes. Different concentrations (0.2 g/mL, 0.4 g/mL, 0.6 g/mL, 0.8 g/mL, 1 g/mL and 2 g/mL) of the plant extracts were introduced into each of the test tubes and incubated for 24 h at 37ºC. The MIC was taken as the least concentration that inhibited the growth of the test organism.

Determination of the minimum bactericidal concentration (MBC)
The method of Kamba and Hassan[12] was adopted in determining the MBC of the respective extracts. One (1) mL was taken from all the tubes in the MIC study and plated on freshly prepared nutrient agar plates using pour plate technique under aseptic conditions. The agar plates were incubated at 37°C for 24 h. The MBC was regarded as the least concentration of the extract that prevented the growth of any microbial colony on the agar plate.

Multiple antibiotic resistance (mar) index
The multiple antibiotic resistance (MAR) index was calculated as described by Krumperman (1983).[14]

MAR index = a/b

Where; a = number of antibiotics to which a particular isolate was resistant, b = total number of antibiotics tested

MAR index higher than 0.2 indicated that the *E.coli, Proteus sp, Klebsiella sp* and *Salmonella sp* have originated from high-risk sources of contamination, where antibiotics are often used.[15]

Animals
Twelve Swiss albino rats (180-190 g) were obtained from the Animal house of Faculty of Life Science, University of Benin. The animals were fed with standard pellet feed (growler mesh) and had free access to water. They were also maintained under standard conditions of humidity, temperature and 12 hours light/darkness cycles. The animals were acclimatized for two weeks before the commencement of the study.

Ethical consideration on the use of experimental animals
Benson Idahosa University Directorate of Research and International Programmes approved the use of animal for this research with approval number DRIP01.

Liver toxicity testing
The rats were divided into two groups of 6 animals each. Rats in group 1 (Test group) were administered Aju Mbaise decoction (2 mL) orally every morning for 42 days. Rats in group 2 (Control) were not treated but allowed free access to feed and water. The weights and clinical signs of toxicity, including death were monitored. Two rats (one from each group) were sacrificed each week by chloroform euthanasia. Blood was collected from the heart by cardiac puncture and placed in EDTA and lithium heparin tubes. The liver was placed in formalin for histology.

Haematological analysis
The serum was prepared by centrifuging the blood in the EDTA tubes for 10 mins at 3000 rpm. The clear supernatant was used for the haematological analysis.[16]

Biochemical analysis
The blood in the lithium heparin tubes was spun in a centrifuge and serum was collected into a plain tube which was used for the estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). These were estimated with a spectrophotometer using Redox reagent enzyme kit as described by Reitman and Frankel.[17]

Histopathological studies
Liver were embedded in formalin, sectioned at 5 microliter and stained with haematoxylin and eosin. Detailed microscopic examination of the section of the above organ was carried out.[18]

Statistical analysis
Data were expressed as Means ± Standard Deviation. Were applicable data were subjected to one-way analysis of variance (ANOVA) and differences between means were regarded as significant at P value < 0.05.

Results and Discussion

The results of the phytochemical screening of the aqueous and ethanol extracts of "Aju Mbaise" are represented in Table 1. Alkaloids, flavonoids, tannins, cardiac glycoside, saponins, reducing sugar and coumarins were present in the aqueous and ethanol extracts. Steroids, and terpenoids were absent in the aqueous extract but present in the ethanol extract. These findings agrees with the report of Ezejindu and Iro,[4] who found flavonoids, tannins, alkaloids, saponins and cardiac glycosides highly present in "Aju Mbaise".
Basically, the phytochemicals found in this study have been severally proven to be active against human pathogens.[19,20] Aside their potential antimicrobial activity, bioactive phytochemical compounds found in this study such as alkaloids are known antimalarial agents, analgesics and can act as stimulants.[20] Glycoside moieties such as saponins, anthraquinones, and cardiac glycosides can inhibit tumour growth, act as an antiparasitic agent and can be used as an antidepressant.[21] In this study, it was observed that the ethanol extract possessed more phytochemicals than the aqueous extracts. These results supported the evidence in previous studies that alcoholic solvents like ethanol and methanol are more suitable than other solvents such as water in extracting a component of medicinal plant.[21-23]

The antimicrobial susceptibility pattern of selected test isolates is presented in Table 2. All isolates tested were resistant to Ceftazidime, Cefuroxime, Ceftriaxone, Cloxacillin and Amoxicillin. *Escherichia coli* was susceptible to Erythromycin, Gentamycin and Ofloxacin with 17, 19 and 25 mm zone of inhibition, respectively. All isolates tested were susceptible to Ofloxacin. The multiple antibiotic resistance index (MAR index) for *Klebsiella* sp, *Proteus* sp, *Escherichia coli*, and *Salmonella* sp were 0.9, 0.8, 0.6, and 0.8, respectively.

