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Original Research Article

Hexane Fraction of *Annona muricata* (Sour Sop) Seed Inhibits 7,12-Dimethylbenz(A)Anthracene-Induced Mammary Gland Tumorigenesis in Rats

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Abstract

Traditional medicine uses *Annona muricata* to treat a variety of illnesses. This study aimed to assess the anti-proliferative effects of the hexane fraction of *Annona muricata* (HFAM) seed in MCF-7 cells and 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary gland tumorigenesis in female rats. Forty *Wistar* rats were divided into five equal groups *in vivo*. The control group was group 1, the DMBA (50 mg/kg) was assigned to group 2, the DMBA (50 mg/kg) + HFAM (100 mg/kg) was given to group 3, the DMBA (50 mg/kg) + HFAM (200 mg/kg) was given to group 4, and the HFAM (200 mg/kg) was given to group 5. The HFAM inhibited growth, elicited anti-inflammatory, antioxidative, and pro-apoptotic activities in MCF-cells. The HFAM decreased IL-1β, MPO, LPO and increased SOD, CAT, BAX, Caspases-3 and-9 in MCF-7 cells by 45%, 82%, 46%, 44%, 41%, 168%, 22% and 17%, respectively. *In vivo*, DMBA decreased body weight gain and increased organo-somatic weight of the mammary gland by 35% and 92%, respectively. Also, DMBA decreased the activities of mammary catalase, glutathione-s-transferase, glutathione peroxidase, and superoxide dismutase by 40%, 66%, 45% and 41%, respectively, while lipid peroxidation increased by 61%. The GC-MS analysis revealed Tirucallol as the most abundant compound. Histology showed glands with malignant epithelial cells and high nucleocytoplasmic in DMBA-administered rats. Interestingly, HFAM decreased inflammation, oxidative stress, and restored the cyto-architecture of glands in DMBA-treated rats. Taken together, HFAM confers protection against DMBA-induced mammary toxicity via anti-inflammatory, antioxidative, and pro-apoptotic activities.

Keywords: Mammary gland, Antioxidants, Oxidative stress, *Annona muricata,* Inflammation, Cancer.

1. INTRODUCTION

Breast cancer is frequently diagnosed in women, and it ranks second globally in terms of cancer-related deaths, after lung cancer (Grabinsky and Brawley, 2022). It (cancer cells) reproduces abnormally, causing apoptosis to be suppressed, inflammation to increase, and metastasis to occur (Lakshmi and Subramanian, 2014). According to Bray *et al.,* (2022), there are an expected 400,000 deaths and 1.5 million new cases of breast cancer each year. Chemotherapy, radiation therapy, and surgery are currently used to treat breast cancer; each of these approaches has a different level of efficacy in treating metastatic illness. Nevertheless, a growing amount of data suggests that unique disease recurrence patterns represent a significant obstacle to breast cancer treatment (Yu *et al.,* 2022). Research into more effective treatment options has drawn a lot of attention due to the higher death rate from breast cancer.

Annona muricata, also known as Sour Sop, is only found in the warmest regions of the tropics of South and Central America, Western Africa, and Southeast Asia. It is a tree that reaches a height of about 58 m, its diameter is 1583 cm, and it features a round, open canopy covered with large, glossy, dark green leaves (Santos *et al*, 2023). It has green, edible fruits that measure 1520 centimeters in diameter and are heart-shaped. They have

