EFFECT OF ETHANOL STEM BARK EXTRACT OF *Annona muricata* ON SOME INVIVO ANTIOXIDANT POTENTIALS OF ALLOXAN INDUCED DIABETIC RATS.

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ABSTRACT

Antioxidant properties of ethanol stem bark extract of *Annona muricata* (soursop)in *alloxan* induced diabetic rats were investigated. Thirty (30) wistar albino rats ranging between 130-150g were carefully shared into five (5) groups of six (6) rats each. Group B-E was intraperitoneally administered with 60mg/kg b.w single dose of *alloxan* monohydrate, whereas group A was normal control and was given distilled water and feed. Group C was further administered with 5mg/kg glibenclamide to act as positive control, while D and E groups were administered with 100 and 400mg/kg b.w of *Annona muricata* stem bark extract. After administration, the rats were sacrificed and blood samples collected via ocular puncture, then invivo antioxidant enzymes and phytochemstry were assayed. The result showed that *Annona muricata* ethanol stem bark had phenol, alkaloids, saponins, cardiac glycoside, anthocyanin and tannin in appreciable quantity. Antioxidant enzymes analyzed showed that CAT, GPX and SOD had significant reduction between groups B-E when compared to group A. In conclusion, the plant contains some phytochemicals and invivo antioxidants enzymes which could assist in mopping up free radicals generated as a result of *Diabetes mellitus*.

**Keywords**: *Annona muricata, Diabetes mellitus*, Antioxidant, Alloxan.

 **INTRODUCTION**

*Diabetes mellitus* (DM), usually referred to as hyperglycemia, is a chain of ailment in which there is elevated blood sugar rise over a prolonged period. Hyperglycemia is the insufficiency in production or action of insulin by the body (Maritim *et al*., 2003). Symptoms include constant urination, high thirst, and high hunger. If left untreated, diabetes can cause numerous complications. Severe complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death (Kitabchi *et al*., 2009: Ayepola *et al.,* 2014). Long-term serious complications are cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and eyes damage. According to *International Diabetes Federation* (2017), there is an estimated 425 million diabetic sufferers globally by a projected rise of 629 million sufferers by the year 2045. In 2017, the global maximum healthcare expenditure by people with diabetes (20-79 years) rises by 54 billion US dollars (from US$673 in 2015 to US$727 in 2017) (International Diabetes Federation, 2017). Research for suitable, safe and cheaper drugs for the elimination of diabetes is ongoing and effort is being shifted to medicinal plants. Nowadays, the search for curative antidiabetic agents has been shifted to plants because of fewer side effects (Shori, 2015).

*Annona muricata* (soursop), is a species of *Annonaceae* family that has been commonly studied in the last decades due to its therapeutic potentials (Elizangela *et al*., 2013). The plant is specifically scattered across the world. *Annona muricata* is a thin, small, and cold-intolerant tree, generally reaching heights of 4-6 meters. It is found in places where there is high humidity and moist weather of 5°C (41°F). In North America, the bark, root and leaves are used for the treatment of diabetes as well as sedatives (Chauhan, 2015; Soheil *et al*., 2015; Olugbuyiro *et al*., 2017).

Antioxidants of plant extract have been found to defend the body from molecules that produces free radicals. (Muthu and Durairaj, 2015; Orak *et al*., 2019).

The study is designed to determine the effect of ethanol stem bark extract of *Annona muricata* on some invivo antioxidant potentials of alloxan induced diabetic rats.

**MATERIALS AND METHODS**

**Chemicals used:** All the chemicals used were of analytical grade and were obtained from reputable manufacturers and distributors.

**Equipment used:**

All the equipment used in this study were all standard equipment from reputable manufacturers. They were obtained from Biochemistry laboratory, Chukwuemeka Odumegwu Ojukwu University, Uli Campus and D-Light diagnostic laboratory Onitsha.

