

## Toxicological Evaluation of Terpenoid Fraction of Root Extract of *Physalis angulata*

<sup>1</sup>Bawa A., <sup>2</sup>Njoku O.U., <sup>2</sup>Nwodo O.F.C., <sup>3</sup>Ugwu Okechukwu P.C., <sup>2</sup>Ogugofor Martins Obinna, <sup>4</sup>Salihu Suleiman and <sup>1</sup>Buba M.

<sup>1</sup>Department of Science Laboratory Technology Federal Polytechnic, Mubi, Adamawa State, Nigeria.

<sup>2</sup>Department of Biochemistry, University of Nigeria, Nsukka.

<sup>3</sup>Department of Biochemistry, Ebonyi State University Abakaliki, Nigeria.

<sup>4</sup>Department of Chemistry, Federal University, Dutse, Jigawa State, Nigeria.

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### ABSTRACT

This study was carried out to investigate the toxicological properties of terpenoids fraction of *Physalis angulata* in male albino rats. These were determined using spectrophotometric methods. The rats were randomly divided into four groups of five (5) rats each. Group 1: was not administered with anything (Normal Control); Group 2: was administered with 50 mg/Kg b. w of vitamin C (standard drug); Group 3: was administered with 25 mg/Kg b. w of the isolated terpenoids; Group 4: was administered with 50 mg/Kg b. w. of the isolated terpenoids; Group 5: was administered with 100 mg/Kg b. w. of the isolated terpenoids for the period of 30 days. All the rats were given free access to water and commercial poultry feed *ad libitum*. Results of the analyses were expressed as mean  $\pm$  standard deviation. The acute toxicity study of the root extract was performed using the acute oral toxicity up and down method and the result of the study showed no mortality of the animals used even at the highest dose of 5000 mg/Kg b.w after 14 days. The results of effects of terpenoids on the liver function biomarkers such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) indicated a non-significant ( $p > 0.05$ ) increase when the serum levels of AST and ALT activities of the groups that received the graded doses of the terpenoids and vitamin C were compared to the normal control. The evaluation of serum level of bilirubin showed a significant ( $p < 0.05$ ) decrease when the group that received vitamin C (standard drug) was compared to the normal control. In conclusion, the study has shown that *Physalis angulata* terpenoid fraction is safe and could be used in the management of liver related disorders.

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Keywords: Terpenoid fraction, *Physalis angulata*, Liver function and Acute toxicity

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### INTRODUCTION

The cells of the liver, which are known as hepatocytes carry out many biochemical activities. Some of these biochemical activities carried include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification and lipid metabolism [1]. It is therefore very obvious that any disease condition or adverse physiological conditions, which affect the hepatocytes, will cause concerted and tremendous metabolic derangement. In such conditions also, there will be an increase in the serum activities of the mitochondrial-bound

liver enzymes since hepatocytic damage causes their release into the serum. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activities of the liver enzymes so as to ensure the hepato-protectiveness of such food or drug [2]. This can be achieved through liver function tests, which include estimation of plasma protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin and bilirubin [3].

Herbs have been used in the preparation of medicines or treatment of various

human and animal diseases [4]. Reduced efficiency of synthetic preparation due to various reasons has resulted in a global interest in the preparation of therapeutic medicines from plants [5]. In addition, plant extract, either as pure compounds or as a standardized extract has provided unlimited opportunities for new drug discoveries because of the unmatched availabilities of chemical discoveries and diversities [6].

According to the WHO, more than 80% of the world's population relies on traditional medicine for primary health care needs. The use of herbal medicine represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [7].

Due to some adverse effect and microbial resistance to the chemically synthesized drugs, a shift to ethno-pharmacognosy has been observed. This is due to the presence of a wide array of phytochemicals from plants which are safe and broadly effective when used as drugs. Many beneficial biological

activities such as anticancer, antimicrobial, antioxidant, antidiarrhoeal, analgesic and wound healings activities of various plants have been reported.