The antibacterial activity of the ethanol extract of Aju Mbaise is presented in Table 3. All concentrations (0.2, 0.4, 0.6, 0.8, 1, 2 g/mL) of the ethanol extract had an inhibitory effect on all the test organisms at 2 g/mL with the highest zone of inhibition (30 mm) observed against *Klebsiella* sp. The lowest zone of inhibition (6 mm) was observed against *Salmonella* sp at 0.2 g/mL. The antimicrobial activity of the aqueous extract of Aju Mbaise is also presented in Table 3. From the results, all concentrations used also had an inhibitory effect on all the test organisms at 2 g/mL with the highest zone of inhibition (25 mm) observed against *Salmonella* sp. There were zones of inhibition of 8, 10, 14, 15, 18, and 19 mm against *Escherichia coli* at 0.2, 0.4, 0.6, 0.8, 1, and 2 g/mL, respectively. The lowest zone of inhibition (8 mm) was observed against *Klebsiella* sp, *Proteus* sp, *Salmonella* sp, and *Escherichia coli* at 0.2 g/mL. Zones of inhibition were observed to increase with increasing concentration of both the ethanol and aqueous extracts.

The minimum inhibitory concentration of the aqueous extract was 0.8 g/mL while the minimum bactericidal concentration was 2 g/mL. The minimum inhibitory concentration and the minimum bactericidal concentration of the ethanol extract of Aju Mbaise were 0.6 and 1.0 g/mL, respectively.

Antimicrobial activity screening of Aju Mbaise showed that the extracts are effective though at varying degrees to all the test organisms. This result is in agreement with that of Enabulele and Ehiagbonare[24] and Karou *et al*,[25] who reported that susceptibility of bacteria to plant extracts based on zones of inhibition varies according to strains and species. It has been reported that Aju Mbaise inhibited *Escherichia* coli with an inhibition zone of 7.5 mm and *Salmonella sp* with an inhibition zone of 8.0 mm at various concentrations.[4,8] In this study, it was observed that at various concentrations, the ethanol extract was more active against the test organisms than the aqueous extract. The test organisms were observed to be mostly resistant to the antibiotics used in this study. Comparing the antibacterial activity of Aju Mbaise extracts with the antibiotic susceptibility pattern, the extracts inhibited the test organisms as much as the antibiotics used. This further buttress the efficacy of the herbal extracts as antimicrobial agents.

A comparative difference in the weight, as well as alanine transaminase (ALT) and Aspartate aminotransferase (AST) in albino rats fed with Aju Mbaise decoction for six weeks and the control are presented in Table 4. The results showed a reduction in weight from the first week (180 g) to the last week (50 g) in the extract fed-rats. The control showed an increase in weight from the first week (190 g) to the last week (260 g). The reduction in weight of the extract fed-rats is in agreement with traditional folklore which suggests that Aju Mbaise decoction aid in weight reduction. Research by Ezejindu and Iro[4] showed that nursing mothers who drank the decoction also had significant weight loss.

The AST of rats fed with the decoction of Aju Mbaise was 10 u/L at first and fifth week, while at the second, third, fourth and sixth week, was 13 u/L. For the ALT of the extract fed-rats the first, second and fifth week was 12 u/L while the third, fourth, and sixth week was 8 u/L. For the control, AST value was 10 u/L, while the ALT value was 12 u/L. There was no significant increase in the liver enzymes; alanine transaminase (ALT) and Aspartate aminotransferase (AST) as compared to the control for the six (6) week administration period.

