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Evaluation of Antioxidant Activities of Terpenoid Fraction of Root Extract of *Physalis angulata*

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ABSTRACT

This study was carried out to investigate the antioxidant properties of terpenoids fraction of *Physalis angulata* in male albino rats. *In vitro* antioxidants activities were carried out using standard biochemical methods while *In vivo* analyses were determined using spectrophotometric methods. Twenty (20) albino rats were randomly divided into four groups of five (5) rats each after one week acclimatization. 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. All the rats were given free access to water and commercial poultry feed *ad libtum*. The study lasted for 30 days after which, blood samples were collected from all the rats through ocular puncture and then used for the analyses. Within the concentration 20-100 µg/ml, the terpenoid fraction produced non-significant ($p>0.05$) increase in nitric oxide generation. The result of the effect of the terpenoids on percentage 2,2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity indicated a non-significant ($p>0.05$) variation as the concentration of the terpenoids increased from 50 to 1000 µg/ml. Similarly, terpenoid fraction caused non-significant ($p>0.05$) increase in ferric ion assessed reducing power. In the phosphomolybdate assay, the *in vitro* antioxidant activity of terpenoid fraction increased with the dose (20-100 µg/ml). In addition, significant ($p<0.05$) increases in the activities of antioxidant enzymes, (CAT and GPx) were observed while there was a non-significant ($p>0.05$) increase in superoxide dismutase (SOD) and MDA in the groups that received the graded doses of the terpenoids and vitamin C (standard drug) compared to the normal control. In conclusion, the study has shown that *Physalis angulata* terpenoid fraction is safe and has antioxidant activities.

Keywords: *In vitro, In vivo,* Antioxidants and *Physalis angulata*

INTRODUCTION

Plant medicine has been utilized successfully for thousands of years and is of great value in the field of treatment and cure of disease. In recent times, demand for herbal drugs is increasing throughout the world. As per the World Health Organization (WHO), 80% of the population of developing countries relies on traditional medicines for their primary health care needs. It is necessary to investigate the rationality of folklore use of medicinal plants for various ailments in modern scientific method in spite of sound traditional back ground and information available in the ancient literature [1], [2].

Free radicals are molecules containing unpaired electrons are highly reactive and react with other molecules by taking or giving electrons, involved in wide variety of pathological effects such as DNA damage, carcinogenesis and various

degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases [3]. There is a dynamic balance in the amount of free radicals generated in the body and antioxidant to quench them and protect the body against the harmful effects. It necessitates maintaining balancing of antioxidants in the body. Many plant extracts and plant products known to have significant antioxidant activity to scavenge free radicals [4].

Physalis angulata L. is an edible and medicinal species of Solanaceae family. It is native of the American continent [5] but, at present, it grows as an introduced plant in several Asian and African countries [6]. Currently, *P. angulata* is a wild species, for which recent plantations have been established in Mexico [7] and Brazil [8]. The species is considered as an alternative crop for its edible fruits and for its diversity of secondary metabolites, which have an important potential in the bio-products industry [9]. In Mesoamerica, this species is used in traditional medicine since pre-Columbian times [10], and presently, forms part of the Chinese traditional medicine, recognizing for it an important capacity as inhibitor of tumor metastasis and angiogenesis [11], and antinociceptive properties [12], among others. Different medicinal properties have been associated with different parts of *P. angulata*. For instance, antinociceptive properties were first recognized for roots [13] and more recently for stems [14], whereas cytotoxicity against murine leukemia cell line P-388, epidermoid carcinoma of the nasopharynx KB-16 cells, and lung adenocarci-noma A-549 has been determined for leaves [15]. For this reasons, evaluating the antioxidant potentials of terpenoid fraction of root extract of *Physalis angulata* in male albino rats was initiated. This was therefore aimed at evaluating the antioxidant activities 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 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.

Determination of 2,2 diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity

The method described by [16], was used. The stable DPPH radical was used for determination of free radical scavenging activity of test samples.

Determination of Ferric ion (Fe^{3+}) Reducing Power

The reducing power of the extract was determined by the slight modification of the method of [17]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Determination of Total Antioxidant Activity using Phosphomolybdate Assay

The total antioxidant capacity of the terpenoid fraction was determined by phosphomolybdate method using butylated hydroxytoluene (BHT) as the standard compound as described by [18]. The total antioxidant capacity was expressed as µg equivalents of butylated hydroxytoluene (BHT) by using the standard BHT graph.

