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Original Research Article

SUB-ACUTE TOXICITY PROFILE OF AQUEOUS EXTRACT OF *Aspilia africana* (PERS) ADAMS IN MICE

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ABSTRACT

Aspilia africana has been treasured in ethnopharmacology. However, there is paucity of information on its relative safety. Hence this study was undertaken to elucidate the sub-acute toxicity profile of the herb with the view to having a scientific validation of the use of this plant among the locals in this clime. A total of twelve mice were used and were divided into four groups of three mice. Group 1 was normal control while groups 2-4 received 10, 100 and 1000 mg/kg bw of *Aspilia africana* for eight days. The results of the phytochemical analyses showed that reducing sugars, alkaloids, total phenolics, flavonoids were significantly ($P<0.05$) higher than terpenoids, tannins, glycosides and steroids. This order was observed: steroids<glycosides<tannins<terpenoids<reducing sugars<alkaloids<total phenolics< flavonoids. The Gas Chromatography Mass Spectrometry identified different biomolecules that were present in the aqueous extract of *Aspilia africana*. They range from 9-Eicosene to hexadecanoic acid to arsenous acid to 1,2-Benzenedicarboxylic acid to other numerous bioactive compounds. There was a non-significant ($p>0.05$) increase in alanine aminotransferase activities of treatment groups 2-4 compared to normal control. With respect to aspartate aminotransferase, a significant ($p<0.05$) increase in activities were seen in groups 2 and 4 compared to normal control while group 3 showed a non-significant ($p>0.05$) decrease in activity compared to group 1. Alkaline phosphatase showed a non-significant ($p>0.05$) decrease in activities of groups 2-4 compared to group 1. In bilirubin levels, a non-significant ($p>0.05$) decrease was seen in conjugated bilirubin while non–significant ($p>0.05$) differences were observed in the total bilirubin and unconjugated bilirubin of treated groups compared to normal control. The sections of the liver presented in the microhistomorphology of group one showed that there was normal hepatic histomorphology. However, mild multifocal areas of leukocytic infiltrations were observed. On the other hand, the sections of the liver presented in groups two and three showed multifocal areas of hepatocellular necrosis and mild to moderate acute cell swelling involving the centrilobular and occasionally, the mid-zonal hepatocytes in the hepatic lobules. Also, the sections of the liver presented in group four demonstrated that there was normal hepatic histomorphology. However, mild to moderate leukocytic infiltration in the portal areas were observed. The above results therefore, suggested relative safety of the sample with respect to the liver cells' integrity but a red flag of a possible toxicity to some major organs at this sub-acute level though no death was recorded.

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INTRODUCTION

Humans have relied heavily on different plants to tackle almost an unending array of diseases. Even in epidemic, endemic conditions and pandemic occurrences, the World Health Organization's efforts seem grossly inadequate in tackling these daring health challenges. The paucity in medical knowledge and personnel shortage have continuosly aggravated quality health care delivery especially in rural populace, low income communities and highly less educated populations. Forkloric and legend accounts of the medicinal potencies of different plants flora are well known, preserved and transmitted from one generation to another. This has led to increased survival rates, improved resistance to bacterial, viral, protozoal, and fungal agents. Increased scientific validations of these medicinal plants will no doubt improve quality health deliveries in different climes. Mohamed *et al* [1] had suggested that medicinal plants are replete with phytochemicals and that they are potent against different pathogenic microorganisms.

Resource distributions, level of literacy among different populace, forkloric and legendary accounts of different climes, among other factors have made ethnomedicine to be prevalent in contemporary time. Different plants have been employed in handling different ailments. Some of these plants are highly toxic and sometimes have led to morbidity and mortality. Although these plants have been reported to be portent in ameliorating different disease conditions, their relative safety levels have not been adequately elucidated. Hence, this study was undertaken to investigate the toxicity profile of Aspinia Africana with the view to establishing its relative safety for a better use of this plant in ethnopharmacology.

