Blood Glucose – Lowering Potentials of Annona muricata Leaf Extract in Alloxan–Induced Diabetic Rats

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ABSTRACT

The blood glucose lowering potentials of ethanol leaf extract of Annona muricata were studied. Thirty wistar albino rats were divided into six groups of five rats per group. Group 1 served as “Normal control” animals and received normal rat pellets and water. Diabetes mellitus was induced in Groups 2, 3, 4, and 5 by intraperitoneal injection of alloxan (130 mg/kg). Group 6 rats were administered with 400 mg/kg daily of the extract without induction; group 3 rats were treated with glibenclamide (5 mg/kg body weight), groups 4 and 5 received 200 mg/kg and 400 mg/kg body weight of A. muricata leaf extract daily respectively throughout the duration of the experiment of 14 days. Group 2 rats were induced but not treated with any drug, thus it served as the “Negative control” group. Quantitative phytochemical analysis of the leaf extract was carried out using the Association of Official Analytical Chemists (AOAC) methods. Acute toxicity test of the leaf extract of A. muricata was determined using 12 rats by Lorraine’s toxicity testing method. The blood glucose levels of the animals in each group were determined using Accu-chek test strip method. The weights of the animals were determined using a standard electronic weighing balance. The result of the quantitative phytochemical analysis of the leaf showed that the ethanol leaf extract contains the following: phenols (74 mg/100 g), flavonoids (3.70 mg/100 g), tannins (2.95 mg/100 g), oxalate (6.48 mg/100 g), terpenoid (13.88 mg/100 g), saponins (6800 mg/100 g), alkaloids (570 mg/100 g), cardiac glycoside (1690 mg/100g). Acute toxicity studies showed that LD₅₀ was 3807.89 mg/kg body weight. The results of the average blood glucose levels (mg/dl) of the rats in each group were group 1, 82.6071±7.7524, group 2, 309.3571±163.6923, group 3, 226.7143±132.8182, group 4, 146.5000±140.1465, group 5, 150.4783±81.8340, and group 6, 22.5844±12.5329 for each group respectively. The average body weights of the animals were determined using a standard electronic weighing balance. The results of the average body weights of the animals were group 1, 185.7143±33.6759, group 2, 177.1429±36.6750, group 4, 219.2857±21.2908, group 5, 119.5455±25.5993, and group 6, 191.7857±25.2475. The findings from this study suggest that ethanolic leaf extract of A. muricata has notable effect in lowering blood glucose levels in diabetic rats and is a more potent drug in the treatment and management of diabetes mellitus and oxidative stress-related diseases.

Keywords: Annona muricata, Acute Toxicity testing, Blood glucose-lowering, Quantitative phytochemicals.

I. INTRODUCTION

Over many centuries ago, the use of medicinal plants for the treatment of diseases has proved to be potent and this has led to an increased study of medicinal plants all over the world. In recent years there has been an increase in the study and use of medicinal plants most especially in developing countries where many people rely on traditional medicine for the treatment of different disease conditions and health challenges. Hence, according to the World Health Organization [35], more than 80% of world’s population relies on traditional medicine for their primary health care needs. This renewed interest by scientists especially biochemists and pharmacologists in the use of medicinal plants may be attributed to its availability, cheapness and accessibility by the local populace, high incidence of side effects of synthetic medicines and most especially the environmental friendliness of natural plant extracts to the body [13]. Medicinal plants are considered to be the main source of biologically active compounds that can be used for the treatment of various ailments including cancer, diabetes mellitus, hypertension, and ulcer etc. thus out of the wide array of plant species on earth, only about 10-1% has been studied chemically and pharmacologically for their potential medicinal value especially for chemotherapeutic effects [35].
Diabetes mellitus which is a metabolic disorder characterized by hyperglycemia, glucosuria, hyperlipidemia etc., resulting from a defect in insulin secretion and insulin action or both has been on the rise in the world population. It is estimated that about 177 million people worldwide are diabetic as of the year 2000 and it is expected that the number will increase up to 300 million in the year 2025 [27]. According to Abdullah et al. [1], diabetes mellitus together with cancer are two disease conditions that have bedeviled humanity in the recent past and in most cases have led to the death of over 100 million people worldwide. The increase in the prevalence of diabetes mellitus has in recent years become a public health concern. The reason for this could be attributed to many factors ranging from social and economic cost of the disease and the side effects of synthetic oral blood glucose-lowering agents [29]. To reduce the prevalence rate of diabetes mellitus as have been stated above, many anti-diabetic drugs have been developed from plant and further studies are still being carried out, for a more efficient anti-diabetic drug (blood glucose lowering drug) that can as well reduce the oxidative stress caused by diabetes mellitus and by extension ameliorate the disease conditions caused by excessive free radical accumulation such as cancer, arthritis, atherosclerosis etc. As a result of this, it is therefore pertinent to develop therapeutic drugs using extracts of medicinal plants for the treatment and/or management of diabetes mellitus and by extension other disease conditions resulting from diabetic complications.

