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# Phytochemical Screening and Evaluation of the Phytoestrogenic, Hypocholesterolemic and Antioxidant activity of Ethanolic Extract of Sour Sop (*Annona muricata*) Seeds in DMBA-Treated Female Wistar Rats

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## Abstract

**Background of study**: *Annona muricata*; commonly known as sour sop has been reported to possess ethno medicinal values including anti-tumor and antioxidant activity.

**Aim**: This research was aimed at evaluating the phytochemical composition and the phytoestrogenic, hypocholesterolemic and antioxidant activity of *Annona muricata* seed extract in 7, 12-dimethylbenzeneanthracene (DMBA) treated female wistar rats.

**Methodology**: Ethanolic extract of *A. muricata* seed was prepared following crude extraction protocol. Qualitative and quantitative screening for the phytochemical constituents was done using standard methods.

A total of thirty rats were randomly divided into six groups with five rats in each group. Group I served as the control group fed on food and water only; group II rats received *A. muricata* seed extract (200 mg/kg per body weight) intraperitoneally (i.p) daily for two weeks; rats in group III were treated with DMBA i.p (20 mg/kg pbw) twice weekly for two weeks; group IV rats were pre-treated with DMBA (20 mg/kg pbw) i.p for two weeks followed by i.p injection of tamoxifen (20 mg/kg pbw); group V rats were pre-treated with DMBA (20 mg/kg pbw) i.p for two weeks followed by i.p treatment with *A. muricata* seed extract (100 mg/kg pbw) for two weeks while rats in group VI received i.p injection of DMBA (20 mg/kg pbw) twice weekly for two weeks followed by i.p treatment with *A. muricata* seed extract (200 mg/kg pbw) for two weeks. Blood samples were collected at the end of the experiment. Serum levels of estradiol and cholesterol were determined by standard methods. Levels of antioxidant parameters superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were also estimated from the serum using standard methods.

**Results:** Phytochemical screening revealed the presence of phenol (26.74 mg/100 g), tannin (21.95 mg/100 g), flavonoids (27.52 mg/100 g), alkaloid (19.25 mg/100 g) and steroid (20.23 mg/100 g). Results obtained from the serum samples of experimental animals showed that there was a significant ($P<0.05$) increase in the estradiol, SOD and CAT levels while there was a significant ($P<0.05$) decrease in MDA and cholesterol levels of the treated rats as compared to the control groups.

**Conclusion**: The results obtained in the study suggest the phytoestrogenic, hypocholesterolemic and antioxidant activity of *A. muricata* extract in DMBA-treated rats.

**Keywords:** Phytoestrogen; DMBA; Antioxidant; Hypocholesterolemic; *Annona muricata*

## Introduction

Cancer has been defined as a multi-step process in which cells undergo alterations due to accumulation of modifications in the genes that control cell proliferation which then results into the formation of a mass of deregulated cells [1]. Breast cancer has been known to be initiated in the luminal epithelial cells of the mammary gland and various endogenous factors have been implicated in its etiology and progression [2]. Studies have revealed that the cumulative exposure to endogenous estrogen all through a woman's lifespan contributes to and may be an etiologic factor in breast cancer [3,4]. Estrogen replacement therapy or hormone replacement therapy using synthetic estrogen has been employed in the management of some types of breast cancer but this has led to a relatively increased risk of breast cancer as reported by Fournier et al. [4]. As an addendum, high cholesterol levels have also been linked to increased endogenous estrogen production as estradiol which is the most potent form of estrogen in females is synthesized from cholesterol. Studies have implicated cholesterol in the initiation and progression of tumors [5]. Exposure of humans to contaminants like 7,12-dimethylbenzeneanthracene (DMBA) can lead to the accumulation of free radicals; resulting in oxidative stress which inevitably leads to depletion of the endogenous antioxidant parameters in the body system and this further promotes the progression of cancer. Due to recent health risks posed by the use of synthetic estrogen in hormone replacement therapy, there is a need for more conventional, potent, natural alternative sources of estrogen with similar functions to natural estrogen and with lesser side effects because estrogen has other benefits in the body system.

