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# TOXICOLOGICAL EFFECT OF TERPENOID FRACTION OF PHYSALIS AGULATA ON LIVER AND RENAL FUNCTION BIOMARKERS

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**Abstract**

*The aim of the research was to investigate the toxicological effects of terpenoid fraction of Physalis angulata on male albino rats. Acute toxicity of the extract was determined according to the OECD guideline No.425 (Acute oral toxicity-Up and Down-procedure). The effect of the fraction on liver function was determined by analyzing the serum level of aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using standard methods. Also, the effect of the extract on kidney function was determined by analyzing the serum level of urea and creatinine. The results of the acute toxicity studies showed that the fraction is safe for consumption (to be used as drug). The results of the fraction's effect on liver function biomarkers showed that there is no significant (P>0.05) increase in the serum levels of AST and ALT in groups treated with the fraction compared to the normal control, but there was significant (P<0.05) decrease in the serum level of ALP in fraction-treated group compared to the normal control. The effect of the fraction on kidney function biomarkers revealed that there was a significant (P<0.05) decrease in the serum level of urea and creatinine in the fraction-treated group compared to the normal control. All these results suggest that the terpenoid extract of Physalis angulata has no negative effects on the function of liver and kidney, and is also safe for use as medicine.*

***KEYWORDS:***
*toxicological effects, terpenoid fraction, Physalis angulata, Albino rats, biomarkers*

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## INTRODUCTION

The use of herbal medicine represents a long history of human interactions with the environment. Herbs have been used in the preparation of medicines or treatment of various human and animal diseases (Cos, Vlietinck, Berghe, & Maes, 2006). According to WHO, more than 80 % of the world's population relies on traditional medicine for primary health care needs. Reduced efficiency of synthetic preparation due to various reasons has resulted in a global interest in the preparation of therapeutic medicines from plants (Zhang & Arnold, 2005). 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 (Cos et al., 2006). More so, Due to some adverse effect and microbial resistance to the chemically synthesized drugs, a shift to ethno-pharmacognosy has been observed

Traditionally, plants are used to treat chronic as well as infectious diseases (Sasidharan, Chen, Saravanem, Sundram, & Latha, 2011). This may be due to the presence of a wide array of phytochemicals from plants which are safe and broadly effective when used as drugs. These phytochemicals were found to have anticancer, antimicrobial, antioxidant, antidiarrhoeal, analgesic and wound healings effects.

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 (Smith, Shenvi, & Widlansky, 2004). It is used as herbal medicine for the treatment of various human ailment like malaria, hepatitis, asthma, dermatitis and rheumatism (Soares, Dinis, Cunha, & Ameida, 2003; Renjifo-Salgado, 2013). Infusions of Physalis angulata have been used to treat earache and postpartum infection. P. angulata leaf has been reported for central nervous system (CNS) depressant action and it also possesses an antitumour activity. In addition, the constituent of antitumour glycoside myricetin -3 – 0 neohespenidoside of Physalis angulata has been reported (Ismail & Alam, 2001). With these benefits in mind, this

research was aimed at the toxicological evaluation of the terpenoid fraction of Physalis angulata so as to ascertain its safety for use as drug.

## MATERIALS AND METHODS

### Materials

### Animals

Adult male albino rats (100-120 g) were obtained from the Animal House of the Department of Zoology and Environmental Studies, University of Nigeria, Nsukka, Enugu State. The animals were housed in steel cages within the Laboratory Animals Facility of Brain-Phosphorylationship Scientific Solution Services, Number 9 Ogui Road, 5th Floor, Right Wing, Enugu, Enugu State and maintained on standard feed and clean drinking water ad libitum. They were allowed to acclimatize to laboratory condition for a period of four weeks before use. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

### Chemicals and Reagents

The chemicals used for this study were of analytical grade and products of Sigma Aldrich (USA), British Drug House (BDH, England) and Andalucia (Spain). They included methanol, ethanol, ethylacatetate, petroleum ether etc. The reagents used for the biochemical assays were commercial kits and products of Randox (USA) and Teco (USA).