creamy white flesh that smells and tastes delicious. Many indigenous cultures in Africa and other tropical and subtropical parts of the world use *A. muricata* in folk medicine extensively. Traditional medicine uses many parts of this plant to treat various ailments and diseases, including inflammation (Abdul-Wahab *et al*, 2018), rheumatism (Mishra *et al*, 2013), diabetes (Moghadamtousi *et al*, 2015), and hypertension (Sokpe, 2020). A number of investigations have confirmed these activities, including anticancer (Syed *et al*, 2016; Ilango *et al*, 2022; Nambooz *et al*, 2024), anticonvulsant, anti-arthritic, antimalarial, hepatoprotective, and antidiabetic (Moghadamtousi *et al*, 2015). An earlier phytochemical study of *A. muricata* found high concentrations of secondary class metabolite compounds, including alkaloids, saponins, terpenes, flavonoids, coumarins, tannins, cardiac glucosides, phenols, and phytosterols (Gavamukulya *et al*, 2014; Nambooz *et al*, 2024). There have been several studies that reported the antiproliferative effects of acetogenins (Annonaceous acetogenins) in plants and their isolated extracts on various cancer cell lines (Astirin *et al*, 2013; Gavamukulya *et al*, 2014; Syed *et al*, 2016; Ilango *et al*, 2022; Nambooz *et al*, 2024), but few of these investigations have explored the mechanism of action behind the effects. In addition, *in vivo* studies conducted by our research group confirmed that extract from *A. muricata* seeds protected against testosterone-induced benign prostatic hyperplasia in rats via mechanisms that involved the induction of apoptotic and antioxidative pathways, and inhibition of inflammation (Adaramoye *et al*, 2019). The current study aimed to determine the effect of n-hexane extract of *A. muricata* seed on an established cancer cell line (MCF-7) and DMBA-induced mammary gland damage in rats.

2. MATERIALS AND METHODS

2.1. Plant material extraction and GC-MS analysis of the extract:

The *A. muricata* fruits were purchased commercially. The fruits were washed, hulled, and the fleshy tissue squeezed to extract the seeds, which were then cleaned and air dried for 28 days. The fruits' seeds were removed and air-dried to room temperature. The seeds' rigid outer shells were manually fractured, revealing their interior fleshy core, which was air-dried. Approximately 970 grams of dried seeds were ground into a fine powder. N-hexane was extracted using soxhlet extractors. The extracts were concentrated using a rotary evaporator, then oven dried at 40ºC. After extraction, the n-hexane extract yielded 20% and was refrigerated until used. Our earlier publication (Adaramoye *et al.,* 2018) included a gas chromatography-mass spectrometry (GC-MS) investigation of the n-hexane fraction in *A. muricata* seeds. The hexane extract was examined with gas chromatography-mass spectrometry. The following were the experimental conditions for the system. The TR 5-MS capillary standard non-polar column featured a film thickness of 0.25 µm, a dimension of 30 Mts, and an ID of 0.25 mm. The mobile phase flow rate (carrier gas: helium) was fixed to one milliliter per minute. The oven temperature in gas chromatography was initially set at 40°C and gradually increased to 250°C at a rate of 5°C per minute. One microlitre was used for injection. The sample was run between 50 and 650 m/z, and the data were compared using the Wiley Spectral library search tool.

2.2 Chemicals

DMBA (7,12-dimethylbenz(a)anthracene) was purchased from Sigma, St. Louis, MO, USA, and stored at 4°C in the dark. Before use, DMBA was dissolved in corn oil. Other chemicals and reagents were of analytical grade and the purest available.

2.3 Experimental Rats:

We got female Wistar rats weighing 30-40 grams from the Central Animal House, Department of Veterinary Medicine, at the age of five weeks. Animals were housed in plastic cages in five groups with the following conditions: 25 ± 3°C, 60 ± 10% humidity, and 12 hours of daylight. Every day during the experiment, the animals were fed experimental diets and given water. The University of Ibadan Animals' Ethics Committee authorized the experimental procedure, as well as the handling and care of rats (UI-ACUREC/App/2015/061).

2.4 Design of the study:

Five sets of eight female rats each were put together. Before the experiment, the animals were acclimated for fourteen days. Group 1 was given maize oil as a control; Group 2 was given 50 mg/kg of DMBA; Group 3 was given 50 mg/kg of DMBA and 100 mg/kg of HFAM; Group 4 was given 50 mg/kg of DMBA and 200 mg/kg of HFAM; and Group 5 was given 200 mg/kg of HFAM. At 7 weeks old, a single dosage of DMBA was given intraperitoneally. For twelve weeks, the HFAM was delivered via oral gavage three times each week. After administering the final dose of HFAM, we starved the rats overnight and sacrificed them the following day.

2.5 The isolation, preservation, and processing of tissues

We removed the mammary glands, cleaned them with an ice-cold 1.15% KCl solution to eliminate bloodstains, dried them, and weighed them. A piece of the mammary gland was fixed in 10% formalin before being histologically examined. The post-mitochondrial fraction (PMF) was obtained as part of the biochemical analysis by homogenizing the gland in 4 volumes of 50 mM phosphate buffer solution at pH 7.4 for 15 minutes and centrifuged at 10,000g for 15 minutes. The entire procedure was conducted at 4 degrees Celsius.

