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# Antioxidant Property and Oral Glucose Tolerance of Stem Extract of *Andrographis paniculata* – Nees

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author EOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AOO and TTA managed the analyses of the study. Author EOA managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

This research aims to investigate the antioxidant property and oral glucose tolerance of stem extract of *Andrographis paniculata* using standard methods. The antioxidant activity of the extracts were assessed using total phenol, total flavonoid, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), nitric oxide, GSH (gluthatione), super oxide, DPPH [1,1-di-phenyl-2-picryl hydrazyl] and ascorbic acid. The oral glucose tolerance of methanol stem extract of *A. paniculata* was carried out using wistar albino rats. The Wistar albino rats were divided into six groups consisting of five rats each. Group 1 was given water only, group two to group five were induced with 2g/kg of glucose. After 30 minutes, group 3 and 4 were treated with the doses of 15 mg/kg and 30 mg/kg of the extract while group 5 was treated with glibenclamide respectively. The blood glucose level of each group was monitored for 2hours at 30 minutes interval and compared with the control that was given water only. The result shows that the extracts were able to scavenge free radicals but methanol

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stem extract of *A. paniculata* performed better in scavenging free radicals as compared to others. The blood sugar levels of the rats treated with doses of 15 and 30 mg/kg of methanol stem extract of *A. paniculata* were significantly lower than the group of rats that were fed with only glucose and the group of rats treated with glibenclamide. The glucose-lowering efficiency of the extract was between 60-120 minutes. Findings from this present study revealed that *A. paniculata* is a potential plant that can scavenge free radicals and also possess hypoglycemic activity.

*Keywords: Antioxidant; free radicals; glucose tolerance; hypoglycemic.*

## 1. INTRODUCTION

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals [1]. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts [2]. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. If these free radicals are not removed, they will be released into the cell and lead to degenerative diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration [3].

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favour of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes [4]. Diabetes mellitus is recognized as one of the leading causes of morbidity and mortality in the world. About 2.5-3% of the world's population suffers from this disease, a proportion which in some countries can reach 7% or more. According to W.H.O projections, the prevalence of diabetes is likely to increase by 35%, currently, there are over 150 million diabetic people worldwide and this is likely to increase to 300 million or more by the year 2025 [4]. Moreover, the World Health Organization (WHO) estimates that 80% of people in developing countries depend on traditional medicine for their health needs, and 85% of traditional medicine involves the use of plant extracts. In other words, about 4 billion people in the world rely on the plants as source of drugs. This has led researchers to continue their search for the "miracle drug" for the treatment of diabetes from plants. Medicinal plants are considered useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. The plant extracts have been developed and proposed for use as antimicrobial substances. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need.

*Andrographis paniculata* belonging to the family Acanthaceace is commonly known as King of Bitters. It is distributed in tropical Asian countries, often in isolated patches Tanwer and Vijayvergia [5]. Native population of plants are spread throughout South India and Sri Lanka which perhaps represent the centre of origin and diversity of the species. *Andrographis paniculata* has been reported as having antibacterial, antifungal, antiviral, anticancer and hypoglycemic effects Tanwer and Vijayvergia [5].

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Identification

*Andrographis paniculata* (Awere) was collected from the forest in Owo, Ondo State and authenticated at the herbarium section of the Department of Botany, Obafemi Awolowo University, Osun State, Nigeria.

#### 2.1.1 Preparation of plant samples

The stem back of *Andrographis paniculata* was separated from the leaf, and likewise the fruit of *Morinda lucida*. They were air dried for about three weeks and ground with a blender to obtain fine powder after which they were stored in an air tight container prior to use as described by Nweze et al., [6].

## 2.2 Experimental Animals

Healthy wistar Albino rats of either sex were purchased, their weight was recorded, separated into groups and kept in well-ventilated cages at room temperature (28-30°C) under controlled light.

## 2.3 Feeding of Experimental Animals

The animals were fed with standard pellet diet for a period of seven days to acclimatize them to the environment prior to the commencement of the experiment while water was given at libitum. For experimental purpose, the animals were kept fasting overnight but allowed for access to water.