In the light of these, medicinal plants are being screened for anti-cancer lead compounds which can prevent or abrogate tumors as a therapy against cancer. There is new evidence for the recent attention on phytoestrogens as a viable alternative to synthetic estrogens in hormone replacement therapy and also its potential in precluding estrogen-

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dependent cancers like breast cancer [6]. Phytoestrogens refer to a varied group of plant substances with semblance to the structure of 17β estradiol which also mimics estrogenic actions by binding to estradiol receptors [7,8]. Examples of compounds categorized as phytoestrogens include isoflavonones, lignans, coumestans and stilbens [9,10]. They have been found to regulate the cell cycle and apoptosis [11].

*Annona muricata* popularly called sour sop; a member of the Annonaceae family is an evergreen, terrestrial, erect tree (5-8 m) in height with the fruits large, oval shaped which contains smooth hard, black seed [12]. Extracts of sour sop has been employed in allopathy and have been found to possess antiviral, anti-inflammatory and also anti-tumor and anti-cancer properties [13]. DMBA is a polycyclic aromatic hydrocarbon that has been used extensively as a model carcinogen in cancer research [14]. Tamoxifen is one of the selective estrogen receptor modulator that can act as an anti-estrogen in mammary tissue and has been found to reduce the risk of breast cancer in women at high risk [15]. There is paucity of information on the phytoestrogenic, hypocholesterolemic and antioxidant activity of *Annona muricata* seed in DMBA-treated female rats and the dire need for a natural potent therapeutic drug that can readily be a substitute for synthetic estrogen which will be of great relevance in the prevention and management of breast cancer necessitated this study. This study was aimed at evaluating the phytoestrogenic, antioxidant and hypocholesterolemic activity of *Annona muricata* ethanolic extract in DMBA-treated female rats.

## Materials and Methods

### Chemicals

Estrogen and cholesterol assay kits were purchased from Randox diagnostics. DMBA was purchased from Sigma chemical company. All other chemicals and reagents used were of analytical grade and highest purity.

### Plant collection and identification

*Annona muricata* fruits were purchased from a local market in Lagos and the seeds were removed. The sample was identified and authenticated at the Department of Botany, University of Lagos, Lagos, Nigeria and assigned the voucher number LUH 7551.

### Plant preparation and extraction

*A. muricata* seeds were removed from the fruit, washed and air-dried afterwards for two weeks. The dried samples were later blended into powder with an electric blender sterilized with 70% ethanol. 743.99 g of the seed powder was soaked in 4 L of 70% ethanol for 72 h. This was later filtered and the filtrate was concentrated using a rotary evaporator to obtain the crude extract which was later freeze-dried for proper storage. The concentrate was then used to prepare the different concentrations used in the study and for phytochemical screening.

### Qualitative and quantitative phytochemical screening of *A. muricata* seeds ethanolic extract

Chemical tests were carried out on the ethanolic extract of *A. muricata seeds* using standard procedures to identify the constituents in accordance to the method of Sofowara [16], Trease and Evans [17] and Harborne [18] as described by Edeoga et al. [19]. Some selected phytochemicals among the ones detected were then quantitatively measured.

**Flavonoid determination:** This was done following the method of Bohm and Kocipai-Abyazani [20]. 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

**Determination of total phenols by spectrophotometric method**: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

**Tannin determination:** This was done using the method described by Van-Burden and Robinson [21] method. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

**Alkaloid determination:** Carried out using the method described by Harborne [18]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

**Steroid determination:** This was done using the method described by Madhu et al. [22]. 1 ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4 N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2°C for 30 min with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

### Experimental design

**Experimental animals:** Thirty adult, healthy, female wistar rats were used for the study. They were acquired from the University's Animal House, College of Medicine, University of Lagos, Nigeria. All the animals were housed in well ventilated, standard clean cages made of plastic and wire gauze. Wood shavings were used as beddings to keep each compartment dry. Here, normal standard ambient conditions of temperature between 28-31°C, relative humidity between 50%-60% and a photoperiodicity of 12 h natural light and 12 h dark were maintained. The animals were allowed to acclimatize for two weeks for proper adaptation to their new environment and were weighed weekly. They had access to pelletized feed and tap water ad libitum. All the experimental procedures were carried out in accordance to the guidelines of the Institutional Animal Ethics Committee (IAEC). All the ethical and humanity considerations as well as euthanasia of the animals were considered and performed.

**Experimental design and treatment:** At the end of the acclimatization period, thirty (30) adult female wistar rats weighing between 120 g and 180 g, were divided into six groups, of five rats in

each. The experimental groups received different concentration of ethanolic extract of *A. muricata* seed with respect to the $LD_{50}$ results.