Natural products especially those derived from plants have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in medicine has stimulated significant scientific interest in the biological activities of these substances [22]. Despite these studies, a restricted range of plant species has experienced detailed scientific inspection and our knowledge is comparatively insufficient concerning their potential role in nature. In a pharmaceutical landscape, plants with a long history of use in ethnomedicine are a rich source of active phytoconstituents that provide medicinal or health benefits against various ailments and diseases. One of such plants with extensive traditional use is Annona muricata (sour sop) which belongs to the family of Annonaceae and is predominantly found in South-Eastern part of Nigeria and Africa in general, though it has also been reported that the plant is widely cultivated in West Indies, China, Southern and Northern America [9]. Annona muricata commonly known as sour sop (English), ‘Ebo’ in Yoruba, ‘Shawshopu’ in Igbo, ‘Tuwon Biri’ in Hausa, ‘Graviola’ (Portuguese) belong to the family Annonaceae comprising at approximately 130 genera and over 70 species [21] that has been widely studied in the last decade due to their therapeutic potentials.

It is a small, upright, evergreen tree that can grow to about 5-8 meters (13 ft) tall. Its young branches are usually hairy, and its leaves are oblong to oval in shape of about 8 centimeters (3.1 inches) to 16 centimeters (6.3 inches) long and 3 centimeters (1.2 inches) to 7 centimeters (2.8 inches) wide. The leaves are glossy dark green with no hair above, and paler with fine lateral nerves and has a strong pungent odour, the leaf stalks are 4 millimeters (0.16 inches) to 13 millimeters (0.5 inches) long and without hairs. The fruits are usually dark green and prickly. They are ovoid and can be up to 30 centimeters (12 inches) long with a moderately firm texture. Their flesh is juicy, acidic, whitish and aromatic and the plant is highly tolerant to poor soil texture and prefers low land areas between the altitude of 0 meters (0 ft) to 1200 meters (3900 ft) but it cannot stand frost [25].

Annona muricata is a medicinal plant that has been used as a natural remedy for a variety of illnesses. Several studies by different researchers have demonstrated that traditionally as is used by the locals, the bark as well as the leaves are used as anti-hypertensive, vasodilator, anti-spasmodic (smooth muscle relaxant) and cardio-depressant (slowing of heart rate), it has also been observed that sour sop is used as anti-cancer, anti-diabetic, anti-bacterial, anti-fungal, anti-malarial, anti-mutagenic (cellular protector), emetic (induce vomiting), anti-convulsant, sedative, insecticidal and uterine stimulant (helps in child birth), it is also believed to be a digestive stimulant, anti-viral, cardio tonic (tones, balances and strengthen the heart), febrifuge (cures fever), nervousness (balances/calms the nerves), vermifuge (expels worm), pediculocide (kills lice), and as an analgesic (pain reliever) [31]. This plant (Annona muricata) has been used widely for the alleviation and treatment of several ailments traditionally but in some instances, it has been abused due to ignorance of the medicinal properties of this plant, hence the prevalence of diabetes mellitus and its attendant complications have continued to affect the general health conditions of people. Though lots of researches have been carried out to develop a curative drug therapy that will cushion and ameliorate these health challenges, yet the prevalence continues to increase. Therefore, in the light of this, this research is intended to ascertain the blood glucose-lowering efficacy of Annona muricata ethanol extract prepared from the leaves of the plant species grown in the South East of Nigeria.