*Physalis angulata* is a plant of the family Solanaceae, widely distributed throughout the tropical and sub-tropical region of the world. It is distributed as a weed in gardens, waste lands, along roads, in the forest, along sea levels and in cultivated fields [8]. The picture of *Physalis angulata* is shown in fig. 1. All over the world, *Physalis angulata* is used as herbal medicine and for the treatment of various human ailment like malaria, hepatitis, asthma, dermatitis and rheumatism [9], [10]. Infusions of *Physalis angulata* have been used to treat earache and postpartum infection. *P. angulata* leaf has been reported for CNS depressant action and it also possesses an antitumour activity. In addition, the constituent of antitumour glycoside myricetin -3 - 0 neohesperidoside of *Physalis angulata* has been reported [11].

This study was therefore designed to evaluates the toxicological properties of terpenoid fraction of root extract of *Physalis angulata*

## MATERIALS AND METHODS

### Materials

Equipment, Chemicals and Reagents used in this study were of analytical standards and grades

### Collection and Preparation of Plant Materials

Fresh roots of *Physalis angulata* were collected from Yola South Local Government Area of Adamawa State, Nigeria. The plant material was identified and authenticated by Mr. Usman Gala of Botany Department, Ahmadu Bello University, Zaria Nigeria. The plant was assigned the voucher number: ABU2051. The root sample was washed and cut into smaller pieces and dried under direct sunlight. The sample was later pulverized to coarse powder using a hammer mill (Gallenkamp, U.S.A.).

### Extraction of Plant Material

A known weight (6.952 kg) of the air-dried root powder was extracted with analytical grade ethanol in a soxhlet at 65°C. The mixture was vacuum-filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan) to afford 52.503g (0.755% w/w) of the extract. The extractive yield was calculated using the relation:

### Fractionation of *Physalis angulata* Root Extract

The extract (52.50 g) was subjected to solvent-guided fractionation in a silica gel (60-120 mesh size) column (2 × 70 cm) successively eluted with 20% ethyl acetate

in n-hexane, followed by 30, 40, 50, 60, 70, 80 and 100% ethyl acetate. The solvent fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. Fractions that gave positive reaction to terpenoids were pulled together and concentrated in rotary evaporator under vacuum to yield (E: nH-F; 14017.50 mg: 26.70% w/w) fraction. A small quantity of the fraction (E: nH-F) was developed using percolated silica thin layer chromatography plates in a mixture of n-hexane: ethylacetate: methanol in different ratios, but (3:2:1) ratio which gave the best resolution (showing three distinct terpenoids  $T_1$ ,  $T_2$ ,  $T_3$  and one steroid  $S_1$  chromatographic spots) served as the solvent mixture for the final elution in the second column. The third terpenoid chromatographic spot ( $T_3$ ) which appeared insoluble in n-hexane: ethylacetate: methanol (3:2:1) mixture, was eluted with 20% acetic acid in ethyl acetate. Consequently, the E:nH-Fraction (14017.50 mg) was subjected to further separation in silica gel (60-120 mesh size) column eluted with mixture of n-hexane : ethylacetate : methanol (3:2:1), followed by 20% acetic acid in ethyl acetate. The sub-fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. The first 300 ml sub-fraction contained a mixture of  $T_1$  and  $S_1$ , while the rest of n-hexane: ethyl acetate: methanol (3:2:1) sub-fractions contain only  $T_2$  the largest amount of terpenoid. However, 20% acetic acid in ethyl acetate was used to elute  $T_3$  and trace  $T_2$ . The sub-fractions were concentrated in rotary evaporator under vacuum to yield ( $T_2$ ; 3.15 g: 22.471% w/w), ( $T_3$  and  $T_2$ ; 0.938 g: 6.69% w/w), sub-fractions.

### Acute Toxicity Studies

For acute toxicity of the extract, was determined according to the OECD (2008) Guideline No. 425 (Acute Oral Toxicity-Up and Down-Procedure). Twenty (20) male albino rats (120 - 150 g) were used for this study. In stage one of the test, two groups of animals received oral administration of 1000 and 2000 mg/kg

respectively (n = 5) of the extract and were observed for 24 h for deaths. As no death occurred in any of the groups in the first stage of the test, 3000 and 5000 mg/kg doses of the extract were respectively administered to two groups of animals (n = 5). The treated rats were under observation for 14 days for mortality and general behaviour.