2.6 Serum Preparation:

Blood was obtained via ocular puncture into simple centrifuge tubes and left to coagulate for one hour. Serum was produced by centrifugation at 3000g for

10 minutes in a bench centrifuge. The clear supernatant was used to conduct biochemical investigations.

2.7 Cell proliferation and viability test

MTT test was performed following Zhou *et al.,* (2014) methods.

2.7.1 Biochemical parameters on cell lysates

The effects of HFAM on biochemical parameters were investigated by seeding MCF-7 cells in 96-well plates. After a 24-hour incubation period, the cells were treated with 90.5 µg/mL (IC_{50}) with HFAM for 24 hours. To separate the supernatant from the cell debris, cells were then extracted, washed, and lysed using lysis buffer before being centrifuged for 10 minutes at 10,000 x g. Biochemical tests were conducted using the cell lysates.

2.7.2 ELISA immunoassay

Using an enzyme-linked immunoassay kit (Thermo Scientific, Waltham, MA; Cayman Chemical, Ann Arbor, MI; and Thermo Scientific, Waltham, MA, respectively), biochemical indices in serum and cell lysates were tested for BAX, caspase 3, caspase 9, and interleukin-1β (IL-1β) antibodies following the manufacturer's instructions The samples were put into the micro ELISA plate wells that had already been coated with antibodies against the rat proteins BAX, caspase 3, caspase 9, and IL-1β. Subsequently, each microplate well was filled with a specific biotinylated detection antibody and an Avidin-Horseradish Peroxidase (HRP) conjugate, which were then incubated. Following the removal of the free components, the substrate solution was applied to each well. The rats' BAX, caspase 3, caspase 9, and IL-1β, as well as the biotinylated detection antibody and Avidin-(HRP) conjugate, made the wells appear blue. The addition of the stop solution stopped the enzyme-substrate reaction, and the color turned yellow. A Biorad microplate reader set to 450 nm was used to determine the optical density. By extrapolating from the standard curves, the optical density values were determined and are proportional to the quantities of the rat BAX, caspase 3, caspase 9, and IL-1β proteins in the samples.

2.8 *In vivo* Biochemical tests

2.8.1 Protein determination level

Protein levels in serum and mammary tissue were measured using the Lowry et al. (1951) technique.

2.8.2 Assessment of biomarkers of mammary oxidative stress

The activity of superoxide dismutase (SOD) was measured using the McCord and Fridovich technique (1969). The reduced glutathione (GSH) was measured at 412 nm using Moron et al.'s (1979) technique. Catalase (CAT) activity was evaluated using hydrogen peroxide as a substrate, following Aebi's (1974) approach. Glutathione-s-transferase (GST) activity was measured using the procedures published by Habig *et al.,* (1974), with CDNB as a substrate. The activity of glutathione peroxidase (GPx) was measured using the method described by Rotruck *et al.,* (1973). Lipid peroxidation was measured as MDA using the method given by Buege and Aust (1978). The mammary nitrite concentration was measured using a sodium nitrite curve and expressed as µM of nitrites/mg protein, as published by Palmer *et al.,* (1978). The activity of myeloperoxidase (MPO) in mammary tissues was evaluated using the Trush *et al.,* (1994) method. Total sulphydryl (TSH) levels in mammary tissue were assessed using Ellman's (1979) method, with minor modifications by Adefisan *et al.,* (2019).

2.9 Histology

Mammary tissue sections fixed in 10% formalin were dehydrated in 95% ethanol, then cleaned in xylene before being embedded in paraffin. Micro sections (3 µm) were produced, stained with haematoxylin and eosin (H&E), and inspected under a light microscope by a histopathologist unaware of treatment groups.

2.10 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of eight rats per group. One-way analysis of variance (ANOVA) was used to analyze the data. Statistical significance was determined at $p<0.05$.