Experimental design for Chronic Toxicity 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 libtum*. 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; Group5: 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.

Determination of Malondialdehyde Concentration

The malondialdehyde content, a measure of lipid peroxidation was assayed in form of thiobarbituric acid-reactive substances (TBA) by the method of [19].

Determination of Reduced Glutathione Activity

The reduced glutathione content was determined according to the method described by [20].

Assay of Catalase Activity

Catalase activity was assayed by the method of [21], where-in the breakdown of H_2O_2 was measured at 230 nm.

Assay of Superoxide Dismutase Activity
Superoxide dismutase was assayed according to the method of [22].

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 Fraction on *In Vitro* Antioxidant Activities of *Physalis angulata*

The evaluation of effect of the terpenoids on *in-vitro* nitric oxide generation indicated a non-significant ($p>0.05$) increase as the concentration of the terpenoids increased from 20 µg/ml to 1000 µg/ml as shown in Figure 1.

The result of effect of the terpenoids on percentage 2,2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity indicated a non-significant ($p>0.05$) as the concentration of the terpenoids increased from 50 µg/ml to 1000 µg/ml as presented in Figure 2. Also, there was a non significant ($p>0.05$) increase in percentage 2,2 diphenyl-1-picrylhydrazyl (DPPH) scavenging activity as the concentration of terpenoids increased from 100 to 500 µg/ml.The results of effect of terpenoids fractions on ferric Ion reducing power are presented in Figure 3. The evaluation of effect of various concentrations of the terpenoids on ferric ion as an indication of reducing power showed a non-significant ($p>0.05$) increase as the concentrations of the terpenoids increased from 20 to 1000 µg/ml. In addition, a significant ($p<0.05$) increase was observed as the concentrations of ascorbic acid increased from 20 to 1000 µg/ml.

The result of effects of the terpenoids on *in vitro* antioxidant activity (AOA) using phosphomolybdate assay indicated that there was dose dependent increase in the in vitro antioxidant activity as the concentrations of the terpenoids increased from 20 to 1000 µg/ml as shown in Table 1.