**Methodology**

**Plant Materials/Extraction**

The stem bark of *Annona muricata* (soursop) plant was obtained from Nnadozie’s Compound at Umudioma Village in Uba Ifakala, Mbaitoli LGA Imo State, Nigeria and it was identified by a botanist from Federal University of Technology Owerri (FUTO), Imo State. The *Annona muricata* (soursop) stem bark was transported to the Laboratory using a sterile polyethylene bag. It was washed with sterile water and placed onto a sterile laboratory bench top so as that it could dry. It was air dried for three months with occasional turning. After three months, the stem bark of *Annona muricata* was chopped into smaller pieces and then blended using a mechanical blender. Then 250g of the ground sample (*Annona muricata*) stem bark was soaked in 1L of 80% ethanol. The mixture was stirred at 1hr interval and was allowed to extract for 72hrs. At the expiration of the 72hrs, the sample was sieved with muslin clothe and then filtered using whatman filter paper.The filtrate was concentrated at 40oC using rotary evaporator. *Annona muricata* stem bark yielded 28.32g of extract. The extract was stoppered in a universal bottle and stored in a refrigerator at -4oC until required for use.

**EXPERIMENTAL DESIGN AND ADMINISTRATION**

Thirty (30) male wistar albino rats weighing between 130-150g were used for the study. The rats were procured from Chris Animal farm, Mgbakwu in anambra state. The animals on arrival were weighed to obtain initial weight and were acclimatized for 14 days in the animal house of department of Biochemistry. The rats were given rat pellets and water *ad libitum*. Diabetes was induced in the rat by injecting intraperitoneally with 60 mg/kg b.w dose of Alloxan Monohydrate. Forty-eight hours after induction, blood glucose was assessed using ACCU-CHEK active glucometer and animals with blood glucose >200 mg/dL (11.1 mmol/L) was selected for the antidiabetic study. A positive control group was also administered with 5.0mg/kg glibenclamide. Plant extract (*Annona muricata)* was administered to diabetic groups at 100mg/kg and 400mg/kg respectively for 28 days to check for any curative measures. Oral route of administration of extract was via an orogastric tube. Doses of extract administered was determined from LD50.

The regimen for the animal grouping and administration of plant extract (*Annona muricata*) stem bark extract are shown below.

**Group 1** (Normal control group no diabetes) + rat pellets + water *ad libitum.*

**Group 2** (Negative control group, diabetic untreated rats) + rat pellets + water *ad libitum.*

**Group 3** (Positive control group, diabetic rats) + 5.0 mg/kg of gilbenclamide + rat pellets + water *ad libitum.*

**Group 4** (Diabetic rats) + 100 mg/kg b.w of ethanol stem bark extract of *Annona muricata+* rat pellets + water *ad libitum.*

**Group 5** (Diabetic rats) + 400 mg/kg b.w of ethanol stembark extract of *Annona muricata* + rat pellets + water *ad libitum.*

**Acute toxicity test of ethanol extracts of *Annona muricata* (soursop) stem** **bark**

The method of Lorke (1983) was used for the acute toxicity test of the ethanol stem bark extracts of *Annona muricata* (soursop). Thirteen (13) albino rats were utilized in this study. The test involved two stages. In stage one, the animals were divided into three (3) groups of three rats and were given 10, 100 and 1000 mg/kg b.w of the extracts respectively. The second stage involved the administration of 2000, 3000, 4000, 5000 mg/kg b.w of extract to 4 groups of 1 animal each.

**Blood collection**: At the end of the experiment, the rats in each group were sacrificed by cervical dislocation while under mild anesthesia and blood collected by cardiac puncture into plain bottle. The blood was centrifuged at 3500rpm for 10 min and serum was stored frozen until needed for antioxidant assay.

**ASSAY OF INVIVO ANTIOXIDANT ENZYMES**

**Assay of Superoxide Dismutase (SOD)**

SOD was assayed according to the method of Kakkar *et al.* (1984).

**ASSAY OF CATALASE (CAT)**

Catalase activity was assayed following the method of Luck (1974).

**ASSAY OF GLUTHIONE PEROXIDASE (POD)**

The method proposed by Reddy *et al*. (1995) was adopted for assaying the activity of peroxidase.