3. RESULTS

3.1 Effects of HFAM on growth, apoptosis and inflammation in the MCF-7 cell line

Cell viability was tested on MCF-7 cell line to assess the anti-proliferative effect of HFAM. Administration of HFAM (5, 25, 50, and 100 μg/mL) led to concentration-dependent increase in growth inhibition of the MCF-7 cell line at 72h (Figure 1). The IC_{50} value of 90.5 µg/mL was obtained for HFAM against MCF-7 cell line at 72h. The mechanisms of cell death induced by HFAM was investigated by observing its effects on protein regulators of apoptosis and inflammation (Figures 2-4). The HFAM at IC_{50} value inhibited oxidative and inflammatory reactions within the MCF-7 evidenced by the reduction in the levels of LPO, myeloperoxidase and IL-1β by 46, 82 and 45%, respectively (Figure 2), Furthermore, HFAM increased the levels of apoptotic markers; caspases-3 and -9 and, BAX by 22, 17 and 168 %, respectively (Figure 4) and was accompanied by induction of antioxidative enzymes; superoxide dismutase and catalase (Figure 3).

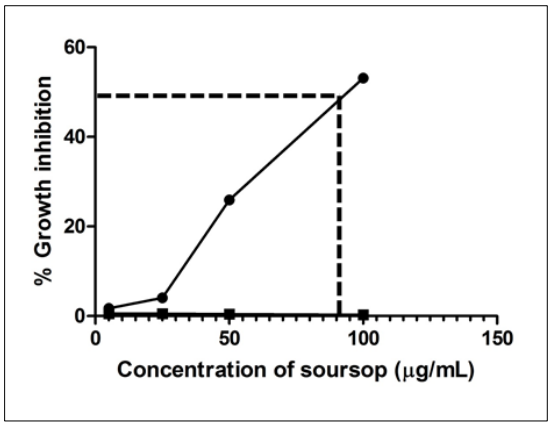


Figure 1: Effect of Sour sop (HFAM) on growth inhibition in MCF-7 cell line showing the minimum inhibitory concentration (IC_{50})

HFAM= hexane fraction of *Annona muricata*

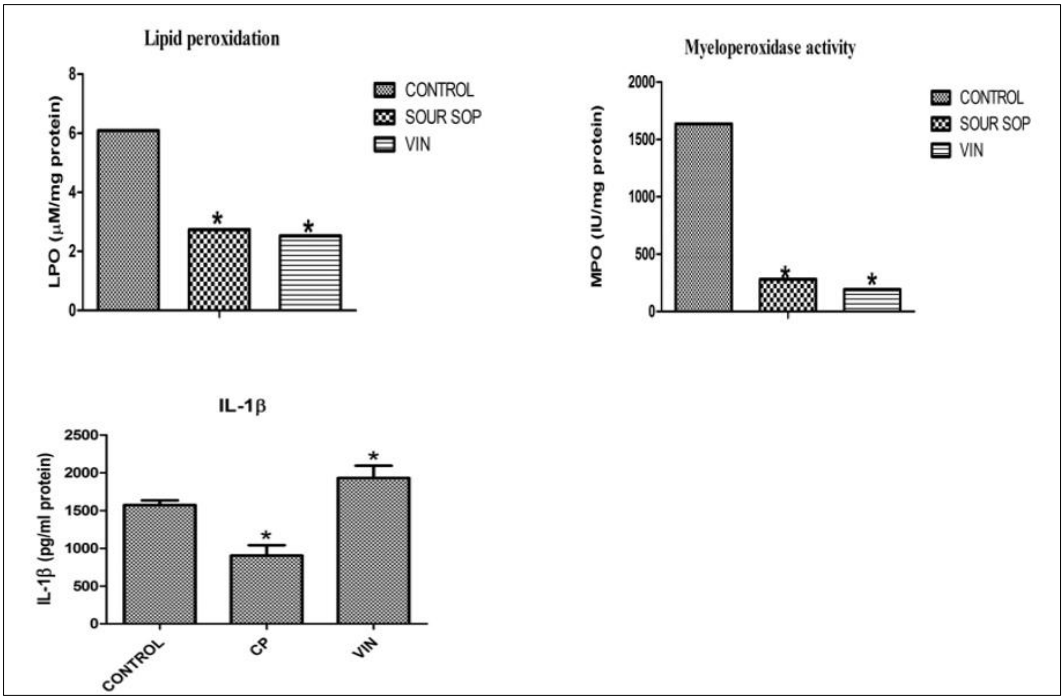


Figure 2: Effects of Sour sop (HFAM) on the process of lipid peroxidation, myeloperoxidase and interleukin 1beta activities in MCF-7 cell line

