EFFECTS OF METHANOL EXTRACT OF AMARANTHUS SPINOSUS LEAF ON SOME SELECTED KIDNEY AND HAEMATOLOGICAL PARAMETERS IN RATS

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ABSTRACT

Amaranthus spinosus is one of the edible vegetable leaf cultivated in Africa for human and animal nutrition. The methanol leaf extract of Amaranthus spinosus gave a percentage yield of 3.64%. The phytochemical test carried out showed that the methanol extract of Amaranthus spinosus contains a high amount of alkaloids, which show that they can take part in nitrogen fixation in the body system, and also could be effective in the treatment of malaria. It also contains a high level of flavonoids and carbohydrate that help in energy supply of the body. The haematological test carried out showed that there was a decrease in PCV and an increase in RBC level in the blood and that it has the ability to reverse anaemia and other blood related diseases in the rat. The extract does not show any significant difference in the levels of creatinine and urea when compared to the control (p>0.05) meaning that the kidney can be able to perform its filtration function without being impaired by the level of creatinine and urea in the blood. This preliminary study strongly support the claims that the plant is an important source of blood in the body system.

Keywords: Amaranthus spinosus, Blood, Kidney markers, haematological parameters and anaemia.
INTRODUCTION

Amaranthus has many species and they are used for different economic purposes when processed and dried. The vegetable type is a herbaceous annual with upright growth habit, cultivated for both its seeds which are used as grain and its leaves which are used as a vegetable or green. Both leaves and seeds contain protein of an unusually high quality. The grain is milled for flour or popped like popcorn. The leaves of both the grain and vegetable types may be eaten raw or cooked. Amaranths grown principally for vegetable use have better tasting leaves [1]. In recent research, it was noticed that the species, *Amaranthus spinosus* has many nutritional values in the body, and also little biochemical effect in some vital organs like the kidney, heart, and the liver when eaten in large quantity. But in this research, the biochemistry of the action of the methanol leaf extract of *Amaranthus spinosus* in the kidney of an albino rat was properly analyzed.

According to [2], about 80% of the world’s populations depend mainly on traditional medicine and the use of plant extracts are mainly involved in the traditional treatment [3]. Medicinal plants constitute the major component of the traditional medicine practised worldwide due to the economic viability, accessibility and ancestral experience [4]. Therefore, the search for safe and more effective agents from plants origin has continued to be an important area of active research [5].

*Amaranthus spinosus* are used as anti-inflammatory, anti-malarial, antibacterial, antimicrobial, antidiuretic, antiviral and in hepatic disorders [6]. Water extract of plant showed significant immuno-stimulating activity and stem extract showed antimalarial activities. *A. spinosus* have several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, betalains, b-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids. The betalains in stem bark of *Amaranthus spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin and kaempferol glycosides [7]. It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside, betaine such as glycinebetaine and trigonelline. Betalains are well known for their antioxidant, anticancer, antiviral and antiparasitosis properties. Many betalain containing species are used as popular medicinal plants to treat various kinds of ailments such as hepatic disorders, malaria, jaundice and scanty urine or to cure wounds [8].
Overview of *Amaranthus spinosus*

*Amaranthus spinosus* originates probably from lowland tropical South and Central America and was introduced into other warmer parts of the world from about 1700 AD onwards. At present it occurs in all tropical and subtropical regions, including tropical Africa, often gregariously and as a weed. It is sometimes collected for home consumption as a cooked, steamed or fried vegetable, especially during periods of drought. The leaves are occasionally found for sale on the market [1].

In Uganda and Kenya, it demands a lower price, for example, because of its spines and because it is not much liked. Its use is declining, and it is acquiring the status of a famine food. It has a bitter taste and is usually eaten in small quantities as a substitute when no other vegetables are available. *Amaranthus spinosus* is also used as forage and said to increase the yield of milk in cattle. However, the spines can cause injury to the mouths of grazing animals and cases of poisoning in cattle have also been reported.

In Uganda, the ash of burnt *Amaranthus spinosus* plants is used as a tenderizer in cooking tough vegetables such as cowpea leaves and pigeon peas. The ash is also used as a vegetable salt and in southern Africa it is used as a snuff, alone or with tobacco [9].

*Amaranthus spinosus* has numerous medicinal uses. The root is known as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea and is also applied as an emmenagogue and antipyretic. In many countries, including those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of eczema, burns, wounds, boils, earache and haemsap is used as an eye wash to treat ophthalmia and convulsions in children [10].

In Malaysia, *Amaranthus spinosus* is used as an expectorant to relieve breathing in acute bronchitis. In mainland South-East Asia, it is also used as a sudorific, febrifuge, antidote to snake poison, galactagogue, and to treat menorrhagia. Some tribes in India apply *Amaranthus spinosus* to induce abortion [1].

Nonetheless, the outcomes of well controlled and well documented clinical studies are still clearly of great value. In many culture throughout the tropic, differentiation between food and medicinal uses of plant eg, bark, leave, nut, seed, tubers, root, flower, is very difficult since
plant uses both categories and is deeply ingrained in the traditions and the fabric of community [11].