Table 1: Phytochemical composition of "Aju Mbaise" aqueous and ethanol extracts

Phytochemical	Inference	
	Aqueous extract	Ethanol extract
Flavonoids	+	+
Tannis	+	+
Cardiac glycosides	+	+
Saponins	+	+
Steroids	-	+
Terpenoids	-	+
Reducing sugar	+	+
Alkaloids	+	+
Coumarins	+	+

KEY: + = Present, - = Absent

High level of transaminases such as ALT and AST is a sign of hepatic damage and are measured clinically as a part of a diagnostic evaluation of liver function test.[26]

Tables 5 and 6 show the haematological parameters of the Aju Mbaise-fed rats. Throughout the administration period, the Haemoglobin (Hb) concentration for the first, second, third and fifth week was 18 g/dL while at the fourth and sixth week the Hb concentration was 17.6 g/dL and 16 g/dL, respectively. The lowest white blood cell count was observed at the fourth and sixth week. The neutrophils were 5% at the second, third, and fifth week, while at the first, fourth and sixth week, it was 20%, 5.8% and 33%, respectively. For lymphocyte, the lowest value (65%) was observed at week six. For platelets count, the fifth week had the lowest value with 208,000 g/L while the first week had the highest value with 730,000 g/L.

Cellular vacuolization is a frequently observed phenomenon upon exposure to pharmaceutical agents and other chemicals and has been extensively reported.[27] Cytoplasmic vacuolization of hepatocytes can be transient or irreversible. Transient vacuolization is observed only during the exposure to an inducer and reversibly affects the cell cycle and migration.[28] In contrast to transient vacuolization, irreversible vacuolization marks cytopathological conditions leading to cell death, as long as the cytotoxic stimulus is present. Vacuolization of the cytoplasm of the hepatocytes seen in the liver of Aju Mbaise-fed rats is a sign of hepatic injury as against the control (untreated) rats where no such abnormality was observed (Figure 1).

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Such toxicity testing is relevant for risk evaluation as changes in the haematological parameters have high predictive value for human toxicity when data are translated from animal studies.[29] It can also be used to explain blood related functions of chemical compounds or plant extract.[30] Haematology results, however, showed a surge in white blood cell count during the period of administration.

Basophils, eosinophils and neutrophils are white blood cells that play a role in keeping the immune system functioning correctly.[31] The unstable values recorded for the white blood cell counts in treated rats as compared to the control (untreated) rats suggest an inflammation or injury to the rats. There was a slight spike in white blood cells, monocytes and a reduction in platelet count with treatment time. These observations suggest a toxic effect.

Table 2: The antimicrobial susceptibility pattern of selected antibiotics against the test isolates

ISOLATES	ANTIBIOTICS/ZONE OF INHIBITION (mm)								
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	MAR INDEX
Escherichia coli	R	R	19	R	17	R	25	R	0.6
Klebsiella sp	R	R	16	R	R	R	30	R	0.9
Proteus sp	R	R	R	R	17	R	21	R	0.8
Salmonella sp	R	R	17	R	R	R	30	R	0.8

Key: R = Resistance, MAR = Multiple antibiotic resistance, CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamicin, CTR = Ceftriaxone, ERY = Ervthromvcin. CXC = Cloxicillin. OFL = Ofloxacin. AUG = Amoxvcillin/Clavulanate.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Antibacterial activity of ethanol and aqueous extracts of Aju Mbaise

Conc. (g/mL)	Ethanol extract Zone of inhibition (mm)				Aqueous extract Zone of inhibition (mm)			
	Escherichia coli	*Klebsiella* sp	*Proteus* sp	*Salmonella* sp	*Escherichia coli*	*Klebsiella* sp	*Proteus* sp	*Salmonella* sp
0.2	8	8	8	6	8	8	8	8
0.4	10	14	10	8	10	12	10	12
0.6	12	16	15	10	14	13	14	13
0.8	14	18	19	15	15	15	16	15
1.0	16	20	20	17	18	18	18	18
2.0	20	30	25	19	19	20	20	25

Table 4: Comparative difference in the weight and liver enzymes in rats fed with Aju Mbaise decoction and the control

		WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	(Mean ± SD)
	Weight of Extract fed Rats(g)	180	150	120	100	80	50	121.7 ± 49.2 g
Liver Enzymes (Treated rats)	AST (u/L)	10	13	13	13	10	13	12.0 ± 1.5 u/L
	ALT (u/L)	12	12	8	8	12	8	10.0 ± 2.2 u/L
	Weight of Control Rat (g)	190	220	230	240	250	260	217.5 ± 27.9 g
Liver Enzymes (Control rats)	AST (u/L)	10	13	10	10	13	13	1.7 ± 4.1 u/L
	ALT (u/L)	12	12	12	12	12	12	2.0 ± 4.9 u/L