HFAM= hexane fraction of *Annona muricata*

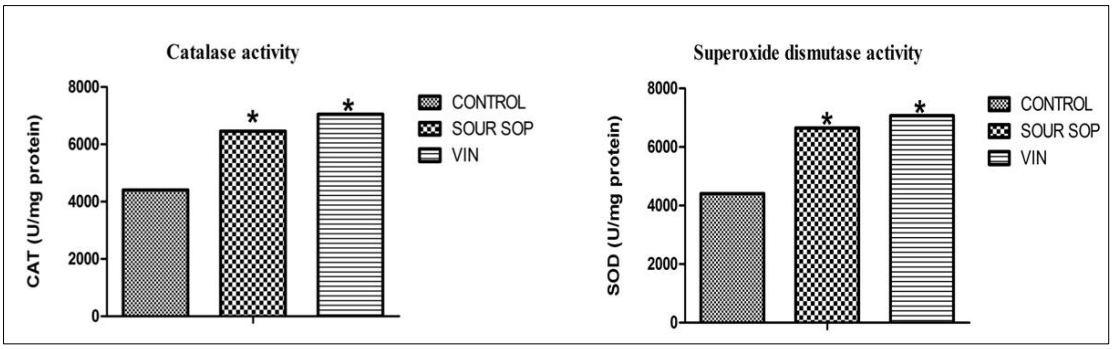


Figure 3: Effects of Sour sop (HFAM) on the activities of superoxide dismutase (SOD) and catalase (CAT) in MCF-7 cell line