### 2.3.1 Free radical scavenging activity by DPPH assay

The free radical scavenging activities of the samples by DPPH method were determined according to the method reported by Brand-Williams et al. [7]. In this method, 2.4 mg of DPPH free radical was dissolved in 100 mL of methanol to prepare its stock solution, which was kept at 20°C until required. The working solution of DPPH was obtained by diluting its stock solution with methanol till the absorbance was noted to be 0.980 ± 0.02 at 517 nm. Then, 3 mL of the working solution was mixed with 100 µL of a sample (1 mg/mL). After incubating the mixture in the dark for 30 min, absorbance was measured at 517 nm. The scavenging activity was calculated by using the formula:

% inhibition = [(Absorbance of blank - Absorbance of sample)/Absorbance of blank] × 100

% inhibition = 100-(ABS)*100

### 2.3.2 Nitric oxide scavenging activity

The reaction was initiated by adding 2.0 ml of sodium nitroprusside, 0.5 ml of PBS, 0.5 ml of leaf extracts (50mg) and incubated at 25°C for 30 minutes. Griess reagent (0.5 ml) was added and incubated for another 30 minutes. Control tubes were prepared without the extracts. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer

NO radical scavenging activity = {(Abs control – Abs sample) / (Abscontrol)} × 100

Where; Abs control is the absorbance of NO radical + methanol; Abs sample is the absorbance of NO radical + sample extract or standard [7].

### 2.3.3 ABTS scavenging effects

The antioxidant effect of the leaf extracts was studied using ABTS (2,2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay

**Procedure:** ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use.

Aliquots (0.5ml) of the extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the per cent inhibition was calculated using the formula [7].

Inhibition (%) = (Control – test) × 100 / Control

### 2.3.4 Superoxide radical scavenging activity

To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of the extracts, the compound and standard in dimethyl sulphoxide (DMSO), 1 ml of alkaline DMSO (1 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm [7].

Inhibition (%) = (Control – test) × 100 / Control

### 2.3.5 Estimation of ascorbic acid

Ascorbic acid was analysed by the spectrophotometric method.

Standard ascorbate ranging between 0.2-1.0 ml and 0.5 ml and 1.0 ml of the supernatant was taken. The volume was made up to 2.0 ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours resulting in the formation of osazone crystals. The crystals were dissolved in 2.5 ml of 85% sulphuric acid, in cold. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. The tubes were cooled in ice and the absorbance was read at 540 nm in a spectrophotometer. The

concentration of ascorbate in the samples were calculated and expressed in terms of mg/g of sample [7].

### 2.3.6 Total flavonoid content

The total flavonoid contents in the samples were determined following the method reported by Brand-Williams et al., [7]. In each experiment, 1.5 mg of the extracts were dissolved in 5 mL of methanol, from which 300 µL were transferred into 3.4 mL of 30% aqueous methanol. To this mixture, 150 µL each of 0.5 M $NaNO_2$ and 0.3 M $AlCl_3.6H_2O$ were added and mixed thoroughly. After the interval of 5 min, 1 mL of 1 M NaOH was also added. The absorbance was observed immediately at 506 nm against a blank

### 2.3.7 Total phenolic content

The total phenolic content in the crude extracts was determined according to a well-cited protocol Brand-Williams et al., [7]. Briefly, 1.5 mg of each extract was dissolved in 5 mL of methanol from which 40 µL was taken and dissolved in 3.16 mL of distilled water. To this 200 µL of Folin-Ciocalteu reagent was mixed and, after an interval of 8 min, 20% of 600 µL sodium carbonate solution was added. The mixture was incubated at 40°C for 30 min and absorbance was measured at 765 nm on UV/Visible spectrophotometer. The standard calibration curve was prepared with gallic or tannic acid standard solution (50 to 500 mg/L) following the same procedure.

## 2.4 Oral Glucose Tolerance Test (OGTT) in Normal Rats

Normal rats were tested for the Oral Glucose Tolerance Test (OGTT) of the various concentrations of *Andrographis paniculata* extract and standard drug glibenclamide. Albino Wistar rats of either sex weighing 250-300 gm were divided into 5 groups consisting of 5 rats in each group. In this study, glucose solution (2 g/kg) was administered orally and blood glucose was monitored at 0, 30, 90 and 120 min after glucose load. The blood glucose level was estimated by using Accucheck glucometer [8].