Ahamefula *et al* [2] reported the presence of flavonoids, terpenoids, steroids, phenolics and suggested antibacterial activity of *Aspilia Africana*. Kulate *et al* [3] reported the presence of essential oils from *Aspilia africana*. This could account for its aroma, on approaching the plant in its natural habitat. The unique aroma is retained beyond the natural habitat to its shade drying point.

Oko *et al* [4], on the other hand, reported acute toxicity of *Aspilia africana* after 96 hr. They opined that toxicity of the plant was dose, route and extractant dependent. From their findings, aqueous extract seemed to be more toxic compared to chloroform extract. Okoli *et al* [5] has evaluated the possibility of using *Aspilia africana* in the care of wound experimentally.

Adeniyi and Odufowora,[6] reported the anti-microbial property of *Aspilia africana*. Different agents can induce hepatic challenges. They include carbon tetrachloride, xenobiotics of different nature and origin, among others [7]. Aspartate aminotransferase is found in the liver, kidneys, cardiac and skeletal muscles in large amounts while small amounts can be found in brain, pancreas and lungs. Alanine aminotransferase is mainly found in the liver with only very small amount in other organs. Oluyemi *et al* [8] submitted that methanolic extract of *Aspilia africana* significantly reduced oestrous cycle and was found to be toxic histologically.

MATERIALS AND METHODS

Plant Material

The plant Aspilia africana was harvested from Amaozalla village in Nsukka Nsukka Local Government Ara of Enugu State Nigeria and identified by Isaac Ossai of Biodiversity Conservation Programme (BDCP) Nsukka. A voucher specimen was dropped in the Herbarium. *Aspilia africana* the collected leaves were picked and shade dried. After shade drying the leaves were pulverized and soaked in water for 72 hrs to get the aqueous extract.

Animals

Albino mice were purchased from the Animal House of Dr Nwankwo of Biochemistry Department University of Nigeria Nsukka and were acclimatized in the Reseach House of Shalom Laboratory Nsukka. They were housed in aluminum cages and exposed to 12 hr light/dark cycle and were fed *ad libitum*. The work was done in strict compliance of ethics for laboratory animals.

Experimental Design

Twelve Albino mice were used for the study. They were grouped as follows:

Group one: 3ml/kg water (Normal control)

Group two: Received 10 mg/kg bw aqueous leaves extract of *Aspilia africana*

Group three: Received 100 mg/kg bw aqueous leaves extract of *Aspilia africana*

Group four: Received 1000 mg/kg bw aqueous leaves extract of *Aspilia africana*

The study lasted for eight days. The administration was by oral intubation. At the end of the work blood was collected using ocular puncture.

Phytochemical Analyses

Qualitative Phytochemical Analyses

The phytochemical analyses were done using the method of Harborne [9]; Trease and Evans [10]. The extract was tested for the presence of alkaloids, glycosides, reducing sugar, steroids, total phenolics, terpenoids, tannins, saponins.

Quantitative Phytochemical Analysis

The quantities of the following phytochemicals were tested using the methods described by Harborne [9]; Trease and Evans [10]; flavonoids, total phenolics, total tannins, alkaloids, terpenoids, steroids and reducing sugar.

GC-MS Analysis of Aqueous Leaves Extract of *Aspilia africana*

A gas chromatography from Agilent USA hyphenated to a mass spectrophotometer (5975 C) with triple axis detector equipped with an auto injector (10 µl syringe) was used. Helium gas was used as a carrier gas.

All chromatographic separation was performed on a capillary column having the following specification: length: 30 m; internal diameter 0.2 µm, thickness 250 µm, treated with phenyl methyl silox. Other GC-MS conditions were ion source temperature (EI), 250 ^{0}C, interface temperature: 300 ^{0}C, pressure: 16.2 psi, out time, 1.8 mm, 1 µl injector in split mode with split ratio 1:50 with injection temperature of 300 ^{0}C. The column temperature started at 35 ^{0}C for 5 minutes and changed to 150 ^{0}C at the rate of 4 ^{0}C/min. the temperature was raised to 250 ^{0}C at the rate of 20 ^{0}C/min and held for 5 minutes. The total elution was 47.5 minutes. Ms Solution software provided by supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NIST11).