II. MATERIALS AND METHODS

A. Collection, Identification and Preparation of Leaves

Fresh leaves of Annona muricata were collected from a home garden in Ebikoro, Uratta in Owerri North Local Government Area of Imo State, Nigeria and identified by Mrs BibianAziagha, a taxonomist in the Botany Department of Nnamdi Azikiwe University, Awka. The leaves were separated from the stalk, washed, and air-dried at room temperature. The leaves were constantly weighed using electronic weighing balance (JA-P Metra) until constant weights were observed, after which the dried leaves were milled to fine powder using a mechanical grinder.

B. Animal Procurement and Management

Wistar albino rats (Rattus norvegicus) of both sexes weighing between 150-220 g were obtained from the Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu State and housed in the animal house of the Anatomy Department, Imo State University Owerri under standard conditions of light, temperature, and humidity of 12 h lights and 12 h Dark period at room temperature. The animals were given free access to standard commercial rat pellets and drinking tap water ad libitum and were kept for 7 days to acclimatize. Acute toxicity test of the leaf extract was also
determined using modified Locke’s toxicity testing method as reported by Aroma and Enegide [6].

The rats were divided into 6 groups of 5 rats each. 3 groups served as control groups, i.e., (Normal control, Negative and Positive control) while the other 3 groups served as the test groups and they were maintained under standard laboratory conditions throughout the period of the study.

C. Chemicals and Solvent

All chemicals and solvent used for the study were of Analytical grades and were purchased from Sigma Aldrich Co. UK, through Bristol Scientific, Ikeja Lagos, Nigeria.

D. Extraction of the Plant Leaves

Ethanol extract of the plant leaves was prepared by a modified method as described by Usunobun et al. [33]. One Hundred (100) grams of the dry powdered plant leaves was soaked in 1000 ml of 70% ethanol solution at room temperature for 48 h (for thorough extraction). The extract was cold macerated (filtered) using muslin cloth, and then filtered through Whatmann filter paper No.4. The extract was thereafter concentrated using a water-bath at 60 ºC to one-tenth of its original volume. The percentage yield of the crude extract was 5.2%. The crude extract was then stored at 4 ºC in the refrigerator and subsequently used for the studies. An aliquot portion of the crude plant extract residue was weighed and used for phytochemical screening while the rest of the extract were dissolved in distilled water for use on each day of the experiment.

E. Quantitative Phytochemical Analysis

Quantitative phytochemical screening of A. muricata leaf was performed using standard procedure:

1. Total Alkaloid Determination

Alkaloid content was determined according to the method of Harborne [15]. Five grams (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25 ºC. This was filtered with Whatmann’s filter paper no. 42 and the filtrate was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide (NH₄OH) was added drop wise to the extract until the precipitate was complete. The whole solution is then allowed to stand in the dark for 4 hours (for thorough extraction). The extract was cold macerated (filtered) using muslin cloth, and then filtered through Whatmann filter paper No.4. The extract was thereafter concentrated using a water-bath at 60 ºC to one-tenth of its original volume. The percentage yield of the crude extract was 5.2%. The crude extract was then stored at 4 ºC in the refrigerator and subsequently used for the studies. An aliquot portion of the crude plant extract residue was weighed and used for phytochemical screening while the rest of the extract were dissolved in distilled water for use on each day of the experiment.

2. Total Flavonoid Determination

The flavonoid content was determined by the use of a slightly modified colorimetry method described by Barros et al [8]. A 0.5 ml aliquot of appropriately (2 mg/2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % NaNO₂ solution. After 6 min, 0.15 ml of 10% AlCl₃ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared with catechin equivalents. The analyses were performed in triplicate. The results were expressed as mg Catechin equivalents per 100 g of sample (mg CE/100 g).

3. Total Terpene Determination

Terpene was determined according to the method of Narayan et al. [23]. Exactly 0.5 g of sample was homogenized with 3.5 ml of ice-cold 95% methanol and then left at room temperature in dark for 48 h. The sample was centrifuged at 400 g for 15 min and the supernatant was collected. Then 200 µl of the supernatant was mixed with 1.5 ml of chloroform, mixed and allowed to stand for 3 min. This was followed by the addition of 100 µl of conc. H₂SO₄. This was allowed to stand in the dark for 90 min after which the supernatant was carefully decanted and the red precipitate dissolved with 1.5 ml of 95% methanol. The absorbance was taken at 538 nm against 95% methanol as blank. Linalool was used as standard. The result is calculated and expressed as mg Linalool equivalent per 100 g of the sample.