**PHYTOCHEMICAL ANALYSIS OF STEM BARK OF *Annona muricata***

**Oxalate determination by Titration method:** The method of (Harborne, 1993) was used for oxalate determination.

**Alkaloids Determination.** The method as described by (Harborne, 1993; Obadoni and Ochuka, 2001) was used for the determination of alkaloid.

**Flavonoids Determination:** The method of Boham and Kocipai (1974) was adopted for flavonoid determination.

**Determination of Saponin.** The method of Obadoni and Ochuko (2001) was adopted for saponin determination.

**Cardiac Glycosides Determination.** A.O.A.C (1975) method was used for the assay of cardiac glycoside.

**Tannin Determination by Follins dennis Titration.** The follin dennis titration method as described by pearson (1974) was used.

**Phytate Determination.** Phytate contents were determined using the method of Young and Greaves (1940) as adopted by lucas Markakes (1975).

**Phenol Determination**: The quantity of phenol was measured by the spectrophotometric method.

**Heamaglutinin Determination:** The spectrophotometric method was used to analyse the *Annona muricata* stem bark.

**Cynogenic Glycoside.** The Acid Titration Method was used to determine the cynogenic glycoside of *Annona muricata* stem bark.

**Determination of Anthocyanin:** The method of Harborne (1973) was adopted for anthocyanin determination.

**Determination of steroid content:** The method of Obadoni and Ochuko (2001) was adopted for determination of steroid.

**RESULTS:**

TABLE 1: Preliminary and Quantitative phytochemical analysis of stem-bark of *Annona muricata* (soursop) plant.

|  |  |  |
| --- | --- | --- |
| Phytochemical | Quantitative | Quantitative Constituents (%) |
| Flavonoids | ++ | 1.461% |
| Alkaloid | ++ | 4.097% |
| Saponins | ++++ | 7.157% |
| Cardiac glycoside | +++ | 5.748% |
| Phytate | + | 0.552% |
| Cyanogenic glycoside | + | 0.540mg/g |
| Anthocyanin | +++ | 4.229% |
| Oxalate | + | 0.193mg/g |
| Tannin | ++ | 3.810% |
| Steroid | ++ | 2.705mg/g |
| Phenol | ++ | 3.501mg/kg |
| Hemaglutin | + | 0.385mg/kg |

Keys: +=trace, ++=moderately present, +++=Highly present, ++++=Excessively present

Table.1 shows the phytochemical constitutents of ethanol stem bark extract of *Annona muricata*. It was evident that all the phytochemical analyzed qualitatively revealed the presence of flavonoids, alkaloids, saponins, cardiac glycoside, phytate cyanogenic glycoside anthocyanin, oxalate, Tanin steroid, phenol and hemaglutin. Saponins, Cardiac glycoside and Anthocyanin were highly present in the ethanol stem bark extract.

Table 2: Effect of ethanol stem bark extract of *Annona muricata* on invivo antioxidant potentials of alloxan induced diabetic rats.

|  |  |
| --- | --- |
| Groups/Treatment | Invivo Antioxidant Concentration |
|  | CAT(Umole/ml) | GPX (Umole/ml) | SOD (%) |
| A(Normal Control) | 29.190±35.295 | 120.876±0.556 | 53.095±13.041 |
| B(Diabetic Untreated) | 32.681±4.288 | 116.425±2.323 | 68.465±6.153 |
| C(Diabetes +5mg/kg) glibenclamide | 29.400±0.580 | 124.260±0.447 | 76.251±11.533 |
| D(Diabetes +100mg/kg) extract | 24.074±0.002 | 124.291±0.001 | 66.541±0.001 |
| E(Diabetes +400mg/kg) extract | 29.164±0.198 | 112.808±1.462 | 54.572±10.397 |
|  |

Values are mean ± SD of 3 determinations.

Table.2 shows the invivo antioxidant concentration of *Annona muricata* stem bark of alloxan induced diabetic rats. The catalase activity was higher in group B but lesser in group A and other groups. Glutathione peroxidase was higher in groups C and D when compared to the control group. Groups B and E were found to be reduced as compared to group A. Percentage of superoxide dismutase in group A was lesser than other groups. Highest SOD activity was observed in group C. Highest GPX and CAT activities were also observed in groups D and B respectively.