**Taxonomic classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
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<tbody>
<tr>
<td>Order</td>
<td>Caryophyllales</td>
</tr>
<tr>
<td>Family</td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Amaranthus</td>
</tr>
<tr>
<td>Species</td>
<td>A. Spinosus</td>
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</table>

**AIMS AND OBJECTIVES**

**AIMS**

This study was aimed at assessing the biochemical and the haematological effect of the methanol extract of the *Amaranthus spinosus* in the kidney of an adult wistar rat.

**OBJECTIVES**

- To determine the effect of methanol extract of *Amaranthus spinosus* in the blood of a wistar albino rats.
- To determine the biochemical effect of the methanol extract of *Amaranthus spinosus* on the kidney markers of wistar albino rats.

**METERIALS AND METHODS**

**Materials**

**Plant**: Fresh leaves of *Amaranthus spinosus* were harvested from the garden in Orba village, Nsukka, Enugu State of Nigeria and was properly washed and dried for research uses.

**ANIMALS**

Sixteen (16) adult albino rats of both sex weighing between 117 – 200 g were purchased and housed in the animal house at University of Nigeria, Nsukka. The animals were acclimatized for one week with adequate feed and clean water regularly given to them before the commencement of the research.

**CHEMICALS/REAGENTS**

All chemicals used in this study were of analytical grade.

| CHEMICALS | MANUFACTURER |
Methanol
Distilled water
Sodium chloride
urea reagents
creatinine reagent
Fehling’s solution A and B
Ferric chloride
Ethyl acetate
Picric acid solution

BDH
STC, UNN
BDH England
Randox
Randox
Teco USA
Merck Darmstadt Germany
BDH England
Lab tech chemicals

INSTRUMENTS/EQUIPMENT

Weighing balance
Volumetric flasks
Refrigerator
Oral Intubation Tube
Spatula
Test tubes
Measuring cylinder
Filter paper
Syringes
Centrifuge
Micropipette
PCV tubes
PCV reader
Pasteur pippete
Test tubes
Colorimeter
Syringes
Measuring Cylinder
Beaker
Micropipettes
Metler HAS
pyrex
Haier Thermocool
Pyrex
pyrex
Watchman no 1
Lifescan
Pac Pacific Cages
Perfect
Pyrex, England
Pyrex, England
Pyrex, England
Pyrex, England
Pyrex, England
EI Scientific Co. India
Mono-ject China
Pyrex, England
Pyrex, England
Perfeet USA

Phytochemical analysis

Materials: Test tubes, water bath, separating funnel, filter paper, cotton wool, litmus paper.
Reagents: Naphthol in ethanol (Molish reagent), concentrated and dilute sulphuric acid, ethanol, mayer’s reagent (potassium mercuric iodide solution), Dragendorff’s reagent (bismuth potassium iodide solution), picric acid solution, 20% potassium hydroxide solution, lead acetate solution, dilute ammonia solution, fehlings solution, ethylacetate, 20% ammonium chloride solution, chloroform, olive oil and million reagent.

METHODOLOGY
Preparation of Plant Extracts: The fresh leaves of *Amaranthus spinosus* were air dried under atmospheric temperature for over two weeks and was ground into powder with mortar and pestle. 500g of the pulverized leaves were subjected to cold maceration in 1400ml methanol for 48h. The filtrate was concentrated in a water bath for solvent elimination; the residue was reconstituted in distilled water and stored in a refrigerator until when needed.

ESTIMATION OF PERCENTAGE YIELD OF EXTRACT
After evaporating the solvent (methanol), the extract was obtained in the slurry form. The extract was weighed with an electronic weighing balance and the weight recorded. The percentage yield was calculated as shown below:

\[
\text{Percentage yield} = \frac{\text{weight (g) of the extract}}{\text{Weight (g) of pulverised leaves}} \times 100
\]

The phytochemical analysis of the plant extract was carried out using [11] methods.

Haematological parameters of packed cell volume (PCV) and red blood cell (RBC) were determined using [12] methods.

The concentration of serum urea was determined using the method of [16] as outlined in Randox kits, UK.

The concentration of serum creatinine was determined using the method of [16] as outlined in Randox kits, UK.

EXPERIMENTAL DESIGN
Sixteen Wistar albino rats were randomly divided into four (4) different groups as follows:

**Group 1**: Normal control rats and were given 0.5ml of normal saline orally.

**Group 2**: Received 100 mg/kg body weight of *Amaranthus spinosus* extract orally.

**Group 3**: Received 200mg/kg body weight of the *Amaranthus spinosus* extract orally.

**Group 4**: Received 400 mg/kg body weight of the *Amaranthus spinosus* extract orally.
RESULTS

Percentage Yield of Extract

\[
\text{Percentage yield} = \frac{12.3}{337.8} \times 100 = 3.64\%
\]

Table 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *AMARANTHUS SPINOSUS*

<table>
<thead>
<tr>
<th>Component</th>
<th>Relative Abundance</th>
</tr>
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<tbody>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+++</td>
</tr>
<tr>
<td>Fats and Oil</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
</tr>
<tr>
<td>Acidic compounds</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key**