4. Total Cardiac Glycosides Determination

Cardiac glycoside content was determined by using the as described by Osagie [26]. To 1 ml of the extract was added 1 ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1 ml of 5% aqueous NaOH. It was boiled for 2 min (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was noted before filtration. The filter paper with the absorbed residue was dried in an oven at 50 ºC till dryness and weight of the filter paper with residue was noted [26].

The cardiac glycoside was calculated as a percentage value, as follows:

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\text{% cardiac glycoside} = \frac{\text{weight of filter paper + residue} - \text{weight of filter paper}}{\text{Weight of sample analyzed}} \times 100
\]

5. Total Saponin Determination

Saponin content was determined according to the method of Harborne, [15]. Exactly 5 g of each sample was put in 20% acetic acid in ethanol and allowed to stand in a water bath at 50 ºC for 24 h. This was filtered and the extract concentrated using a water bath to one-quarter of the original volume. Concentrated NH₄OH was being added drop wise to the extract until the precipitate is complete. The whole solution is then allowed to settle, and the precipitate collected by filtration and weighed. The saponin content was weighed and calculated as a percentage.

6. Total Phytate Determination

Phytate contents were determined using the method of Wheeler and Ferrel [34]. About 0.2 g of the sample was weighed into 250ml conical flasks. The sample was soaked in 100 ml of 2% concentrated HCl for 3 h. The sample was then filtered. Exactly 50 ml of the filtrate was placed in 250 ml beaker and 100 ml distilled water added to it. Then 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (iii) chloride solution which contained 0.00195 g iron per ml. the percentage phytic acid was calculated using the formula:
where 0.00195 and 1.19 are both Constants.

7. Total Oxalate Determination by Titration Method

This was determined according to Osagie [26]. Potassium permanganate, KMnO₄ is a strong oxidizing agent. Permanganate, MnO₄⁻ is an intense dark purple color. Oxalate reduces MnO₄⁻; Cr₂O₇²⁻ in the presence of H₂SO₄.

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.19}{2} \times 100$$

8. Total Phenol Determination

The total phenol content of the samples was determined using the method of Barros et al. [8]. The extract solution (1 ml) was mixed with Folin and Ciocalteu’s phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used to make standard curve (0.01-0.4 mM; Y = 2.8557X-0.0021; R² = 0.9999) and the results was expressed as mg of gallic acid equivalents (GAEs) per g of extract.

F. Animal Treatment

1. Acute Toxicity Testing

The median lethal dose (LD₉₀) of A. muricata ethanolic leaf extract was determined in rat using a modified method of Lorké [18], as reported by Aroma and Enegide [6]. Exactly 12 rats were used for this study. The test was carried out in two phases. In the first phase, nine (9) rats were divided into three (3) groups of three (3) rats each and were given 10, 100, and 1000 mg/kg body weight of the extract respectively, through oral administration. After administration of the extract, observations were made at regular interval to check for the onset of adverse effect, time of death or time of recovery. This period lasted for 24 h. In the second phase, three (3) rats were divided into three (3) groups, the dose levels of the extract were increased higher at the dose of 1600, 2900 and 5000 mg/kg body weight of the extract. They were observed for 24 h for possible toxicity symptoms as well as for possible delayed toxicity symptoms for 7-14 days. The lethal dose was calculated using the formula:

$$\text{LD₉₀} = \frac{(D_9 \times D_{100})}{2}$$

where D₀ = highest dose that gave no mortality; D₁₀₀ = lowest dose that produced mortality.

2. Animal Grouping

Thirty wistar albino rats were divided into six groups of five rats per group. Diabetes mellitus was induced in Groups 2, 3, 4 and 5 only by intraperitoneal injection of alloxan (130 mg/kg). Group 1 rats served as “Normal control” animals and received normal rat pellets and water. Group 6 was administered with 400 mg/kg daily of the extract without induction; group 3 was treated with glibenclamide (5 mg/kg body weight), groups 4 and 5 received 200 mg/kg and 400 mg/kg body weight of A. muricata leaf extract daily respectively throughout the duration of the experiment of 14 days. Group 2 was induced but not treated with any drug, thus it served as the “Negative control” group.