### Equipment

The equipment used were obtained from the Department of Biochemistry, University of Nigeria, Nsukka; Facility of Brain-Phosphorylationship Scientific Solution Services, Number 9 Ogui Road, 5th Floor, Right Wing, Enugu and other scientific shops in Onitsha Main Market, Anambra State. They included conical flasks (Pyrex, England), water bath (Gallenkamp, England), beakers (Pyrex, England), weighing balance (Metler HAS, U.S.A), filter papers (Whatman), test tubes (Pyrex, England), measuring cylinder (Pyrex, England), glass funnel (Pyrex, England), spectrophotometer

(Spectronic 20D, Germany), refrigerator (Thermocool, England), centrifuge (Vickas Ltd, England) and rotary evaporator.

## Methods

### 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:
Yield (%) = [Weight of extract (g)/Weight of plant material (g)] ×100.

### Determination of Terpenoids Content

The method reported by Ladan et al. (2014) was used for the quantitative determination of terpenoids. Extract (2 g) was weighed and soaked in 50 mL of 95 % ethanol for 24 hrs. The mixture was filtered and the filtrate extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as total terpenoids.

### 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 trial thin layer chromotography 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.

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**Chronic Toxicity Study**

In this study, Sixteen (16) albino rats were used. They were acclimatized for a period of one week and fed with commercial poultry feed and water ad libtum. The animals were divided into four groups of four (4) 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.

Group 1: Normal Control
Group 2: 25 mg/kg b. w of the isolated terpenoids
Group 3: 50 mg/kg b. w. of the isolated terpenoids
Group 4: 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 biochemical analyses. Thereafter, they were anaesthesized with chloroform, sacrificed and then their organs were excised for histological studies.

**Biochemical Studies**

**Blood Collection**

**Procedure:**

Blood (5 ml) was collected by orbital technique. Blood sample for clinical chemistry determinations were collected from the retro-bulbar plexus of the medial canthus of the eye of the rats. A nucrocapillary tube was carefully inserted into the medial canthus of the eye of the rats to puncture the retrobulbar plexus and thus enable outflow of 2 mL of blood into a clean glass test tube. The blood sample was left at room temperature for 30 minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 rpm for 10 minutes using a table centrifuge to enable a complete separation of the serum from the clotted matter. The clear serum supernatant was then carefully aspirated with syringe and stored in a clean sample bottle for the biochemical tests.

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

The method of Reitman & Frankel (1957) was used for alanine aminotransferase (ALT) activity determination using a Quimica Clinica Applicada (QCA) test kit from Quimica Applicada, Spain.

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α-Ketoglutarate + L-Alanine $\xrightarrow{ALT}$ L-Glutarate + Pyruvate - - 1

Pyruvate + Reduced Cofactor + $H^+$ $\xrightarrow{LDH}$ Lactate + Cofactor - - 2

ALT catalyses the conversion of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. In reaction II, LDH catalyses the oxidation of the reduced cofactor to the cofactor itself. The rate of decrease in absorbance of the reaction mixture at 505 nm, due to the oxidation of the reduced cofactor is directly proportional to the ALT activity.

**Serum Aspartate Aminotransferase (AST) Assay**

The method of Reitman & Frankel (1957) was used for Aspartate amino transferase (AST) determination by colorimetric method for in vitro determination of AST in serum using a Quimica Clinica Applicada (QCA) test kit (Quimica Clinica Applicada, Spain).

α-Ketoglutarate + L-aspartate $\xrightarrow{AST}$ L-aspartate + oxalacetate 3

Oxaloacetate + NADH + $H^+$ $\xrightarrow{MAH}$ Malate + $NAD^+$ 4

AST catalyses the reaction of α-ketoglutarate and L-aspartate to L-glutamate and oxaloacetate. In reaction II, malate dehydrogenase (MDH) catalyses the oxidation of NADH to NAD. The rate of decrease in absorbance of the reaction mixture at 505 nm, due to oxidation of NADH is directly proportional to the AST activity.

**Assay of Serum Alkaline Phosphatase Activity**

The method of Babson et al. (1966) 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 Urea Concentration**

The modified method of Berthelot-Searcy for the in vitro determination of urea in serum (Searcy, Reardon, & Foreman, 1967) using the Quimica Clinica Applicada (QCA) creatinine test kit (QCA, Spain). **Principle:**

Urease

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$$\text{Urea} + H_2O \xrightarrow{\text{GLDH}} 2NH_4^+ + CO_2 \quad - \quad - \quad - \quad 5$$

$$2NH_4^+ + 2\ \alpha\text{-KG} + 2\ \text{NADH} \longrightarrow 2\ \text{L-Glutamate} + 2NAD^+ + 2H_2O \quad 6$$

**Serum Creatinine Assay**

The modified Jaffe method for the in vitro determination of creatinine in serum Blass, K.G., Thiebert, R.J. & Lam, L.K. (1974) using the Quimica Clinica Applicada (QCA) creatinine test kit (QCA, Spain).