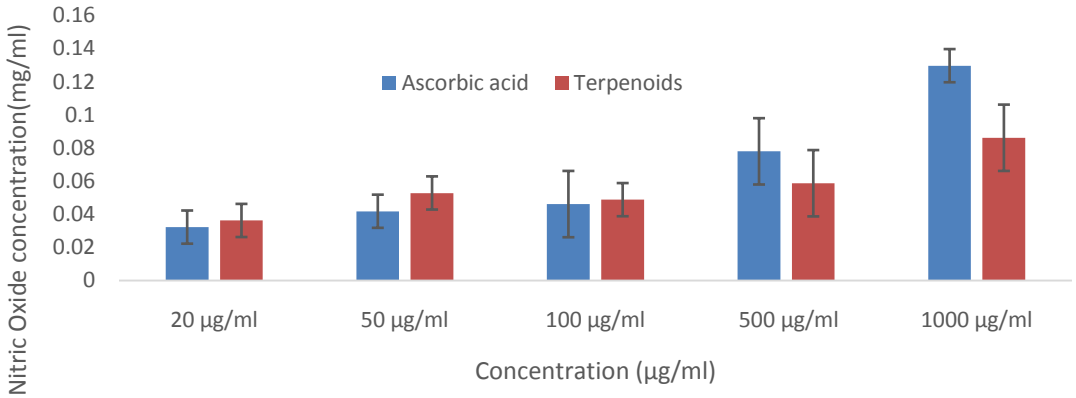

Figure 1: Effect of Terpenoids Fraction on *In vitro* nitric oxide

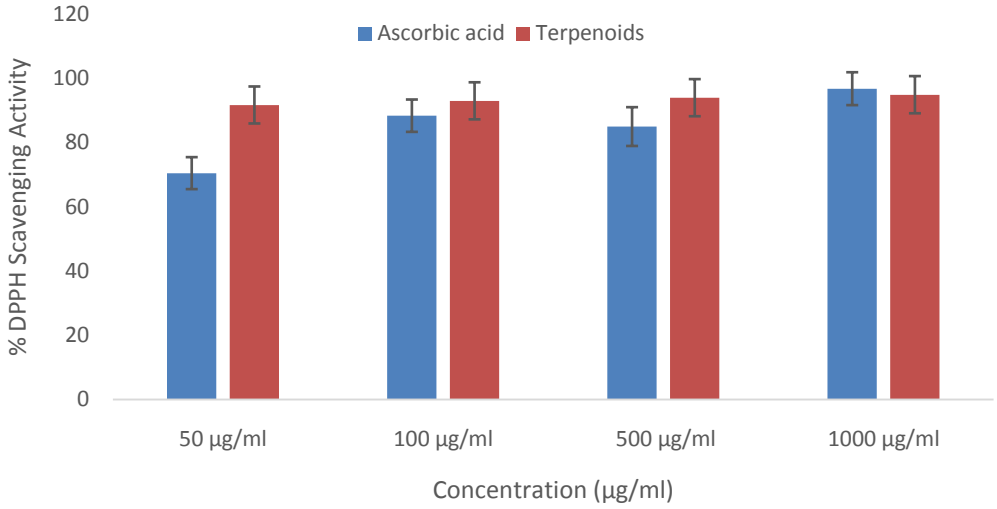

Figure 2: Effect of terpenoids on 2,2 diphenyl-1-picrylhydrazyl (DPPH)

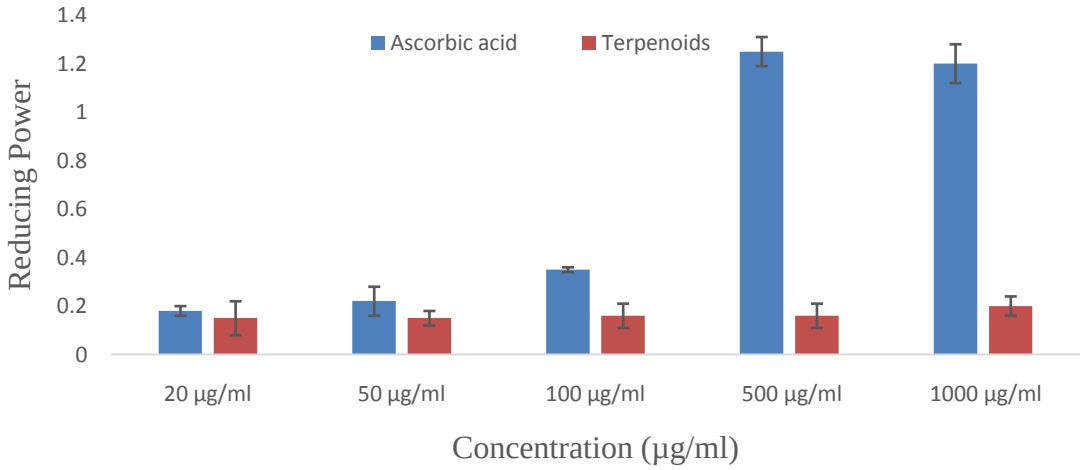

Figure 3: Effect of terpenoids fraction of *Physalis angulata* on ferric ion reducing power

Table 1: Effect of Terpenoids on In-Vitro Antioxidant Activity (AOA) (Phosphomolybdate Assay)

S/N	Concentration of Terpenoids	Antioxidant Activity (AOA)
1	1000	755.67±109.43
2	500	753.54±24.01
3	100	688.12±60.21
4	50	772.00±35.92
5	20	644.88±278.92

Values are expressed as mean±SD. n=3.

Effect of Terpenoids Fraction of *Physalis angulata* on Antioxidant Enzymes

The results of effects of terpenoids on the activities of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase are presented in Table 2. The activities of the serum antioxidant enzymes varied after the administration of the graded doses of the terpenoids and vitamin C. The results showed that there was a significant ($p<0.05$) increase in the serum levels of two of antioxidant enzymes, (CAT and GR) while there was non-significant ($p>0.05$) increase in superoxide dismutase (SOD) when the groups that received the graded doses of the terpenoids and vitamin C (standard drug) were compared with the normal control.