2.6 Determinations of Different Liver Markers

Determinations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) Activities

Alanine aminotransferase and aspartate aminotransferase were determined using the method of Reitman and Frankel [11] as outlined in Teco Kit.

Determination of alkaline phosphatase (ALP) activity

The ALP was determined using Randox kit as recommended by Deutsche Gesellschaft fur Klinische Chemmie (GSCC) that is German Association of Clinical Chemistry.

Determination of bilirubin concentration

The concentrations of total, conjugated and unconjugated bilirubin were determined using the method of Jendrassik and Grof [12] as outlined in Randox Kit.

Histopathological Examination

The histopathological examination of the tissues of the liver of albino mice was done using the method of Drury *et al* [13].

Statistical Analyses

Data were reported as means±SEM where applicable. One-way analysis of variance (ANOVA) was used to analyze the experimental data. Duncan multiple test range was used to separate the means and differences were considered significant at $p<0.05$ using statistical package for service solution (SPSS) version 23.

RESULTS

Qualitative and Quantitative Phytochemical Compositions of *Aspilia africana*

The results of the phytochemical analyses of *Aspilia africana* showed that reducing sugars, alkaloids, total phenolics, flavonoids were significantly ($P<0.05$) higher than terpenoids, tannins, glycosides and steroids. This order was observed: steroids<glycosides<tannins<terpenoids<reducing sugars<alkaloids<total phenolics< flavonoids.

Table 1 Showing the Results of Qualitative and Quantitative Phytochemical Compositions of *Aspilia africana*

Phytochemicals	Bioavailabilty	Amount Present (Mean±SEM)
Glycosides	+	4.93±0.07^a
Flavonoids	+++	847.53±113.17^d
Reducing Sugars	++	224.23±11.90^b
Alkaloids	++	444.33±11.98^c
Tannins	+	5.36±0.59^a
Terpenoids	+	56.60±5.90^a
Steroids	+	4.30±0.05^a
Total Phenolics	++	838.71±42.96^d

Superscripts that were the same were considered non-significant ($P>0.05$)while those that were different were considered significant ($P<0.05$). + Present in low amount; ++ Present in moderate amount; +++ Present in high amount

Biocompounds Identified by Gas Chromatography Mass Spectrophptometry (GCMS) of Aqueous Leaves Extract of *Aspilia africana*

The Gas Chromatography Mass Spectrophptometry identified different biomolecules that were present in the aqueous extract of *Aspilia africana*. They range from 9-Eicosene to hexadecanoic acid to arsenous acid to 1,2-Benzenedicarboxylic acid to other numerous bioactive compounds.

Some biocompounds identified by Gas Chromatography Mass Spectrophptometry (GCMS) of aqueous leaves extract of *aspilia Africana* include: 9-Eicosene, Nonadecane, 1,2-Benzenedicarboxylic acid, Hexadecanoic acid, Cis Vaccenic acid, 1-Hexadecanol, Pentadecafluorooctanoic acid, 1-Octadecanesulphonyl chloride, Cyclohexane, Bis (2-ethylhexyl) phthalate, Squalene, 2,6- Di-tert-butyl-4-methylphenol, Tetrasiloxane,

Arsenous acid 1,2- Bis (trimethylsilyl) benzene, Tris (tert-butyldimethyloxy) arsane, Silicic acid, and yclotrisiloxane

Effect of Aqueous Leaves Extract of *Aspilia africana* on Liver Markers Enzymes of Albino Mice

The result of the alanine aminotransferase indicated a non-significant (P>0.05) increase in activity compared to the normal control. The peak activity of ALT was observed at 1000 mg/kg bw though non-significantly (P>0.05) higher than 10 and 100 mg/kg bw.