Group I (Positive control): Rats treated with distilled water only.

Group II: Rats treated with 200 mg/kg per body weight *Annona muricata* (A.M) extract only.

Group III (Negative control): Rats treated with 20 mg/kg per body weight (pbw) 7, 12-dimethylbenzeneanthracene (DMBA), twice a week for 2weeks only.

Group IV: Rats treated with 20 mg/kg pbw of DMBA twice a week for 2 weeks+Tamoxifen 20 mg/kg pbw

Group V: Rats treated with 20 mg/kg pbw DMBA, twice a week for 2 weeks+100 mg/kg pbw A.M extract.

Group VI: Rats treated with 20 mg/kg pbw DMBA, twice a week for 2 weeks+200 mg/kg pbw A.M extract.

DMBA and extract were given subcutaneously by intraperitoneal injection. The experiment lasted for ten weeks. The experimental and control animals were carefully checked daily and their weight taken weekly.

At the end of the experiment, rats were weighed and were sacrificed under light anaesthesia by cervical dislocation. Blood samples of the animals were collected at the jugular vein, using a needle and syringe, inside EDTA bottles and centrifuged at 3000 rpm for 15 min to obtain the serum for assay.

## Assay for estradiol

This was done following the manufacturer's instructions inscribed on the standard kit for determining estradiol level from serum. 50 µl each of calibrator (estradiol in a protein-based buffer spiked with estradiol), control (estradiol in a protein-based buffer in which serum has been spiked with estradiol) and specimen samples were pipetted into correspondingly labelled wells in duplicate. 100 µl of the conjugate working solution was also pipetted into each well and incubated on a plate shaker for 1 h at room temperature. The wells were washed three times with 300 µl of diluted wash buffer per well. 150 µl of TMB (tetramethylbenzidine and hydrogen peroxide) substrate was pipetted into each well at timed intervals and then incubated on a plate shaker at room temperature. 50 µl of the stop solution was then pipetted into each well at timed intervals. The absorbance was read within twenty minutes at 405 nm. The estradiol concentration (pg/ml) of the test samples were extrapolated from a calibrator curve of absorbance against estradiol concentration.

## Assay for cholesterol

Assay for serum cholesterol was done following the instructions of the respective test kit used. An enzyme solution was prepared by dissolving the enzyme reagent (cholesterol kit, 100 mL dilution, cholesterol oxidase 12 unit, cholesterol esterase 3.5 unit, peroxidase 6700 unit, 4-aminoantipyrine 17.0 mg/dL) to 100 mL in the cholesterol buffer solution. The blank (3 ml of enzyme solution and 0.02 ml of D.I water), standard (3 ml of enzyme solution and 0.02 ml of standard cholesterol) and sample (3 ml of enzyme solution and 0.02 ml of serum sample) solutions were prepared respectively and then placed in a water bath at 37°C for 5 min. The absorbance of the test and standard solution samples were then measured in a spectrophotometer at 500 nm and recorded. The serum cholesterol level in mg/dl was then calculated using the formula below:

$$\text{Cholesterol level (mg/dl)} = \frac{\text{Absorbance of Sample} \times 300}{\text{Absorbance of Standard}}$$

## Evaluation of antioxidant activity of *Annona muricata in vivo* (SOD, CAT and MDA) levels

**Superoxide dismutase (SOD):** The levels of SOD activity was determined by the method of Misra and Fridovich [23]. 50 µl of sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) in a cuvette. 0.3 ml of epinephrine was added, mixed by inversion immediately and the change in absorbance was monitored every 30 s for 2 min at 480nm in a spectrophotometer. Similar step was done for the blank except that the sample was replaced with distilled water. The percentage inhibition was calculated as shown below and 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the autoxidation of epinephrine.

$$\%\,\text{Inhibition} = 100 - \left[100 \times \frac{\text{(increase in absorbance per minute for sample)}}{\text{(increase in absorbance per minute for blank)}}\right]$$

**Catalase determination:** Catalase activity was determined according to the method of Claiborne [24]. Hydrogen peroxide (2.95 ml of 19 mM solution) was pipetted into a 1 cm quartz cuvette and 50 µl of sample added. The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 min. The activity of catalase in µmole $H_2O_2$/min/mg protein was determined using the formula below:

$$\text{Catalase activity} = \frac{\Delta A_{240/\text{min}} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/ml}}$$