Table 2: Effect of Terpenoids Fraction on Antioxidant Enzymes

S/N	Groups	Catalase (µmoles/mg protein)	Glutathione Reductase (µg/mg protein)	Superoxide Dismutase (µg/mg protein)
1	Normal Control	1.17±0.20^{a}	10.13±3.61^{a}	11.05±0.12^{a}
2	Positive Control	2.43±0.26^{c}	29.13±6.70^{c}	11.29±0.55^{a}
3	25 mg/Kg b.w Terpenoid	1.93±0.21^{b}	23.71±1.72^{c}	11.19±0.06^{a}
4	50 mg/Kg b.w Terpenoid	2.39±0.16^{c}	24.94±2.21^{c}	11.26±0.10^{a}
5	100 mg/Kg b.w Terpenoid	1.22±0.13^{a}	15.79±3.69^{b}	11.21±0.12^{a}

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

DISCUSSION

The result of evaluation of *In vitro* nitric oxide scavenging activity of terpenoid fraction of *Physalis angulata* as shown in Figure 1 revealed that the terpenoids possess *In vitro* nitric oxide scavenging activity. The terpenoids showed a dose dependent elevation of nitric oxide scavenging activity up to 1000 μg/ml (0.09) though lower than that of 1000 μg/ml (0.13) of vitamin C (positive control). Nitric oxide is produced by several different types of cells such as endothelial cells and macrophages. According to [23], [24], although the early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels, the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage.

Phosphomolybdenum assay has been routinely used to evaluate the antioxidant capacity of extracts [25]. The assay is simple and independent of other antioxidant measurements commonly employed. However, the antioxidant capacities of terpenoids extracted from *Physalis angulata* were also evaluated using phosphomolybdenum assay by monitoring their formation of green phosphomolybdenum complex. The formation of the complex was measured by the intensity of absorbance in the terpenoids at various concentration (1000, 500, 100, 50 and 20 μg/ml) as shown in Table 1. According to [26], the phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of green phosphate/Mo(V) complex with the maximal absorption at 695 nm. The assay revealed that the 1000 μg/ml (755.67±109.43) showed the highest phosphomolybdenum reduction. This could be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants [27].

[28], reported that DPPH is a stable radical with a maximum absorption at 517 nm which can easily undergo scavenging by antioxidant. It has been widely used to evaluate the ability of compounds as free-radical scavengers or hydrogen donors and to determine the antioxidative activity of plant extracts and foods [29], [30]. As shown in Figure 2, the scavenging abilities of terpenoids were concentration-dependent. Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation [27].

A number of previous studies have reported that the reducing power could serve as a significant indicator of potential antioxidant activity. According to [28], antioxidants could be referred to as reductants, and inactivators of oxidants. Antioxidative activity has been proposed to be related to reducing power. Thus, the antioxidant potential of terpenoids fraction of *Physalis angulata* was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) as shown in Figure 6. Free radical reducing power assay was used by several authors for the assessment of antioxidant activity of various food product samples [29], [30]. Also, [11] suggested that most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay.

The *In vivo* antioxidant effect of the terpenoids was evaluated by measuring the serum levels of catalase, glutathione peroxidise and superoxide dismutase. Catalase is one of the enzymatic antioxidants which is known to be present in the peroxisome of aerobic cells. Catalase has been reported to be very effective in facilitating the conversion of hydrogen peroxide to water and

molecular oxygen. Glutathione reductase is a seleno-enzyme known for its ability to eliminate peroxides as potential substrates for the Fenton reaction. In the other hand, superoxide dismutase has been reported as one of the most effective intracellular enzymatic antioxidants due to its ability to facilitate the conversion of superoxide anions to dioxygen and hydrogen peroxide.

There was a significant ($p < 0.05$) increase in the concentration of antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) in the groups that received the graded doses of the terpenoids and vitamin C (positive control) compared to the normal control. However, there was a dose dependent significant ($p<0.05$) increase in the serum levels of the antioxidant enzymes from the group that received 25 mg/Kg b.w to 50 mg/Kg b.w terpenoids while a decrease in antioxidant enzymes was observed in the group that received 100 mg/Kg b.w terpenoids. This could be attributed to a possibility of modulation of the effects of the terpenoids resulting in a possible toxicity of the terpenoids at a higher dose. This suggests the ability of terpenoids isolated from *Physalis angulata* to protect against oxidative stress resulting from the activities of free radicals at a dose dependent manner.

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