3. Induction of Hyperglycemia

The rats were fasted for 16h after which diabetes mellitus was induced with alloxan monohydrate, (130 mg/kg (b.wt) dissolved in 0.3 ml of distilled water) intraperitoneally (i.p) [11]. Hyperglycemia was confirmed when elevated blood glucose levels were equal to or greater than 200 mg/dl after 48 h of injection [31]. Prior to induction of diabetes mellitus (hyperglycemia), the blood glucose status was determined using (Accu-Chek) glucometer with compatible glucose test strips for all the animals scheduled for experiment and subsequently on alternate days (every two days) after induction of diabetes mellitus.

4. Administration of Extract

Five thousand milligrams (5000 mg) i.e. (5 g) of the crude extract was reconstituted using distilled 25 ml of distilled water and the concentrated extract at increasing doses of 200 mg/kg (b.wt) and 400 mg/kg (b.wt) based on body weights of the individual rats were administered to the rats through oral gavage. The administration of the extract was carried out for 14 days. Body weights of the animals were measured every two (2) days, after which the animals were sacrificed, and blood samples collected for biochemical analyses.

G. Collection of Blood Sample

Blood samples were obtained by repeated lancet puncture of the tail tip veins. Fasted alloxan administered rats with blood glucose concentration greater than or equal to 200 mg/dl were considered to be diabetic and included in the study using (Accu-Chek) glucometer with compatible glucose test strips for all the animals scheduled for experiment.

H. Statistical Analysis

All the data were expressed as mean ± SD and statistical significance was evaluated by using t-test Assuming equal variances, One way analysis of variance (ANOVA) in Microsoft Excel statistical package 2007 version. The level of significance was considered as (p<0.05).
III. RESULTS AND DISCUSSION

A. Quantitative Phytochemical Analysis of Ethanolic Leave Extracts of Annona muricata

The quantitative phytochemical screening of the ethanolic leaf extracts of Annona muricata (Table I) revealed the presence of Phenols (74.00 mg/100 g), Flavonoids (3.70 mg/100 g), Tannins (2.95 mg/100 g), Oxalates (6.48 mg/100 g), Terpenoids (13.88 mg/100 g), Phytates (130.00 mg/100 g), Alkaloids (570.00 mg/100 g), Cardiac Glycosides (1690.00mg/100g), Saponins (6800.00 mg/100 g) in ethanolic leaf extracts of Annonamuricata.

| Phytochemicals | Concentration (mg/100 g) |
|----------------|-------------------------|
| Total Phenol   | 74.00 ± 1.53            |
| Total Flavonoids | 3.70 ± 0.52             |
| Tannins        | 2.95 ± 0.01             |
| Oxalate        | 6.48 ± 0.38             |
| Terpenoids     | 13.88 ± 0.42            |
| Phytate        | 130.00 ± 0.01           |
| Saponins       | 6800.00 ± 0.42          |
| Alkaloids      | 570.00 ± 0.06           |
| Cardiac Glycoside | 1690.00 ± 0.01        |

The values are means ± Standard deviations.

B. Animal Studies

The result of the effects of ethanolic leaf extract of Annona muricata on alloxan induced diabetic rats and its effect are represented using chart and the data used in plotting the chart were derived from statistical analysis of data (presented as mean ± SD) using Microsoft excel 2007, that were obtained from dose administration of 200 mg/kg body weight and 400 mg/kg body weight of the ethanolic leaf extract of Annonamuricata on alloxan induced diabetic rats. During the study, no death was recorded in group one, 3 rats died in group two, 3 rats died in group three (that were administered 5 mg/kg body weight Glibenclamide), 3 rats died in group four (that were administered 200 mg/kg body weight of the ethanolic extract), 1 rat died in group five (that were administered 400 mg/kg body weight), no death was recorded in group six (that were also administered 400 mg/kg body weight of the extract), The cause of their death is however yet to be ascertained.