Table 5: The mean and standard deviation of the haematological parameters (Haemoglobin, packed cell volume, white blood cell, Neutrophils, Lymphocytes, Basophiles, Monocytes, and Platelets) of the extract fed-rats and the control

Parameter	Extract fed-rats	Control
Hb (g/dL)	18.0 ± 0.8	17.8 ± 0.4
PCV (%)	52.7 ± 2.8	53.7 ± 0.5
WBC (%)	14,518.3 ± 4402.6	8255± 1322.6
NEU (%)	12.3 ± 11.7	21.3 ± 3.4
LYMP (%)	84.9 ± 12.5	53.0 ± 4.0
MONO (%)	3.9 ± 7.4	11.5 ± 1.0
BASO (%)	1.5 ± 0.9	0.7 ± 0.5
EOS (%)	0.5 ± 0.6	0 .3 ± 0.1
PLAT (g/dL)	465000.0 ± 19384.2	490500.0 ± 63213.1

Values are Mean ± SD of six independent measurements

Conclusion

In conclusion, this study has shown that Aju Mbaise extract has antibacterial activity and has shown promising weight reduction effect. However, the vacuolation of the cytoplasm of the hepatocytes may progress to severe liver injury with continuous usage.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors appreciate the management of Benson Idahosa University, and all Laboratory staff for their support to this research

Table 6: Haematological parameters of Swiss albino rats fed with Aju Mbaise decoction and control.

	Aju Mbaise fed rats									Control								
	Hb (g/dl)	**PCV (%)**	**WBC (mm^3)**	**Neut (%)**	**Lymp (%)**	**Mon (%)**	**Baso (%)**	**Eosi (%)**	**Platelets (g/dl)**	**Hb (g/dl)**	**PCV (%)**	**WBC (mm^3)**	**Neut (%)**	**Lymp (%)**	**Mon (%)**	**Baso (%)**	**Eosi (%)**	**Platelets**
Week1	18	54	20,140	20	96.1	0.3	0.1	1.5	730,000	18	53	6200	20	58	12	1	0	390000
Week 2	18	54	12,720	5	74.0	19	1.0	1	638,000	17	53	7370	18	49	11	0	0.1	450000
Week 3	18	54	18,270	5	93.5	0	1.2	0.3	434,000	17.6	54	9420	19	51	10	1	0	496000
Week 4	17.6	53	9,200	5.8	91.0	1	2.2	0	459,000	18	54	8550	22	50	11	0	0.1	500000
Week 5	18	54	16,320	5	90.0	1	2.2	0	321,000	18	54	8220	21	52	12	1	0	544000
Week 6	16	47	10,460	33	65.0	2	2.2	0	208,000	18	54	9770	28	58	13	1.0	0	563000

Key: Hb = haemoglobin, PCV = packed cell volume, WBC = white blood cells, Neut = Neuthrophils, Lymp = lymphocytes, mono=monocytes, Baso, basophils, Eosi = eosinophils