HFAM= hexane fraction of *Annona muricata*

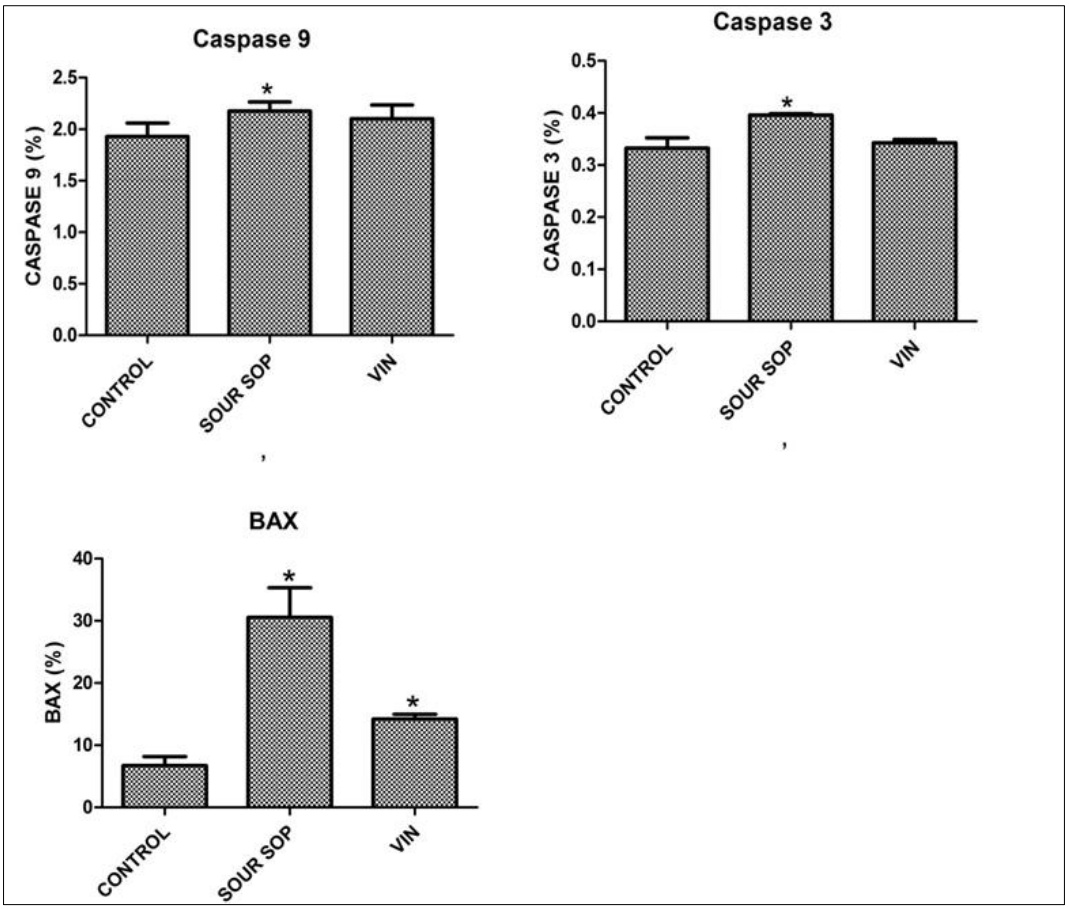


Figure 4: Effects of Sour sop (HFAM) on the levels of caspases-3, -9 and BAX in MCF-7 cell line
HFAM= hexane fraction of *Annona muricata*

3.2 Effects of HFAM on body and organo-somatic weights of DMBA-treated rats

Table 1 presents a comparison of the total weight, organ weight, and organo-somatic weight of rats treated with DMBA and HFAM. Rats given DMBA experienced a 35% reduction in body weight growth in comparison to the control group. Conversely, compared to control rats, the mammary gland weight and organo-somatic weight of DMBA-administered rats increased significantly by 57% and 92%, respectively. Co-treatment with HFAM significantly reduced the animals' organo-somatic weight of the mammary gland and body weight gain to levels close to the control values.

Table 1: Effect of HFAM on organs' and mammary tissue's weight of rats given DMBA

Treatments	Body Weights			Mammary Tissue	Organosomatic
	Initial (g)	Final (g)	Weight Gain (g)	Weight (g)	Weight (as % Body wt)
CONTROL	52.6±14.94	156.2±12.35	103.6±16.33	2.52±0.59	1.62±0.39
DMBA	59.5±5.07	127.3±14.73	67.75±17.13*	3.96±0.45*	3.11±0.33*
DMBA+SS1	55.3±5.72	146.0±12.67	90.67±12.26	2.76±0.92**	1.89±0.52**
DMBA+SS2	56.8±10.05	159.3±5.32	102.5±7.05**	2.80±1.01**	1.76±0.76**
SS2	40.5±5.04	143.0±15.64	102.5±16.71**	3.16±0.43	2.21±0.39

The mean ± standard deviation for each group of eight animals was used to express the values. *= significantly different from control ($P < 0.05$) ** =significantly different from DMBA ($P < 0.05$), SS= Sour Sop= HFAM= Hexane fraction of *Annona muricata* . DMBA= 7,12-dimethylbenzanthracene; SS 1= Sour sop (100mg/kg); SS 2= Sour sop (200mg/kg)

3.3 Effects of HFAM on antioxidant enzymes, apoptosis, and inflammatory indices of DMBA-treated rats

Table 2, Figures 5 and 6 demonstrate that DMBA-administration caused oxidative stress within the mammary gland evidenced by increase in the levels of malondialdehyde by 61 % when compared control, and was followed by a sharp decline in the activities of antioxidative enzymes; superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and levels of reduced glutathione. Furthermore, levels of BAX and caspase-9 were significantly reduced, while caspase-3 and IL-1β were insignificantly affected in DMBA-treated rats (Figures 6 and 7). Treatment with

HFAM (200 mg/kg) increased the activities of superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase and decreased malondialdehyde level in the mammary tissues of the rats. Likewise, BAX and caspase-9 levels were elevated in DMBA-exposed rats that received HFAM.

Table 2: Effect of HFAM seed on antioxidant parameters in mammary tissue of rats given DMBA

Treatments	GPX (Nmol/Mg	LPO (Mg/G Mg Protein)	GSH (Mg/G) Protein/Min)
Control	50.27±13.27	3.65±1.13	170.08±5.76
DMBA	29.67±0.45*	5.87±0.13*	150.00±4.42*
DMBA+SS1	42.08±1.27**	4.07±0.45	147.27±8.25
DMBA+SS2	43.08±4.14**	3.31±0.28**	168.40±7.82**
SS2	52.39±2.83	3.71±1.03	182.64±4.73

Values are expressed as Mean ± Standard deviation of 8 animals per group.
*= significantly different from control (P < 0.05)
** =significantly different from DMBA (P < 0.05), SS= Sour Sop= HFAM= Hexane fraction of *Annona muricata* .
DMBA= 7,12-dimethylbenzanthracene; SS 1= Sour sop (100mg/kg); SS 2= Sour sop (200mg/kg)