1. Median Lethal Dose (LD50) of the Extract

Oral administration of a single dose of the extract at doses 10, 100, 1000, 1600, and 2900 mg/kg body weight did not produce any mortality to the rats, while dose 5000 mg/kg body weight produced mortality during 24 h of observation. The calculated LD50 for ethanolic leaf extracts of Annona muricata at the end of 24 h acute toxicity test was 3807.89 mg/kg body weight.

| Group | Dose (mg/kg) | Body weight | Number of Animals | Number of Death | % Mortality |
|-------|--------------|-------------|-------------------|-----------------|-------------|
| A     | 10           | 3           | 0                 | 0               | 0           |
| B     | 100          | 3           | 0                 | 0               | 0           |
| C     | 1000         | 3           | 0                 | 0               | 0           |
| D     | 1600         | 1           | 0                 | 0               | 0           |
| E     | 2900         | 1           | 0                 | 0               | 0           |
| F     | 5000         | 1           | 1                 | 100             | 100         |

2. Effect of the Leaf Extract on Body Weight

Fig. 1 shows the effect of the ethanolic leaf extract of Annonamuricata on the general body weight. The results showed that the body weight of rats in group four were higher than rats in the other groups used in the study.

Fig. 1. Average Body weights for the various groups of rats measured every two days (data presented as mean ± SD using Microsoft Excel 2007).

From the statistical analysis of the results of the body weight, there was no significant difference (P>0.05) in body weight when the glibenclamide and extract treated groups were compared with the normoglycemic group, and no significant difference (P>0.05) between the extract treated groups.

Fig. 2 Shows the effect of the ethanolic leaf extract of Annona muricata on the final average body weights of the various groups of rats at the end of administration. The result did not show any significant (P>0.05) increase in the body weight of both the treated and control groups, and no significant increase (P>0.05) in body weight between the extract treated groups.

Fig. 2. Final Average weights of the various groups of rats at the end of administration.

There was no significant difference (P>0.05) in increase in the body weights when the Normoglycemic group was compared with all the test groups and Glibenclamide treated group. There was also no significant difference (P>0.05) in the weights when the induced (non- treated) group was compared with all the extract treated groups and Glibenclamide-treated group. Also, there was no significant difference (P>0.05) in body weights when the Glibenclamide-treated group was compared with all the extract treated groups. When all the groups treated with the extract were compared, there was no significant difference in their body weight.
3. Effect of Ethanolic Leaf Extract of Annona muricata on Hyperglycemic Rats

Fig. 3 shows the effect of the Ethanolic leaf extract of Annona muricata on the glucose level of experimental rats. The result showed that a significant (P<0.05) decrease in the glucose levels of the Glibenclamide treated group and A. muricata extract treated groups. There is no significant (P>0.05) difference in glucose levels between the 3 extract treated groups. There is no significant difference (P>0.05) between the Normoglycemic group and the Glibenclamide group. When the diabetic (untreated) group was compared with the Glibenclamide-treated group, there was a highly significant difference (P<0.05) between Glibenclamide-treated group and Normoglycemic. ** Significant difference (P<0.05) between A. muricata treated groups and Diabetic untreated group. # Significant difference (P<0.05) between Glibenclamide-treated group and Normoglycemic group. ## Significant difference (P<0.05) between Glibenclamide-treated group and Diabetic untreated. *Significant difference (p<0.05) between A. muricata treated group and Glibenclamide-treated group.

Fig. 4 shows the effect of the Ethanolic leaf extract of Annona muricata on the glucose level of experimental rats. The result showed a significant (P<0.05) decrease in the glucose levels of the Glibenclamide treated group and A. muricata extract treated groups. There is no significant (P>0.05) difference in glucose levels between the groups treated with the extract.

From the statistical analysis of the result of glucose levels of the rats in each group using Microsoft excel 2007, it was observed that when the Normoglycemic group was compared with the Glibenclamide-treated group, 200 mg/kg treated (induced) group, there was a highly significant difference (P<0.05) in the decrease of glucose level, but there was no significant difference (P>0.05) between the Normoglycemic group and the 400 mg/kg (induced), 400 mg/kg (not induced) group. When the diabetic (untreated) group was compared with Glibenclamide-treated group, 200 mg/kg (induced), 400 mg/kg (induced) and 400 mg/kg (not induced) group, there was a significant difference (P<0.05) in glucose levels as the glucose levels reduced progressively especially with increase in the concentration of the extract. There was no significant difference (P>0.05) in glucose level when the Glibenclamide-treated group was compared with the 200 mg/kg (induced) and 400 mg/kg (induced) group. But there was a significant difference (P<0.05) between the Glibenclamide group and the 400 mg/kg (not induced) group. There was no significant difference (P>0.05) in glucose levels between the 3 extract-treated groups.