### 2.4.1 Grouping of the rats

**Group I -** Normal control received 0.9% saline
**Group II -** received oral glucose only
**Group III -** received oral glucose + Methanol extract of *Andrographis paniculata* leaf (30 mg/kg)
**Group IV -** received oral glucose + Methanol extract of *Andrographis paniculata* leaf (15 mg/kg)
**Group V -** received oral glucose + Standard drug glibenclamide

## 3. RESULTS AND DISCUSSION

From this study, extract of stem of *A. paniculata* exhibited the highest total phenol, total flavonoid, ABTS, nitric oxide, GSH, super oxide, DPPH [1,1-di-phenyl-2-picryl hydrazyl] and ascorbic acid. Methanol extract of *A. paniculata* demonstrated higher scavenging activity as compared to others and this may be due to the fact that the bioactive components responsible for the scavenging activities are present in high quantity in the methanol extract of *A. paniculata.* It has been documented that plant rich in secondary metabolites, including phenolics, flavonoids and carotenoids have antioxidant activity due to their redox properties and chemical structures [9]. A report by Rice-Evans et al., [10] showed that phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox potential of phenolic compounds played an important role in determining the antioxidant potential. Praveen and Awang [9], has conducted a study over 56 different types of plants which showed that methanol extract possessed a higher antioxidant activity compare to non- polar extract.

The blood glucose concentration of control and different doses of *Andrographis paniculata* (15 and 30mg/kg) extract was estimated at 0,30,60,90 and 120 minutes respectively as shown in Fig. 1. Rats induced with glucose caused an increase in blood glucose level and treatment with extract (15mg/kg and 30mg/kg) reduced the blood glucose level significantly as compared with induced. The optimum glucose level was obtained at 1h. The reduction in blood group by the extract could be the result of its hypoglycemic activity [11]. At 30mg/kg, the extract was most effective. Thus the extract enhanced glucose utilization and improves tolerance in glucose loaded rats. This could probably due to the presence of potent phytochemicals like flavonoids, steroids, tannins, Saponin and phenol. Several authors reported that flavonoids, steroids, terpenoids, and phenolic acids are known to be bioactive principle (Issac, 2013) This result suggests that the methanol of *A. paniculata* will be useful in the lowering of blood glucose level.

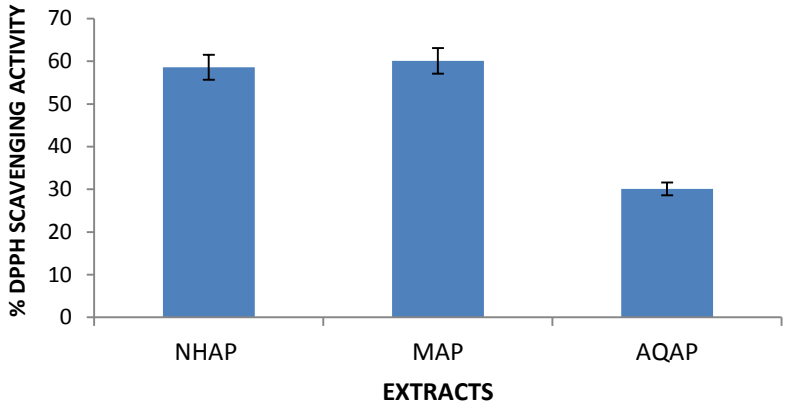


**Fig. 1. Percentage DPPH (2, 2 diphenyl-1-picryl hydrazyl) scavenging activity**
*Keys: NHAP- n-hexane extract of A. paniculata, MAP- methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

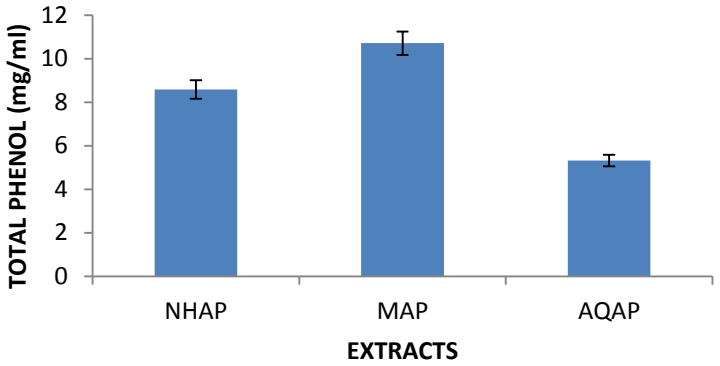


**Fig. 2. Total phenol in different extracts of *A. paniculata***
*Keys: NHAP- n-hexane extract of A. paniculata, MAP- methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