**Malondialdehyde determination:** Lipid peroxidation was determined by measuring the levels of malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale [25]. An aliquot of 0.4 ml of the test sample was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% trichloroacetic acid (TCA) was added. Then 0.5 ml of 0.75% 2-thiobarbituric acid (TBA) was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice to room temperature and centrifuged at 3000 rpm for 10 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein was computed with a molar extinction coefficient of 0.156 $\mu M^{-1}cm^{-1}$ Source.

$$\text{Lipid peroxidation (mM MDA formed)} = \frac{\text{Absorbance} \times \text{Vol.of mixture}}{\sum 532\text{nm} \times \text{Vol.of sample}}$$

## Statistical Analysis

The data obtained in this study were subjected to one-way analysis of variance (ANOVA) and represented as Mean ± SD. The differences between groups were considered statistically significant at $P<0.05$.

## Results

It was observed that animals that were treated with *Annona muricata* seed extract recorded a higher body weight (results not shown) when compared to those in the control and DMBA only groups.

## Qualitative and quantitative phytochemical screening

The result obtained from the qualitative phytochemical screening of the ethanolic extract of *A. muricata* seeds is shown in Table 1. Phytochemical screening revealed the presence of flavonoid, phenol, tannin, terpenoid, alkaloids, cardiac glycoside, steroid and anthraquinone while phlobatannin was not detected.

The results of the quantitative phytochemical screening of selected phytochemicals present in the ethanol extract of *Annona muricata* seeds is shown in Table 2. It revealed that flavonoids was present in the highest amount (27.52 mg/100 g), followed by phenol (26.74 mg/100 g), tannin (21.95 mg/100 g), steroid (20.23 mg/100 g), while alkaloid was found to be in the lowest amount (19.25 mg/100 g).

## Effect of *Annona muricata* seed extract on estradiol and cholesterol levels in DMBA-treated rats

As shown in Table 3, there was a significant (P<0.05) increase in the estradiol levels of all the DMBA-treated groups that received the extract as compared to the DMBA only group and the control group. However, the highest significant estradiol level was observed in the group that received the 200 mg/kg per body weight *A. muricata* seed extract. Also, there was a significant (P<0.05) decrease in the cholesterol levels in all the groups as compared to the control group. The lowest cholesterol level was recorded in the DMBA-treated group that received 100 mg/kg per body weight of *A. muricata* seed extract.

| Phytochemicals | *A. muricata* seed ethanol extract |
|---|---|
| Flavonoid | + |
| Phenol | + |
| Tannin | + |
| Terpenoid | + |
| Alkaloid | + |
| Steroid | + |
| Anthraquinone | + |
| Phlobatannin | - |

**Table 1:** Qualitative phytochemical analysis of *Annona muricata* seeds ethanol extract.

| Phytochemicals | *A. muricata* seed extract (mg/100 g) |
|---|---|
| Flavonoid | 27.52 ± 0.82 |
| Phenol | 26.74 ± 0.43 |
| Tannin | 21.95 ± 0.25 |
| Alkaloid | 19.25 ± 0.22 |
| Steroid | 20.23 ± 0.25 |

Results are expressed as Mean ± SD of three determinations

**Table 2**: Quantitative phytochemical analysis of *Annona muricata* seeds ethanolic extract.

| Group | Estradiol (pg/ml) | Cholesterol (mg/dL) |
|---|---|---|
| I | 4.090 ± 0.057 | 147.6 ± 1.365 |
| II | 21.98 ± 0.509$^{****}$ | 91.21 ± 0.587$^{****}$ |
| III | 2.875 ± 0.248$^{*}$ | 131.6 ± 0.735$^{****}$ |
| IV | 12.25 ± 0.127$^{***}$ | 94.75 ± 0.304$^{****}$ |
| V | 32.20 ± 0.332$^{****}$ | 87.58 ± 0.068$^{****}$ |
| VI | 35.51 ± 0.523$^{****}$ | 95.68 ± 0.636$^{****}$ |

Data are represented as Mean ± SD for five rats (n=5) in each group and are significantly different at $P<0.05$

$^{*}$Statistically different compared to positive control (Group I) at (P<0.05)