- Not detected
+ Minutely present
++ Moderately present
+++ Present in large amount

Effect of *Amaranthus spinosus* extract on the serum creatinine concentration of experimental Rats

In the figure 1 below, after daily administration of *Amaranthus spinosus* extract (100, 200 and 400mg/kg body weights) for two weeks, there was no significant (p>0.05) difference in the serum creatinine concentration of the test groups compared to the control. There was no significant (p>0.05) difference at varying dose of the extract.
Fig. 1: Effect of *Amaranthus spinosus* extract on the serum creatinine concentration of the experimental Rats

**Effect of *Amaranthus spinosus* extract on the serum urea concentration of experimental Rats**

In the fig. 2 below, after daily administration of *Amaranthus spinosus* extract (100, 200 and 400mg/kg body weights) for two weeks, there was no significant (p>0.05) difference in the serum urea concentration of the test groups compared to the control. There was a slight significant (p<0.05) difference at varying dose of the extract.

Fig. 2  **Effect of *Amaranthus spinosus* extract on the serum urea concentration of experimental Rats**

**Effect of *Amaranthus spinosus* extract on the RBC of experimental Rats**

In the fig.3 below, after daily administration of *Amaranthus spinosus* extract (100, 200 and 400mg/kg) for two weeks, there was a significant increase (p<0.05) in the RBC concentration of the test groups compared to the control. There was no significant (p>0.05) difference at varying dose of the extract.
Effect of *Amaranthus spinosus* extract on the PCV of experimental Rats

In the fig. 4 below, after daily administration of *Amaranthus spinosus* extract (100, 200 and 400mg/kg b.w) for two weeks, there was a significant decrease (p<0.05) in the serum PCV concentration of the test groups compared to the control. There was no significant (p>0.05) difference at varying dose of the extract.

**DISCUSSION**

*Amaranthus spinosus* is a medicinal plant that can be grown and consumed in many parts of the world, for the purpose of meeting the human nutritional requirements. Both the leaves and the seed contain protein of an unusually high quality. The leaves of both grain and vegetables may be eaten raw or cooked. It is said to have many nutritional values in the body [1]. Beside the nutritional benefits of the plant, there have been speculations that the plant could
have blood building capacity and some effects in the kidneys, hence the reason for this research.

This study was carried out to determine the biochemical effect of methanol extract of *Amaranthus spinosus* on the kidney parameters and some blood components of rats. The phytochemical test carried out showed that the methanol extract contains a high level of alkaloids. This tells us that it contains nitrogenous compound which can be used to produce compounds like Emetine used as an antiprotozoal Agent [17]

They mainly protect the body of plants and animal against parasites, and also useful in the treatment of malaria. It also showed high level of flavonoids and carbohydrate, which shows that the extract is a highly phenolic compound used in the production of energy in the body system [17].

The evaluation of the biochemical effect of the methanol leaf extract of *Amaranthus spinosus* on the blood parameters showed a significant difference (p<0.05) in the PCV level of the blood. It also showed decreased level of PCV compared to the control. It decreased from 50% to 40%, resulting to low PCV level after 14 days of treatment with the extract. The administration of the leaf extract also caused a significant rise in RBC count from 300 to 350 per litre. This shows that the leaf of *Amaranthus spinosus* was rich in blood supplements in the body. There was no significant difference (p>0.05) in the serum creatinine concentration of the test groups compared to the control. Also in the Urea test, there was no significant difference, (p>0.05) in the serum urea concentration of the test groups compared to control. But in the test groups, there was a little significant difference (p< 0.05) in the doses of 100mg/kg to 400 mg/kg. It decreased from 35 mg/dl to 25 mg/dl. This enables the kidney to determine the amount of urea and creatinine that are been filtered in the bloodstream. In the creatinine chart, increase in the dose of the extract produces a small effect in the kidney [18]. The control even showed high activity than the ones that received the extract. This is to say that there is no harmful effect the leaf of *Amaranthus spinosus* has in the kidneys.

From the earlier pharmacological studies, the leaf extract has been shown to contain high quantities of iron (38.4mg/100g dry weight), and calcium (968.7mg/100g dry weight) [20]. The blood building capacity of the extract could be attributed to high iron and calcium contents. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates . Thus, this experiment showed that the methanol extract of
Amaranthus spinosus can be used to supplement blood and energy to the body system for adequate functioning.

CONCLUSION
In conclusion, this study showed that the methanol leaf extract of Amaranthus spinosus is a potential blood supplement. This is to say that the blood level in human beings can be increased by the intake of the leaf extract of Amaranthus spinosus, thereby making the body to be free from Anaemia or any blood related diseases. The leaf extract cannot lead to any kidney malfunction, so excessive consumption cannot hinder adequate kidney functions. The plant can also be consumed for nutritional purposes.

SUGGESTIONS FOR FURTHER STUDIES
Further studies are however required to establish the safety of the extract.

REFERENCES