The result of aspartate amino transferase (AST) showed that 10 and 1000 mg/kg bw were significantly (P<0.05) higher compared to normal control and 100 mg/kg bw. The lowest concentration (10 mg/kg bw) demonstrated the highest activity compared to other concentrations.

Alkaline phosphatase activity of treatment groups were non-significantly (P>0.05) lower than the normal control as shown in Table 2.

Table 2. The Effect of Aqueous Leaves Extract of *Aspilia africana* on Liver Markers Enzymes of Albino Mice

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group One	9.00±0.58[a]	20.00±1.53[a]	29.33±0.33[a]
Group Two	10.00±1.00[a]	27.00±1.73[b]	27.33±2.03[a]
Group Three	9.67±0.88[a]	17.33±1.45[a]	26.67±2.19[a]
Group Four	11.00±1.00[a]	25.33±0.88[b]	27.67±0.33[a]

Superscripts that were the same were considered non-significant (P>0.05) while those that were different were considered significant (P<0.05).

Effect of Aqueous Leaves Extract of *Aspilia africana* on Total, Direct and Indirect Bilirubin of Albino Mice

The results of total, direct and indirect bilirubin of treatment were non-significantly (P0.05) different from the normal control as shown in Table 3.

Table 3. Effect of Aqueous Leaves Extract of *Aspilia africana* on Total, Direct and Indirect Bilirubin of Albino Mice

Groups	Total Bil (mg/dl)	Direct bil (mg/dl)	Ind. Bil (mg/dl)
Group One	0.79±0.06[a]	0.35±0.07[a]	0.44±0.11[a]
Group Two	0.88±0.04[a]	0.35±0.02[a]	0.53±0.03[a]
Group Three	0.74±0.06[a]	0.25±0.06[a]	0.50±0.10[a]
Group Four	0.84±0.07[a]	0.34±0.01[a]	0.50±0.88[a]

Superscripts that were the same were considered non-significant (P>0.05)while those that were different were considered significant (P<0.05).

Histopathological Examinations of Different Microphotographs of Albino Mice Liver Treated with Different Concentrations of aqueous leaves extract of *Aspilia africana.* **Figure 1** shows the histological examination of Different Microphotographs of Albino Mice Liver treated with different concentrations of Aqueous Leaves Extract of *Aspilia africana.* The sections of the liver presented in group one showed the normal hepatic histomorphology. However, mild multifocal areas of leukocytic infiltrations were observed (arrow). Central vein (V); Portal triad (P). HE x200 as shown in Plate 1. On the other hand, the sections of the liver presented in group two showed multifocal areas of hepatocellular necrosis (arrow) and mild to moderate acute cell swelling involving the centrilobular and occasionally, the mid-zonal hepatocytes in the hepatic lobules. The affected cells appeared swollen, occluding adjacent hepatic sinusoids and show a finely vacuolated pale eosinophilic cytoplasm (*). Central vein (V); portal triad (P). HE x200 as shown in Plate 2

The Sections of the liver presented in group three showed a moderate-to-severe acute cell swelling involving the centrilobular and mid-zonal hepatocytes in the hepatic lobules. The affected cells appeared swollen, occluding adjacent hepatic sinusoids and show a finely vacuolated pale eosinophilic cytoplasm (*). Also, mild leukocytic infiltration (arrow) was observed in the portal areas. Portal triad (P). HE x200 as shown in Plate 3. Also, the sections of the liver presented in group four demonstrated that there was normal hepatic histomorphology. However, mild to moderate leukocytic infiltration in the portal areas were observed (arrow). Central vein (V); Portal triad (P). HE x200 as shown in Plate 4