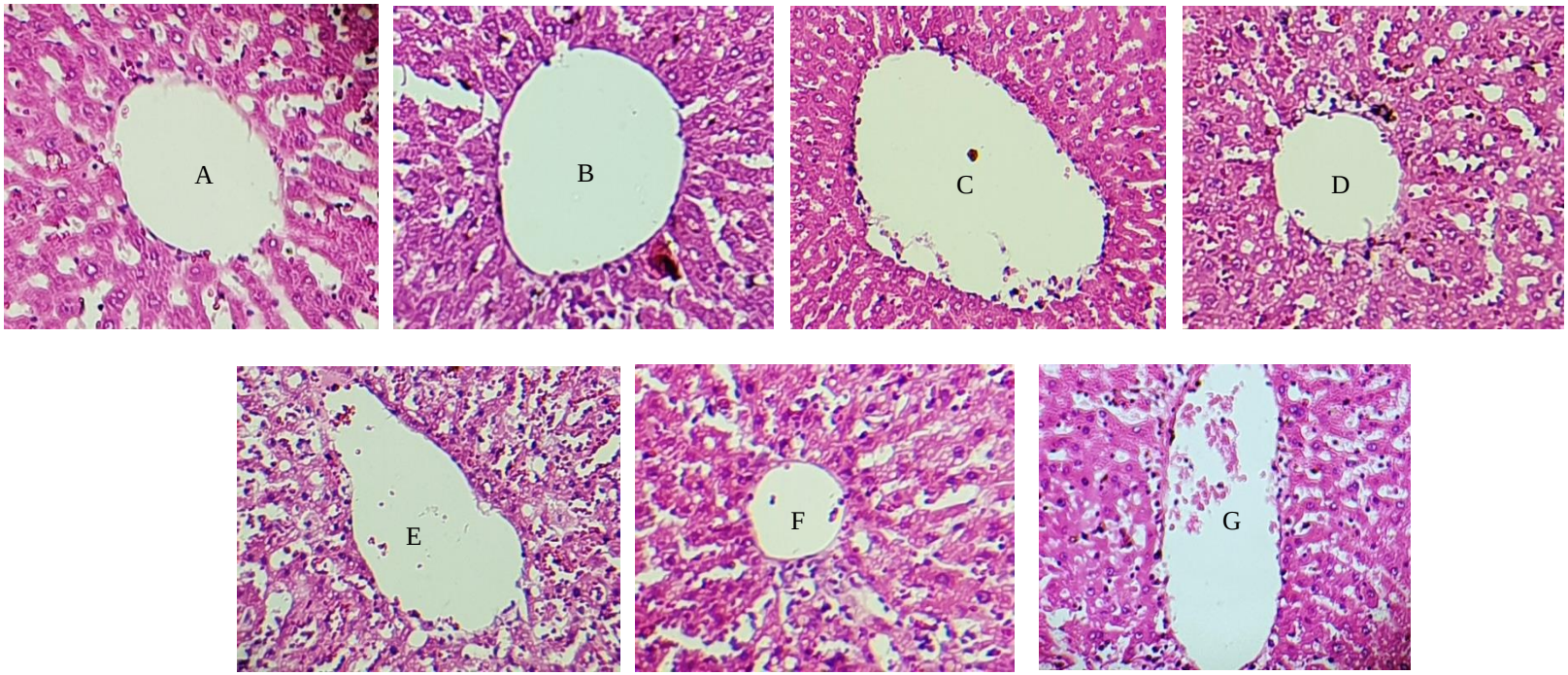

Figure 1: Photomicrograph of liver sections of rats treated with Aju Mbaise decoction. **(A) Control:** Histology shows normal liver architecture. **(B) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears slightly pyknotic with less fatty changes. **(C) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears slightly vacuolated with mild mononuclear infiltrates. **(D) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears vacuolated with prominent fatty changes, mononuclear infiltrates and steatosis. **(E) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears vacuolated with prominent fatty changes, mononuclear infiltrates and steatosis. **(F) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears slightly pyknotic with prominent mononuclear infiltrates and steatosis. **(G) Test:** Histology reveals visible centriole and hepatocytes with nucleus that appears vacuolated with mild mononuclear infiltrates.