IV. DISCUSSION

The phytochemical analysis conducted using ethanolic leaf extract of Annona muricata (Table 1) revealed the presence of Phenols (74.00 mg/100 g), Flavonoids (3.70 mg/100 g), Tannins (2.95 mg/100 g), Oxalates (6.48 mg/100 g), Terpenoids (13.88 mg/100 g), Phtyates (130.00 mg/100 g), Alkaloids (570.00 mg/100 g), Cardiac Glycosides (1690.00 mg/100 g), Saponins (6800.00 mg/100 g). These phytochemicals are known to support bioactive activities in medicinal plants in diverse ways.

The phytochemical result of this study is in agreement with the studies carried out by Usunobun et al. [33], and Usunobun and Okolie [32], that the ethanolic leaf extract of A. muricata contains flavonoids, saponins, tannins, alkaloids, terpenoids, reducing sugars, cardiac glycosides, and that these phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. However according to the study carried out by Arthur et al. [7], the phytochemical analysis of aqueous extract of A. muricata revealed the presence of saponins, tannins cardiac glycosides, flavonoids, terpenoid while alkaloids were conspicuously absent.

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and have remarkable activity in cancer prevention and as antimicrobial agent [28]. The presence of tannins in ethanolic leaf extract of Annona muricata may serve as a potential source of bioactive compound in cancer prevention and treatment and contributes to its antimicrobial properties as similarly reported by Ruch et al. [28] and Edeoga et al. [10].

Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity [24]. From the phytochemical analysis of ethanolic leaf extract of A.muricata, the presence of flavonoid together with other physico-chemicals such as...
alkaloids, tannins, saponins and phenolic compounds in the extract suggests the antioxidant and free radical scavenging potentials of the leaf extract as similarly reported by Lukaciniva et al. [19], thus according to Usunobun and Okolie [32], flavonoids, tannins, terpenoids, alkaloids and phenolic group found in plants are free radical scavengers that prevents oxidative cell damage and induce mechanisms that affect cancer cells, and inhibits tumor invasion. This as reported by Usunobun and Okolie [32] is due to the presence of conjugated ring structures and carboxylic group which inhibits lipid peroxidation.

Alkaloids are beneficial biomolecules or substances to plants, serving as repellent to predators and parasites. The presence of Alkaloids in the ethanolic leaf extract of A. muricata which are known to have antimicrobial, antiviral, anti-hypertensive and antidiabetic effects as reported by Trease and Evans, [30] and McDevitt et al. [20], in the study carried out justifies the blood glucose-lowering effect of ethanolic leaf extract of A. muricata in diabetic rats as similarly reported by Gaikwad et al. [12]. Hence, from the phytochemical results of the leaf extract of A. muricata, the presence of flavonoids, terpenoids, cardiac glycosides, together with alkaloids contributes to the blood glucose lowering potentials of A. muricata leaf extract which can be used in the management and treatment of diabetes mellitus as similarly reported by Gaikwad et al. [12].

Annona muricata leaf has been revealed to contain saponins, known to produce inhibitory effect on inflammation [16]. Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers and as such may be used as anti-cholesterol agents or cholesterol lowering agent [32]. The presence of these phenolic compounds in Annona muricata leaves contributes to their antioxidative properties and thus the usefulness of these plants in herbal medicine as previously reported by Usunobun and Okolie [32].

Cardiac glycosides are important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure [36]. Hence the presence of cardiac glycosides in Annona muricata leaf extract is an indication of the potential of the leaf for the treatment of cardiac infections along with other ailments such as cough, chest pain and diabetes mellitus as previously reported by Usunobun et al. [33] and Gaikwad et al. [12].