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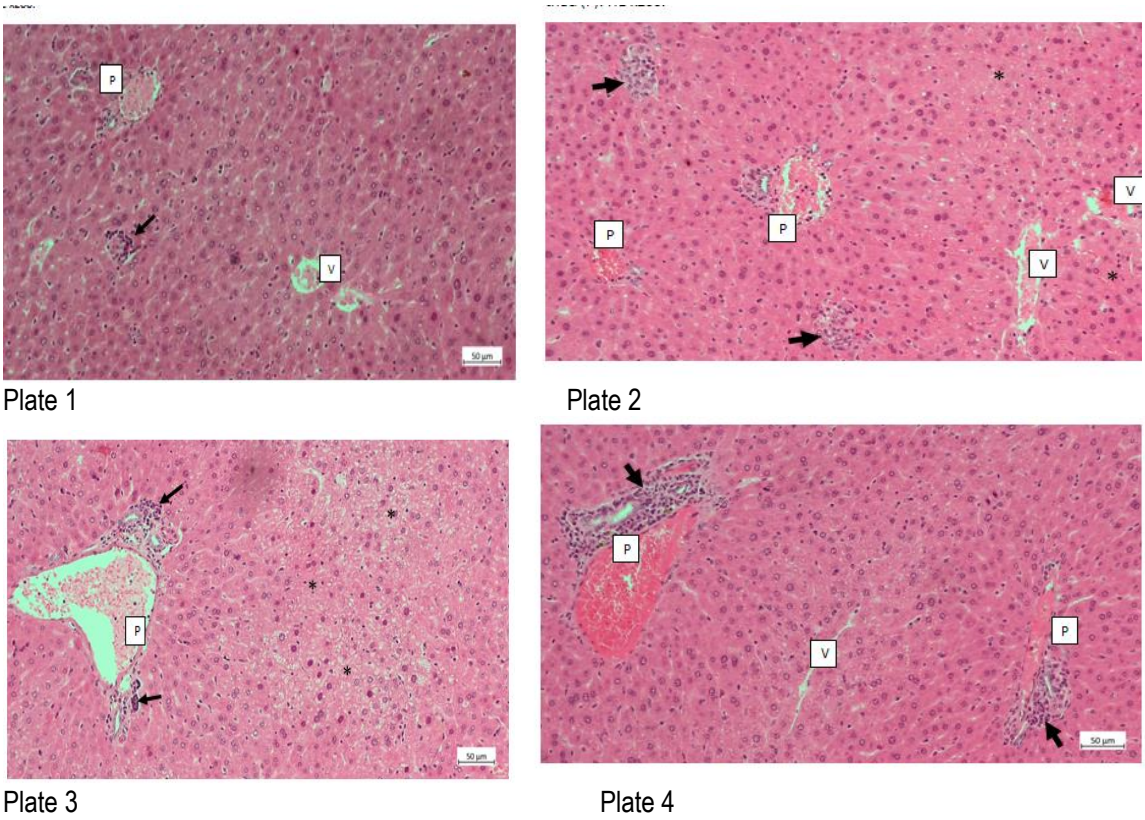

Figure 1. Histological examination of Different Microphotographs of Albino Mice Liver treated with different concentrations of aqueous Leaves Extract of *Aspilia africana*. Plate 1 (Received 3 ml/kg water; microphotograph showing central vein (V); Portal triad (P). HE x 200), Plate 2 (Received 10 mg/kg bw aqueous leaves extract of *Aspilia africana*; microphotograph showing central vein (V); portal triad (P). HE x 200), Plate 3 (Received 100 mg/kg bw aqueous leaves extract of *Aspilia Africana*; microphotograph showing central vein (V); Portal triad (P).HE x 200), Plate 4 (Received 1000 mg/kg bw aqueous leaves extract of *Aspilia africana*; microphotograph showing central vein (V); Portal triad (P). HE x 200)

DISCUSSION

Resource distributions, level of literacy among different populace, forkloric and legendary accounts of different climes, among other factors have made ethnomedicine to be prevalent in contemporary time. Different plants have been employed in handling different ailments. Some of these plants are highly toxic and sometimes have led to morbidity and mortality. Although these plants have been reported to be portent in ameliorating different disease conditions, their relative safety levels have not been adequately elucidated. Hence, this study was undertaken to investigate the toxicity profile of *Aspilia africana* with the view to establishing its relative safety for better use of this plant in ethnopharmacology. The choice of aqueous extraction is to mimic ethnopharmacology. This is germane as the natives that use *Aspilia africana* use water in extracting the plant for various uses in handling different ailments. Oko *et al* [4] reported acute toxicity of *Aspilia africana* after several hours. They were of the opinion that the toxicity of the plant was heightened when water was used as a medium of extraction. From their findings, aqueous extract seemed to be more toxic compared to chloroform extract.