### Experimental design for the Study

In this study, twenty (20) albino rats were used. They were acclimatized for a period of one week and fed with commercial poultry feed and water *ad libitum*. The animals were divided into four groups of five (5) rats each, based on the similarity of their body weights and the extract were orally administered as shown below. The study lasted for 30 days and they were grouped as shown below: Group 1: Normal Control; Group 2: 50 mg/kg b. w of vitamin C (standard drug); Group 3: 25 mg/kg b. w of the isolated terpenoids; Group 4: 50 mg/kg b. w. of the isolated terpenoids; Group 5: 100 mg/kg b. w. of the isolated terpenoids.

On day 30, blood samples were collected from all the rats through ocular puncture and then both serum and plasma were used for antioxidant analyses.

### Biochemical Analysis

#### Assay of Serum Alanine Aminotransferase (ALT) Activity

The method of [12] was used for alanine aminotransferase (ALT) activity determination using a Quimica Clinica Aplicada (QCA) test kit from Quimica Aplicada, Spain.

#### Serum Aspartate Aminotransferase (AST) Assay

The method of [13] was used for Aspartate amino transferase (AST) determination by colorimetric method for *in vitro* determination of AST in serum using a Quimica Clinica Aplicada (QCA) test kit (Quimica Clinica Aplicada, Spain).

### Assay of Serum Alkaline Phosphatase Activity

The method of [14] was used for the determination of serum alkaline phosphate by Colorimetric method for *in vitro* determination of alkaline phosphatase in serum using Quimica Clinica Applicada (QCA) test kit (QCA, Spain).

### Determination of Serum Albumin Concentration

The method of [15] was used for serum albumin determination. Commercially prepared reagent test kit Quimica Clinica Applicada (QCA) (Spain) reagent was used for the analysis.

### Determination of Bilirubin Concentration

Bilirubin concentration was determined according to the method described by [16].

### Statistical Analysis

The statistical analysis was carried out using Statistical Product and Service Solution (SPSS 15.0) version. Statistical differences were evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to detect significant differences among the mean values of the different groups.

## RESULTS

### Acute Toxicity Testing of the Mice

The acute toxicity testing showed that even at extract dose of 5000 mg/Kg body weight of the mice, terpenoid extract did not cause death of any of the experimental mice, however, sluggish movement, raised hair

and loss of appetite were observed in the group administered with 5000 mg/Kg body weight relative to the control. Thus, the LD<sub>50</sub> of this plant extract is greater than 5000 mg/Kg in mice as shown in Table 1.

**Table 1: Result of Acute Toxicity Testing**

GROUPS	DOSAGE (mg/Kg)	A/D	PERIOD	SIGN OF TOXICITY OBSERVED
Group 1	1000	3/0	24 hours	No sign of toxicity
Group 2	2000	3/0	24 hours	No sign of toxicity
Group 3	3000	3/0	14 days	No sign of toxicity
Group 5	4000	3/0	14 days	No sign of toxicity
Group 5	5000	3/0	14 days	No Sign of toxicity

KEY:

A/D = number of mice administered/number of deaths

### Effect of Terpenoids on Liver Function Indices

The results of effects of terpenoids on the liver function biomarkers such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) indicated a non-significant ( $p > 0.05$ ) increase when the serum levels of AST and ALT activities of the groups that received the graded doses

of the terpenoids and vitamin C (standard drug) were compared to the normal control. However, there was a significant ( $p < 0.05$ ) decrease in the serum level of ALP in the groups that received the graded doses of the terpenoids compared to the normal control. There was no significant increase in the serum level of ALP when the group that received vitamin

C (standard drug) was compared to the normal control as shown in Table 2.

when the group that received vitamin C (standard drug) was compared to the normal control.