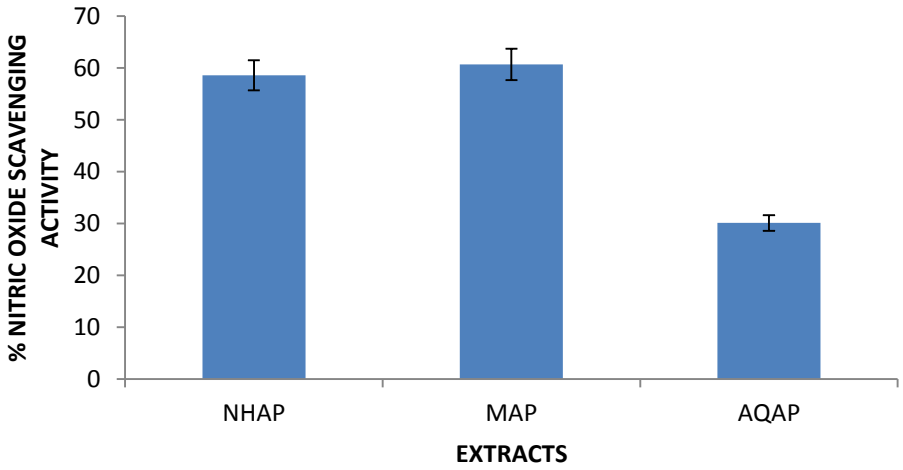


**Fig. 3. Percentage scavenging activity of nitric oxide in *A. paniculata***
*Keys: NHAP- n-hexane extract of A. paniculata, MAP - methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

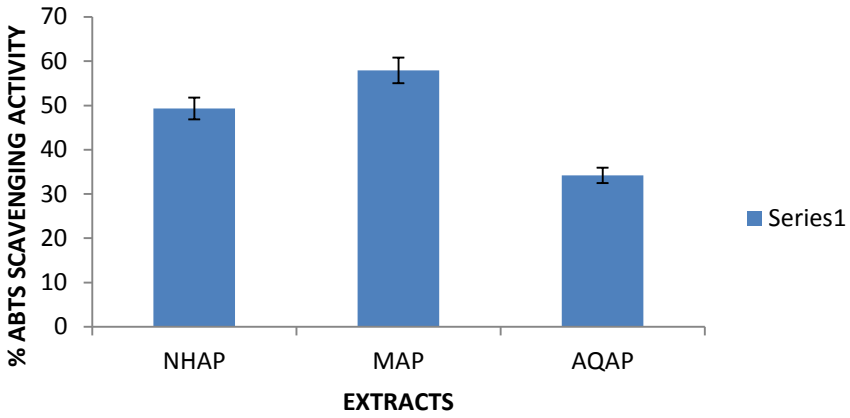


**Fig. 4. Percentage scavenging activity of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) of different extracts of *A. paniculata***

*Keys: NHAP- n-hexane extract of A. paniculata, MAP - methanol extract of A. paniculata, AQAP - aqueous extract of A. paniculata*

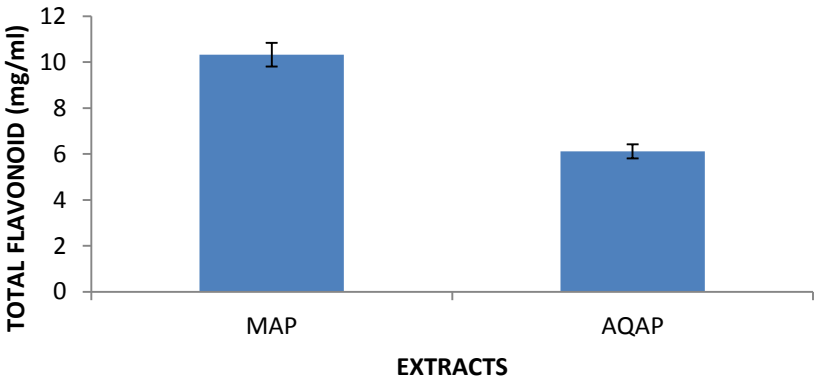


**Fig. 5. Total flavonoid in *A. paniculata***

*Keys: NHAP- n-hexane extract of A. paniculata, MAP- methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

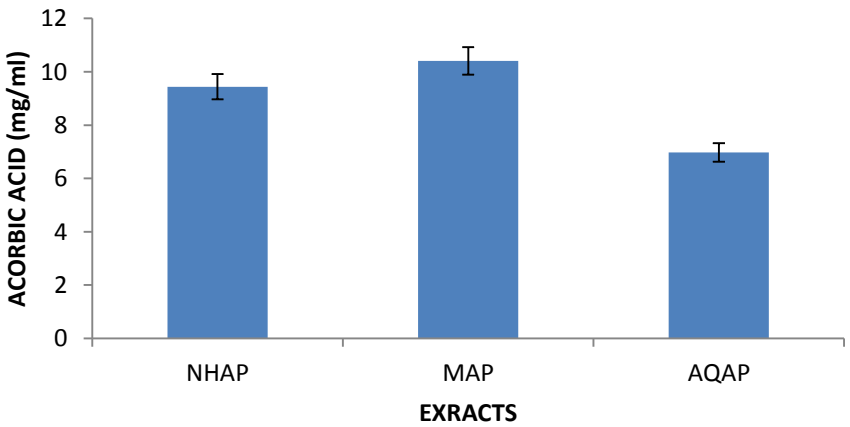


**Fig. 6. Ascorbic acid in *A. paniculata***

*Keys: NHAP- n-hexane extract of A. paniculata, MAP- methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