The results of the phytochemical analyses suggested that the plant was relatively replete with different phytochemicals that could account for the different pharmacological claims of the plant. The presence of tannins, flavonoids and total phenolics could account for the antioxidant potency of Asipnia Africana. Mohamed *et al* [1] had suggested that medicinal plants are replete with potent phytochemicals. Humans have relied heavily on different plants to tackle almost an unending array of diseases. Kulate *et al* [3] reported the presence of essential oils from *Aspilia Africana.* Oils are known to possess different bioactivity as phytochemicals. Ahamefula *et al* [2] reported the presence of flavonoids, terpenoids, steroids, and phenolics that could elicit biological activity.

The result of the GCMS indicated that phenol was present in the plant, further corroborating the antioxidant potential of the plant. The presence of arsenous acid, benzene and phthalate raised a red flag with respect to the safety of the extract as used in ethnopharmacology.

The liver marker enzymes were varied in their activities. alanine aminotransferase, a predominant marker of hepatic challenge, suggested that there was no major hepatic injury caused by the aqueous leaves extract of *Aspinia africana*. However, aspartate aminotransferase activity was significantly ($P<0.05$) increased, suggesting a possible liver injury. It is speculated that a look at the three liver enzymes (ALT, AST, and ALP) simultaneously, rather pointed to a possible of the liver cells while outright injuries or necrosis might have been prevented. Different agents can induce hepatic challenges. They include carbon tetrachloride, xenobiotics of different nature and origin, among others [7]. Hepatoprotective bio-compounds are expected to reverse this injury by possibly neutralizing the toxicant or by protecting the membranes of the hepatocytes. This is vital as the integrity of the cell membranes determines the functionality and viability of the concerned cell. Kulate *et al* [3] reported the presence of essential oils from *Aspilia africana.* This could account for its aroma, on approaching the plant in its natural habitat. The unique aroma is retained beyond the natural habitat to its shade drying point. Essential oils are known to possess antioxidant capacity and hence hepatoprotective potential.

The histopathological findings further reported moderate to severe challenge of the hepatocytes at 10 and 100 mg/kg bw and a relatively normal histomorphology in 1000 mg/kg bw. The presence of arsenous acid, 1, 2 -Benzenedicarboxylic acid, Bis (2-ethylhexyl) phthalate, 1, 2- Bis (trimethylsilyl) benzene and Tri (tert-butyldimethyloxy) arsane, could account for these challenges reported in the histopathological findings [8] submitted that methanolic extract of *Aspilia africana* significantly reduced oestrous cycle and was found to be toxic histologically. Oko *et al* [4] reported acute toxicity of *Aspilia africana* after 96 hr. They opined that toxicity of the plant was dose, route and extractant dependent. From their findings, aqueous extract seemed to be more toxic compared to chloroform extract.

CONCLUSION

Aspinia africana seemed to be mildly toxic at the tested doses and duration, hence caution is seriously needed so as to harness the pharmacological potency of *Aspinia africana* without compromising the issue of safety.

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AUTHORS' CONTRIBUTION

OEA: Experimentation and data analysis; **CGE**: Conceptualization and supervision; **OCE**: Experimentation; **IHE**: Manuscript proofreading; **IEO**: Manuscript drafting; EPE Manuscript drafting; **EAO**: Manuscript drafting

CONFLICT OF INTEREST

The authors declare no conflict of interest

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