The evaluation of serum level of bilirubin showed a significant ( $p < 0.05$ ) decrease

**Table 2: Effect of Terpenoids on Liver Function Indices**

S/N	GROUPS	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	ALBUMIN (g/dL)
1	Normal Control	27.45±0.41 <sup>c</sup>	174.34±2.19 <sup>c</sup>	6.02±1.67 <sup>b</sup>	3.79±0.33 <sup>ab</sup>
2	Positive Control	21.83±1.64 <sup>a</sup>	172.75±2.22 <sup>c</sup>	5.64±1.51 <sup>a</sup>	4.05±0.33 <sup>b</sup>
3	25 mg/kg Terpenoid b.w	24.25±1.46 <sup>b</sup>	124±01.30 <sup>b</sup>	6.23±0.40 <sup>b</sup>	3.99±0.40 <sup>a</sup>
4	50 mg/kg Terpenoid b.w	22.30±2.05 <sup>a</sup>	74.90±5.61 <sup>a</sup>	5.17±0.73 <sup>a</sup>	4.13±0.45 <sup>b</sup>
5	100 mg/kg Terpenoid b.w	23.48±0.29 <sup>ab</sup>	108.16±1.56 <sup>b</sup>	7.13±1.44 <sup>c</sup>	4.28±0.47 <sup>b</sup>

Values are expressed as mean±SD. Values in the same column having different superscripts are significantly different. n= 5.

**Table 3: Effect of Terpenoids on Bilirubin Concentration**

S/N	GROUPS	Bilirubin (mg/dL)
1	Normal Control	0.21±0.05 <sup>b</sup>
2	Positive Control	0.09±0.08 <sup>a</sup>
3	25 mg/kg b.w Terpenoid	0.19±0.03 <sup>b</sup>
4	50 mg/kg b.w Terpenoid	0.11±0.07 <sup>ab</sup>
5	100 mg/kg b.w Terpenoid	0.16±0.06 <sup>ab</sup>

Values are expressed as mean±SD. Values in the same column having different superscripts are significantly different. n= 5.

#### DISCUSSION

The extraction of 1.5 kg of pulverized *Physalis angulata* root with ethanol gave a percentage yield of 4.7 %. The acute toxicity study of the root extract was performed using the acute oral toxicity up

and down method. The result of the study showed no mortality of the animals used even at the highest dose of 5000 mg/Kg b.w after 14 days and hence, an indication

that the plant is safe and could be consumed.

The toxicological profile of different chemical agents can be evaluated by routine toxicological testing which involves the examination of different organs such as the heart, liver and kidney. It has been reported that dose dependent reactions could be revealed in animal experiments. However, the assessment of liver function is ideal for toxicity evaluation of drugs and especially plant extracts as possible toxicities normally result in the alterations of their physiological function biomarkers. The liver function results indicated that normal control has a significant ( $p < 0.05$ ) higher AST and ALP when compared with the groups that received the vitamin C and graded doses of the terpenoids respectively. The normal control also has a higher concentration of ALT though not significant when compared with the groups that received the vitamin C and graded doses of the terpenoids respectively. However, there was a sudden non-significant increase in liver

biomarkers in the group that received 100 mg/Kg b.w terpenoids.

[17] reported that serum levels of AST, ALT, ALP and bilirubin could be used to ascertain the functionality and cellular integrity of the liver. In addition, [15] reported that the serum levels of these aforementioned enzymes are indicators of the status of an organism's internal environment. Whenever there is a liver damage, ALT, AST and ALP are released thereby causing a rise in their serum level above the normal level. According to [16], elevations of serum levels of these enzymes are an indication of damage and hepatocytes inflammation. [17] also reported that increase in serum levels of AST are an indication of hepatic injuries similar to viral hepatitis, infarction, and muscular damages. In the other hand, ALT is specific for liver and thus mediates the conversion of alanine to pyruvate and glutamate [17]. An increase in the serum level of ALT is a suitable biomarker of hepatic injuries. In conclusion, the study has shown that *Physalis angulata* terpenoid fraction is not toxic and could be used in the management of liver related disorders.

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