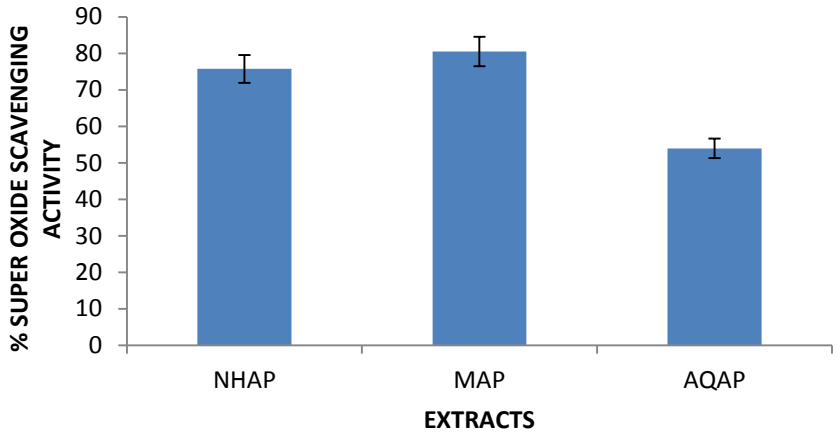


**Fig. 7. Percentage scavenging activity of super oxide in *A. paniculata***
*Keys: NHAP- n-hexane extract of A. paniculata, MAP- methanol extract of A. paniculata, AQAP- aqueous extract of A. paniculata*

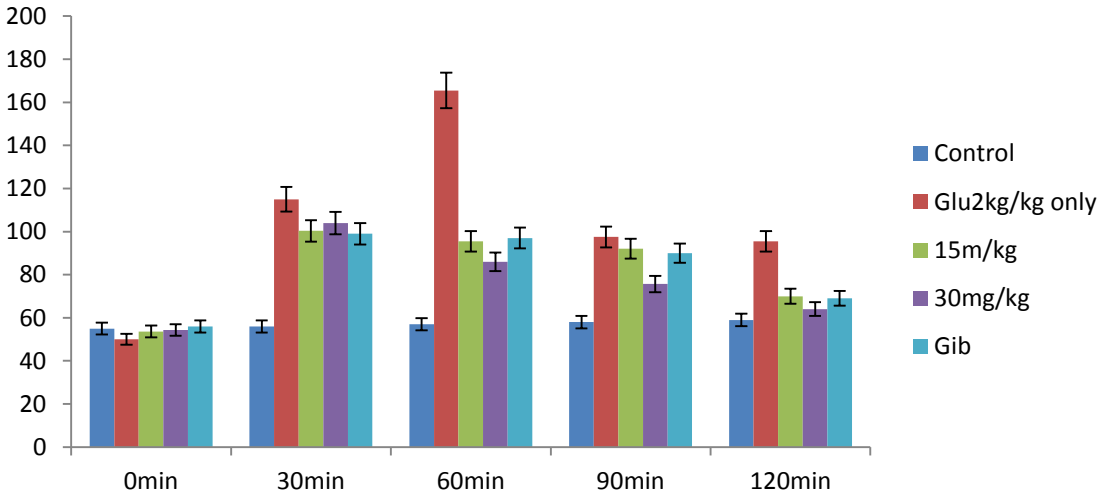


**Fig. 8. Effect of methanol stem extract of *A. paniculata* on glucose induced rats**

## 4. CONCLUSION

The present study revealed that the methanol stem extract of *A. paniculata* is a potential source of antioxidant and also possess hypoglycemic activity. Thus could be useful in the treatment of degenerative diseases as a result of free radicals that is being released into the blood stream when the body natural antioxidant defense mechanism is lowered. More also, the result also suggested that the extract can be used to reduce the blood glucose of the body.

## ETHICAL APPROVAL

The study was approved by animal ethics committee and preserved by the author.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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