**DISCUSSION**

Effect of ethanol stem bark extract of *Annona muricata* on some invivo antioxidant potentials of alloxan induced diabetic rats were investigated and the result of the study showed that there were presence of phytochemical and antioxidant activities of the plant. Phytochemicals are plant derived chemicals. It is a secondary metabolites produced by plants, these metabolites are normally produced as a means of self defense against pests, insects, pathogens and other agents. Foods with antioxidant properties, nutraceuticals, phytonutrients, antinutrients and phytotoxins are capable of protecting the immune system against reactive oxygen species (Chukwuebuka *et al*., 2020).

 Flavonoids are class of polyphenolic secondary metabolites found in plants such as *Annona muricata* stem, and the result showed that flavonoid was present at a value of 1.461%. Flavonoids are important in plant pigmentation and flower coloration, they are also involved in ultraviolet filtration and nitrogen fixation (Chukwuebuka *et al*., 2020). Flavonoids are free radicals which inhibits oxidative cell destruction because of their anticancer potentials (Ugwu *et al*., 2013). Flavonoids has majorly anti-microbial potency attacking different arrays of microorganisms (Sanni *et al.,* 2014). This property can be liken to the ability of the plant to quench free radicals. The carboxylic nature of flavonoid have been reported to inhibit lipid peroxidation (Usunobun *et al.,* 2014).

In Table 1, it was found that the alkaloid content of *Annona muricata* stem bark contained 4.097%. The presence of little amount of alkaloid is for the regulation of plant growth and also preventing insects and chordate animal from been eating. (Chukwuebuka *et al*., 2020). The presence of alkaloid can be used in the manufacturing of antimicrobial drugs.

Saponin are known to mingle alongside cholesterol tissue of carcinogenic cells then disrupting their life span and productivity (Usunobun *et al.,* 2014). Saponins are a subclass of terpenoids and are the largest class of plant extract and they have the potency of agglutinating with haemoglobin (Usunobun *et al.,* 2014). From our findings, it was evident that the saponin content of *Annona muricata* stem bark was found to be 7.157%. It means that saponin content in *Annona muriicata* stem bark can be useful in the development of cosmetics and drugs, this is because it forms foams in aqueous solution and creates hemolysis with its bitter attributes (Okwu, 2004) . Saponin derived from plants can also be used in controlling invasive worm species (Chukwuebuka *et al*., 2020). The saponin content present in this work was also in line with the work done by Usunobun *et al,* (2014) who reported high content of saponin. Medicinal plant constituents of saponin are accountable in much life growth processes in humans with their slow inflammatory effect (Usunobun *et al.,* 2014).

The amount of cardiac glycoside present in the stem bark of *Annona muricata* was found to be 5.748%. Cardiac glycoside is a class of organic compounds that are used for the creation of drugs which are used for the treatment of certain ailment in humans (Chukwuebuka *et al*., 2020). The cardiac glycoside can be extracted from plant based compounds. Cardiac glycoside can be used in the formulation of drugs which can be useful in the treatment of heart related diseases (Weseler, 2010). The presence of these phytoconstituent is in consonance with the work of (Usunobun *et al.,* 2014).

Cyanogenic glycoside, oxalate and hemaglutin were present in the sample in traces and there values were 0.540mg/g, 0.193mg/g and 0.385mg/kg respectively.Cardiac glycosides are known for the elimination of congestive heart diseases (Yukari *et al.,* 1995). The result of this work followed a pattern of result gotten from the work of Usunobun *et al.* (2014) who reported a high presence of phytochemical from *Annona muricata* leaves.

Anthocyanin and Tannin were present in the stem bark of *Annona muricata* at values of 4.229% and 3.810% respectively. Anthocyanin may have a protective role in plants against extreme temperature and can also counter the effect of reactive oxygen species (Chukwuebuka *et al*., 2020). Tannins are class of phenolic compounds that provides defensive properties against herbivores. Tannins are known to be inhibitory for fungal spore germination (Mazid *et al.,* 2011).