References

1. Saslis-lagoudakis CH, Hawkins JA, Greenhill SJ, Pendry CA, Watson MF, Tuladhar-Douglas W. The Evolution of Traditional Knowledge: Environment Shapes Medicinal Plant Use in Nepal. Proc Res Soc. 201**4**; 3(2):281-293.
2. Marayl G. Study of Pharmacological Effect of *Verbena Officinalis* Linn: A Review. Pharm Lett. 2013; 8(9):321–325.
3. Maurya R, Srivastava S, Kulshreshta DK, Gupta CM. Traditional Remedies for Fertility Regulation. Curr Med Chem. 2004; 11(11): 1431–1450.
4. Ezejindu CN and Iro OK. Antibacterial activity, phytochemical properties and mineral content of "Aju Mbaise" decoction administered to nursing mothers. Dir Res J Health Pharmacol. 2017; 5(3):33-38.
5. Falodun A, Imieje V, Erharuyi O, Ahomafor J, Jacob MR, Khan S, Hamann MT. Evaluation of three medicinal Plant extracts against *Plasmodium falciparum* and selected microorganisms. Afr J Trad Compl Altern Med. 2014; 11(4):142-143.
6. Cordell GA. Biodiversity and drug discovery of a symbiotic relationship. Phytochem. 2000; 55(6):463-480.
7. Falodun A. Herbal Medicine in Africa-Distribution, Standardization and Prospects. Res J Phytochem. 2010; 4(3):154-161.
8. Ogueke C, Owuamanam C, Onyedinima C. Antibacterial activity, phytochemical properties and mineral content of Aju Mbaise decoction: A liquid extract administered to nursing mothers. Nig J Nutr Sci. 2015; 37(1):23-25.
9. Salim MN, Masyitha D, Harris A, Balqis U, Iskandar CD, Hambal M, Darmawi**.** Anti-inflammatory activity of *Jatropha curcas* Linn. latex in cream formulation on CD68 expression in mice skin wound. Vet World. 2018 Feb; 11(2): 99–103.
10. Kouakou K, Panda SK, Yang M-R, Lu J-G, Jiang Z-H, Van Puyvelde L and Luyten W. Isolation of Antimicrobial Compounds From Cnestis ferruginea Vahl ex. DC (Connaraceae) Leaves Through Bioassay-Guided Fractionation. Front Microbiol. 2019; 10:705.
11. Idu M, Uwaya D, Ovuakporie-Uvo O. *In Vivo* Antioxidant Activities of *Combretum platypterum* (Welw.) Hutch. and *Combretum racemosum* P. Beauv. (Combretaceae). Appl Med Res. 2016; 2(3):50-55.
12. Kamba AS and Hassan LG. Phytochemical screening and antimicrobial activities of *Euphobia balasamifera* leaves stems and roots against some pathogenic microorganisms. Afr J Pharm Sci Pharmacol. 2010; 1(1):57-64.
13. Vandepitte J, Engbaek K, Rohner P, Piot P. Basic Laboratory Procedures in clinical bacteriology. (2nd ed.). World Health Organisation. Geneva; 2003. 120-136 p.
14. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to indentify high-risk sources of fecal contamination of foods. Appl Environ Microbiol. 1983; 4(6):165-170.
15. Paul S, Bezbaruah RL, Roy MK, Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. Lett Appl Microbiol. 1997; 2(4):169–171.
16. Bowen RA and Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med. 2014; 24(1):31–44.
17. Reitman S and Frankel S. A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase Am J Clin Pathol.1957; 2(8):56.
18. Harris H. On the rapid conversion of haematoxylin into haematein in staining reactions. J Appl Microscop Lab Meth. 1900; 3(7):77–780.
19. El-Mahmood MA. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. J Med Plants Res. 2009; 3(7):498-505.
20. Owolabi OJ, Omogbai E, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of *Kigelia africana* (Bignoniaceae) stem bark. Afr J Biotechnol. 2007; 6(14):1677-1680.
21. Enabulele S, Oboh F, Uwadiae E. Antimicrobial, phytochemical and nutritional properties of *Monodora myristica* seeds. IOSR J Pharm Biol Sci. 2014; 9(4):1-6.
22. Emad MA, Amna SK, Nazlina I. Antibacterial activity of oleo-gum resins of *Commiphora molmol*, and *Boswellia papyrifera* against methicillin-resistant *Staphylococcus aureus* (MRSA). Sci Res Essays. 2009; 4(4):351- 356.
23. Adegoke AA and Adebayo BC. Antibacterial activity and phytochemical analysis of leaf extracts of *Lasiethera africanum* Afr J Biotechnol. 2009; 8(1):077-080.
24. Enabulele SA and Ehiagbonare JE. Antimicrobial, Nutritional and Phytochemical properties of *Perinari excelsa* seeds. Int J Pharmacol Biol Sci. 2011; 2**(**3):B459 – B470.
25. Karou D, Savadogo A, Canini A, Yamoogo S, Traore S. Antibacterial activity of alkaloids from *Cida acuta*. Afr J Biotechnol. 2006; 5(2):195-200.
26. Cordoba J, Cabrera J, Lataif L, Penev P, Zee P, Blei AT. High prevalence of sleep disturbance in cirrhosis. Hepatology 1998; 27:339-345.
27. Aki T, Nara A, Uemura K. Cytoplasmic vacuolization during exposure to drugs and other substances. Cell Biol Toxicol. 2012; 2(8):125–131.
28. Morissette G, Lodge R, Marceau F. Intense pseudo transport of a cationic drug mediated by vacuolar ATPase, procainamide-induced autophagic cell vacuolization. Toxicol Appl Pharmacol. 2008; 22(8):364–377.
29. Olson R, Sivropolou E, Sikkema J, De Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev. 2000; 5(9):201–222
30. Yakubu R, Ekeanyanwu CR, Ugu IG, Nwachuku UP. Biochemical characteristics of the African nutmeg, *Monodora myristica*. Agric J. 2007; 5(5):303-308.
31. LaFleur-Brooks M. Exploring Medical Language: A Student-Directed Approach (7th ed.). St. Louis, Missouri, US: Mosby Elsevier; 2008. 398 p.