Phytic acid (Phytate) is an important phytoconstituent involved in regulating vital cellular functions as in-vivo and in-vitro experiments have demonstrated striking anticancer (preventive as well as therapeutic) effects of phytic acid and in addition to reduction in cell proliferation, phytic acid increases differentiation of malignant cells often resulting in reversion to the normal phenotype [5]. Given the numerous health benefits, phytates participation in important intracellular biochemical pathways, normal physiological presence in our cells, tissues, plasma, urine, etc., the levels of which fluctuate with intake, epidemiological correlates of phytate deficiency with disease and reversal of those conditions by adequate intake, and safety. The presence of phytate in the leaf extract suggests that ethanolic extract of A. muricata is essential for the treatment and management of cancer and this is in agreement with the study carried out as reported by Anonymous 2 [5].

In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca$^{2+}$) and iron (II) (Fe$^{2+}$) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates can form larger kidney stones that can obstruct the kidney tubules. An estimated 80% of kidney stones are formed from calcium oxalate thus, with kidney disorders, gout, rheumatoid arthritis, or certain forms of chronic vulvar pain (vulvodynia) are typically advised to avoid foods high in oxalic acid. Methods to reduce the oxalate content in foods are of current interest [4]. This however suggests the high toxicity tendencies of the leaf extract at higher dose, which could have deleterious consequences and could lead to fatality.

Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. However according to Kaberaet al. [17] pharmacologically, terpenoids has been demonstrated to possess anti-hypertensive, anti-microbial and antinociceptive properties as most plant that have high concentration of terpenoids are used in the treatment of hypertension, microbial infection and as insecticide. This therefore suggests that the high concentration of terpenoid found in the ethanoilic leaf extract of A. muricata is of pharmacological benefit in the treatment and management of hypertension, microbial infection and as insecticide.

Acute toxicity study of the ethanolic leaf extract of A. muricata on experimental rats, showed that the extract was lethal at concentration of 3807.89 mg/kg, and this is in contrast with the study carried out by Arthur et al. [7] that no mortality was observed at all dose levels from the critical 24 h post administration to the end of the seventh day and that the LD$_{50}$ was estimated to be $<5000$ mg/kg. However, this study is in agreement with the study carried out by Adewole and Ojewole [2] that high dose of aqueous leaf extract of A. muricata were found to be toxic and or lethal to the animals. This could however be as a result of the presence oxalate and annonaceous acetogenins both of which possess deleterious consequences at high dose / concentration as reported by Anonymous 1, [4] and Coria-Tellez et al. [9].

From the animal study, it could be observed that the ethanolic leaf extract of A. muricata caused a reduction of the blood glucose level of the extract treated experimental rats and this indicates that the extract is a very potent blood glucose-lowering agent largely comparative to the standard drug (Glibenclamide) used in the study, at a higher dose based on the body weight. These results agree largely with the study carried out by Adeyemi et al. [3] and Arthur et al. [7]. It could be observed from the study that ethanolic leaf extract of Annona muricata is more potent in lowering the blood glucose levels in alloxan induced diabetic rats which is in agreement with the work of Adewole and Ojewole [2] than standard drug (glibenclamide) used for the study, this could be as a result of the presence of antidiabetic phytochemicals such as terpenoids, phenols, alkaloids, flavonoids in the leaf extract as previously reported by Gaikwad et al. [12].

From the animal study conducted, it was observed that there was no significant weight gain in the animals throughout the duration of the study, this is consistent with the study carried out by Usunobun et al. [33], that the leaf extract of A. muricata has increased fiber content, and the no significant difference in weight gain obtained from the
statistical analysis justifies the claims of Arthur et al.[7] that, the ethanolic crude extract at higher doses such as 200 mg/kg and 400 mg/kg per day may be metabolized into toxic end product which interferes with gastric function and decreased food conversion efficiency, thus, this could lead to loss of appetite and decreased body weight. It is therefore suggested that *Annona muricata* leaf extract possess hypoglycemic properties and is useful in the management of diseases such as diabetes mellitus and other diseases resulting from diabetic complications.

V. CONCLUSION

The results of this study suggest that the ethanolic leaf extract of *Annona muricata* has antidiabetic effect and possesses some antioxidant properties that can be of importance in the treatment and management of such disease condition such as diabetes mellitus, cancer, arthritis, and other disease conditions resulting from oxidative stress this could be as a result of the phytochemical constituents of the leaf. The results of the acute toxicity testing LD₅₀ however suggest that the extract is lethally toxic at concentrations greater than 3807.89 mg/kg.

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