Steroid and phenol were found to be present in the stem bark of *Annona muricata* with values of 2.705mg/kg and 3.501mg/kg respectively. Phenolic compounds play a significant role in plant defense against bacteria and fungi. This shows that the plant extract can act as defense mechanism to bacteria and some organisms. (Serghini *et al.,* 2001). The stem bark of *Annona muricata* has shown the presence of photochemical which can be incorporated in the formulation of pharmaceutical products used in combating sickenesses (Yukari *et al.,* 1995).

Table 2 shows the effect of ethanol stem bark extract of *Annona muricata* on invivo antioxidant potentials of alloxan induced diabetic rats.

Antioxidant enzymes are proteins participating in the catalytic transformation of reactive oxygen species and their by-products into stable nontoxic molecules. These antioxidant enzymes are important in defending against oxidative stress that induces cell damage (*Guillermo and Nuria,* 2017).

Antioxidant enzymes includes; Superoxide dismutase, glutathione peroxidase, glutathione reductase, Catalases, just to mention but a few. Antioxidant enzymes are therefore important for maintaining optimal cellular and systemic health and well being, *Praveen and Ashish, (2012*).

In Table 2, Catalase activity, Glutathione peroxide activity and superoxide activity were 29.190±35.295, 120.87±0.556 and 53.095±13.041 respectively in Group A (normal control).

In group B (diabetic untreated), the catalase, Glutathione and superoxide activities were 32.681±4.288, 116.425±2.33 and 68.465±6.153 respectively. This shows that catalase and superoxide activities both increased as compared to the normal control that decreased in their antioxidant activities.

Group C (Diabetes + 5mg/kg glibenclamide also increased in catalase, glutathione and superoxide activities with values of 29.400±0.580, 124.260±0.447 and 76.251±11.533 respectively. These increment is comparable to the normal which did not increase that much.

 Glutathione and superoxide activities were higher in group D (diabetes + 100mg/kg stem bark ethanol extract of *Annona muricata* (Soursop) with values of 124.491±0.001 and 66.541±0.001 respectively as compared with the normal control that had 10.876±0.556 and 53.095±13.041 respectively.

Meanwhile catalase activity in group D was decreased to 24.074±0.002 as compared to the control which had value of 9.190±35.295.

In group E (Diabetes +400mg/kg stem bark ethanol extract), there was a reduction in catalase and glutathione activities with values of 9.164±0.198 and 112.808±1.462 as against the normal control which had catalase and glutathione values of 29.190±35.295 and 120.876±0.556 respectively. SOD activity in group E was 54.572±10.397 as against a reduction of 53.095±13.041 in the normal control.

Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GPX) play important roles as protective enzymes against free radical formation in tissue (*Chinedu and Okwuolisa, 2021).*

The result of our study as regard the antioxidant enzyme is not in line with the work of *Chinedu and Okwuolisa (2021)* who reported a significant reduction in superoxide and catalase activities in mice administered with methanol leaf extract of 50mg/kg – 1000mg/kg of *Annona muricata.*

Superoxide dismutase (SOD) is also known to help carry nitric oxide into hair follicles. SOD combacts the effects of free radicals that are causing hair follicles to die. So increase in SOD activity helps to counter the effect of Nitric oxide *(Praveen and Ashish, 2012). Annona muricata* ethanol stem bark extract can serve as a dietary supplement which can provide adequate supply of superoxide dismutase which in turn is helpful in maintaining overall well being and also protects the body from harmful effects of free radicals.

Glutathione peroxidase (GPX) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides. The high concentration of glutathione peroxidase was an indication of the ability of the extract to fight against free radicals in the body. Glutathione are also responsible for boosting immunity *(Praveen and Ashish, 2012). Annona muricata* extract has very good antioxidant potentials. Glutathione protects the cell against ill effects of pollution and boost immune system (*Praveen and Ashish, 2012*).

CONCLUSION

Effect of ethanol stem bark extract of *Annona muricata* on some invivo antioxidant potentials of alloxan induced diabetic rats were investigated and the result shows that the plant contains major phytochemical and invivo antioxidant potential which could be used in mopping up free radicals and oxidative stress .

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