**Principle:**

Creatinine + Alkaline picrate ⟶ Creatinine-picrate compl 7

An alkaline pH, creatinine reacts with picrate to form a janousky complex. The rate of increase in absorbance at 546 nm due to the formation of the creatinine-picrate complex is directly proportional to the concentration of creatinine in the sample.

**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 dfferent groups.

**RESULTS**

**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.

**Table 1: 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$^{c}$ | 174.34±2.19$^{c}$ | 6.02±1.67$^{b}$ | 3.79±0.33$^{ab}$ |
| 2 | 25 mg/kg b.wTerpenoid | 24.25±1.46$^{b}$ | 124±01.30$^{b}$ | 6.23±0.40$^{b}$ | 3.99±0.40$^{a}$ |
| 3 | 50 mg/kg b.wTerpenoid | 22.30±2.05$^{a}$ | 74.90±5.61$^{a}$ | 5.17±0.73$^{a}$ | 4.13±0.45$^{b}$ |
| 4 | 100 mg/kg b.wTerpenoid | 23.48±0.29$^{ab}$ | 108.16±1.56$^{b}$ | 7.13±1.44$^{c}$ | 4.28±0.47$^{b}$ |

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

**Effect of Terpenoids on Kidney Function Indices**

The evaluation of kidney function biomarkers as shown in Table 2 revealed that there was a significant ($p<0.05$) decrease in the serum level of creatinine in the groups that received the graded doses of the terpenoids when compared to the normal control. Also, there was a significant decrease in the serum level of urea when the group that received 50 mg/kg b.w terpenoids were compared to the normal control.

**TABLE 2: Effect of Terpenoids on Kidney Function Indices**

| S/N | GROUPS | Creatinine (mg/dL) | Urea (mg/dL) | Bilirubin (mg/dL) |
|---|---|---|---|---|
| 1 | Normal Control | 0.18±0.06$^{b}$ | 24.92±1.87$^{b}$ | 0.21±0.05$^{b}$ |
| 2 | 25 mg/kg b.w Terpenoid | 0.12±0.05$^{ab}$ | 22.14±1.79$^{b}$ | 0.19±0.03$^{b}$ |
| 3 | 50 mg/kg b.w Terpenoid | 0.07±0.03$^{a}$ | 8.82±3.45$^{a}$ | 0.11±0.07$^{ab}$ |
| 4 | 100 mg/kg b.w Terpenoid | 0.11±0.06$^{ab}$ | 15.24±1.68$^{ab}$ | 0.16±0.06$^{ab}$ |

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 and kidney 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.

Lavanaya, Rameh, Kavita & Malarvizhi (2011) 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, Rehman et al. (2006) 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 Sood (2006), elevations of serum levels of these enzymes are an indication of damage and hepatocytes inflammation. Abolfathi, Mohajeri, Rezaie, & Mehrdod (2012) 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 (Abolfathi et al., 2012). An increase in the serum level of ALT is a suitable biomarker of hepatic injuries.

Nephrotoxicity has been described as a major complication characterized by morphological damage of intracellular organelles and cellular necrosis with concomitant functional alterations such as the antioxidant defense system depletion and mitochondrial damage (Joy & Nair, 2008). According to Kim & Moon (2002), nephrotoxicity occurs when there is derangement of kidney-specific detoxification and excretion due to the damage or destruction of kidney function by exogenous or endogenous toxicants. Abdulazeez et al. (2010) reported that renal failure causes the retention of

creatinine and other non-protein nitrogenous constituents of the blood. There were significant ($p < 0.05$) decreases in creatinine and urea levels of treatment groups compared to the normal control. The sudden increase in creatinine and urea level observed in the group that received 100 mg/kg b.w terpenoids could suggest that the terpenoid fraction may have a possible toxicity at higher doses.

## CONCLUSIONS

This present study has shown that Physalis anguata is a rich source of terpenoids.

The study has also shown that the terpenoids fraction Physalis anguata is safe within the ranges of amount used, because there were no observable toxicities at the doses used. However, it may not be completely safe at a very high dose.

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