I- Control group; II- 200 mg/kg A.M seed extract only; III- 20 mg/kg DMBA only; IV- 20 mg/kg DMBA followed by Tamoxifen (20 mg/kg); V- 20 mg/kg DMBA followed by 100 mg/kg A.M seed extract; VI- 20 mg/kg DMBA followed by 200 mg/kg A.M seed extract

**Table 3:** Effect of *A. muricata* extract on serum estradiol and cholesterol levels in DMBA-treated female rats.

| Group | SOD (U/L) | CAT (µMol $H_2O_2$ consumed/min) | MDA (mMol) |
|---|---|---|---|
| I | 5.99 ± 1.32$^{****}$ | 0.21 ± 0.09$^{****}$ | 5.71 ± 0.92$^{****}$ |
| II | 6.35 ± 3.34$^{*****}$ | 0.13 ± 0.04$^{*}$ | 8.37 ± 1.52$^{****}$ |
| III | 5.09 ± 0.69$^{****}$ | 0.14 ± 0.04$^{*}$ | 8.43 ± 0.84$^{****}$ |
| IV | 4.68 ± 1.87$^{*****}$ | 0.15 ± 0.05$^{*}$ | 6.72 ± 1.92$^{*****}$ |
| V | 6.25 ± 2.01$^{***}$ | 0.10 ± 0.02$^{*}$ | 6.47 ± 1.41$^{****}$ |
| VI | 6.79 ± 3.75$^{****}$ | 0.16 ± 0.06 $^{*}$ | 7.20 ± 1.85$^{*****}$ |

Data are represented as Mean ± SD and are significantly different at $P<0.05$

I- Control group; II- 200 mg/kg A.M seed extract only; III- 20 mg/kg DMBA only; IV- 20 mg/kg DMBA followed by Tamoxifen (20 mg/kg); V- 20 mg/kg DMBA followed by 100 mg/kg A.M seed extract; VI-20 mg/kg DMBA followed by 200 mg/kg A.M seed extract

**Table 4:** Effect of *A. muricata* seed extract on serum SOD, CAT and MDA levels in DMBA treated rats.

## Effect of *A. muricata* extract on serum levels of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) in DMBA treated rats

As shown in Table 4, there was a significant (P<0.05) higher level of SOD in group VI (20 mg/kg DMBA followed by 200 mg/kg A.M seed extract) when compared to the other groups, followed by the group that received extract only while the lowest SOD value was observed in group IV. There was a significant (P<0.05) decrease in the catalase level of all the groups as compared to that of the control but there was a significant (P<0.05) increase in the CAT level of the group that received DMBA followed by 200 mg/kg A.M extract as compared to the DMBA only group.

Also, a significant (P<0.05) increase in the MDA level was observed in the DMBA only group (Group III) when compared to that of the normal control rats. However, there was a significant (P<0.05) decrease in the MDA level of the groups that were treated with DMBA followed by A.M seed extract had a significant (P<0.05) decrease in MDA level as compared to the DMBA positive control group with the group that received 100 mg/kg per body weight of the extract having a much more lower MDA level.

## Discussion

Breast cancer has been adduced as the commonest type of cancer that is responsible for the death of most Nigerian women has been linked to many etiological factors of which hormones and lifestyle is among. The phytoestrogenic, hypocholesterolemic and antioxidant activities of *A. muricata* seed extract on 7,12-dimethylbenzeneanthracene (DMBA)-induced breast cancer in rat was evaluated in this study. DMBA is a known potent carcinogen which has been used extensively in cancer research. Tumor progression is inevitably followed by an increase in the oxidation process [26].

Phytochemical screening of the *A. muricata* seed extract revealed the presence of flavonoid, phenol, tannin, alkaloid, cardiac glycoside and steroid with the highest being flavonoid. Phytochemicals are biologically active compounds which are not conventional nutrients but which nonetheless contribute significantly to protection against degenerative diseases [27]. It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities [28,29] and they might induce mechanism that affect cancer cells and inhibit tumor invasion [30]. These activities could be attributed to their ability to neutralize and quench free radicals [28,29,31]. It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation [32]. Tannins are

known to be beneficial for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [33-35].

The observed non-significant increased body weight in the groups treated with *Annona muricata* might have resulted from the high caloric value of the seed as reported by Onimawo [36] who found out that *A. muricata* seed has a high carbohydrate content (55.1%) which suggests a high caloric value of the seed.

As observed in this study, *A. muricata* seed extract demonstrated a high phytoestrogenic activity as there was a higher concentration of estradiol in the groups treated with the extract as compared to the DMBA only and control groups. High estrogen levels though have been reported as a risk factor for breast cancer [37], yet other studies showed that estrogen may have a protective effect against breast cancer and might also lower its risk [38,39]. This might be a better substitute for synthetic estrogens as hormone replacement. The phytoestrogenic activity of *A. muricata* seed extract might be attributed to the presence of fair amounts of steroids in the seed extract as they are also closely related to sex hormones in man. Moreover, a scan for the flavonoids present in *Annona muricata* extract revealed rutin as the most abundant flavonoid followed by quercetin-3-glycoside, quercetin and kaempferol as reported by Chunhua et al. [40]. These flavonoids have been reported to contain phytoestrogen which are similar to endogenous steroid hormones, with analogous functions in our body [41]. They could act on cells or tissues by competitively binding to estrogen receptors with endogenous estrogens during developmental and reproductive stages. Actual research shows that phytoestrogens have been shown to have diverse effects depending on their concentrations [42]. A second mechanism of action for phytoestrogens may be their ability to affect the endogenous production of estrogen. The pituitary gland releases gonadotrophins that stimulate estrogen synthesis in the ovaries [43].

High cholesterol levels have been discovered to stimulate a pro-inflammatory reaction that further contributes to the initiation and progression of breast cancer [44]. As observed from our results, post administration of *A. muricata* seed extract in DMBA treated rats resulted in a significant reduction in the cholesterol levels. This is in alignment with a similar study on *Piper aduncum* capsule by Arroyo-Acevedo et al. [45] who found out that the plant has a lipid lowering effect in mice. There was a statistically significant reduction ($p<0.05$) in the level of cholesterol of treated experimental animals compared to the control groups as shown in Table 3. This reduction in cholesterol level might be attributed to the presence of fibre. Results of proximate analysis on *Annona muricata* seeds by Fasakin et al. [46] shows that its crude fiber content is higher (43.44%) when compared to *Ipomea batatas*, *T. triangulare, P. guineensis* and *Corchorus olitorius* [47,48]. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Adequate intake of dietary fibre has been found to reduce the serum cholesterol level and breast cancer [49,50]. Flavanoids may boost the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells [51].

Reactive oxygen species have been implicated in the initiation and progression of breast cancer. Superoxide dismutase and catalase are endogenous antioxidant enzymes that help to scavenge free radicals *in vivo*. Malondialdehyde (MDA) is an index of lipid peroxidation that is a by-product of lipid peroxidation which also leads to the generation of reactive oxygen species. Tumor progression due to DMBA is virtually always accompanied by an increase in the oxidation process. As seen from the results, there was a significant ($p<0.05$) increase in the SOD and catalase levels but a significant ($P<0.05$) decrease in the MDA levels of the DMBA groups treated with *A. muricata* seed extract as compared to the DMBA only group. This suggests an antioxidant activity of *A. muricata* seed extract which justifies its utilisation as an anti-tumor and anti-cancer agent. In a study by Minari and Okeke [52], they reported that there was a reduced lobular alveolar hyperplasia in the breast tissues of the mice treated with the ethanolic extract of *A. muricata* leaves which suggests a chemopreventive effect of the extract on DMBA-induced cell proliferation. The literature is replete with previous reports which confirm the antioxidant potential of the plant [53-55] which is adduced to its rich constituents of phytochemicals most especially flavonoids and phenolics. Phytochemicals have been shown to stimulate synthesis of antioxidants enzymes and detoxification systems at the transcriptional level through antioxidant response elements [56].

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities [28,29] and they might induce mechanism that affect cancer cells and inhibit tumor invasion [30]. These activities could be attributed to their ability to scavenge free radicals [28,29,31]. It can also be adduced to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation [32].

## Conclusion

This study demonstrated the phytoestrogenic, hypocholesterolemic and antioxidant activity of *Annona muricata* seed extract in DMBA-treated rats and suggests a rationale for its potential as a natural hormone replacement therapy and also in the management of other oxidative-stress induced diseases such as diabetes. However, further research is recommended to elucidate its actual mechanism of action and the isolation of the specific lead compounds in the seed that is responsible for its pharmacotherapeutic activities. An assessment of its anti-tumor activity *in vitro* and *in vivo* is also recommended.

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