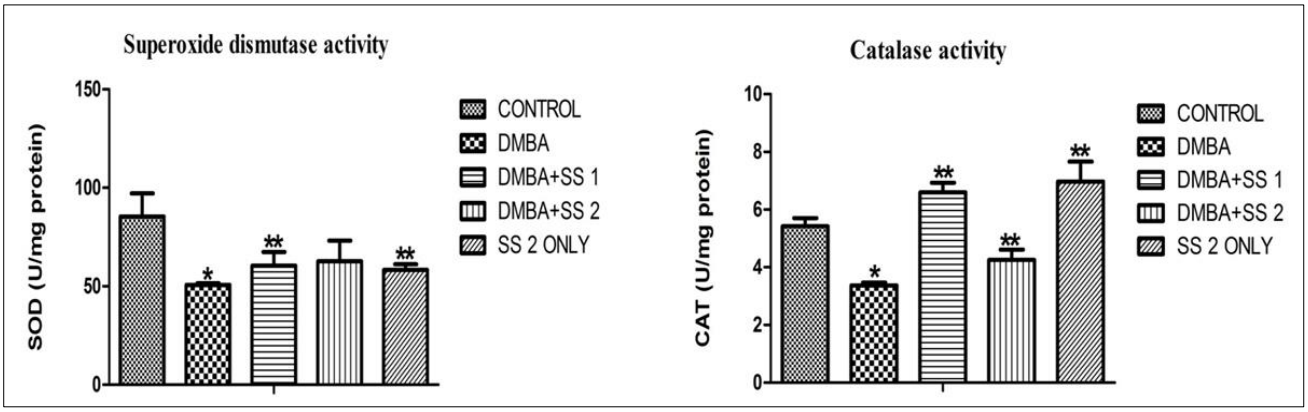


Figure 5: Effects of Sour sop (HFAM) on the activities of mammary superoxide dismutase (SOD) and catalase (CAT) in rats treated with DMBA

HFAM = hexane fraction of Annona muricata, *= significantly different from control (P < 0.05), ** = significantly different from DMBA (P < 0.05)

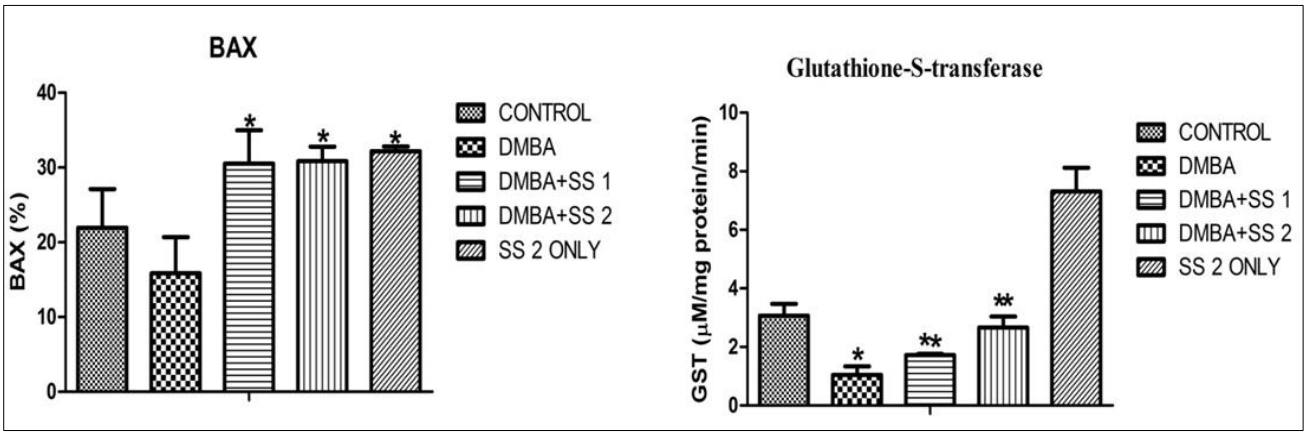


Figure 6: Effects of Sour sop (HFAM) on the level of BAX and activities of mammary glutathione-s-transferase (GST) in rats treated with DMBA

HFAM = hexane fraction of Annona muricata, *= significantly different from control (P < 0.05), ** = significantly different from DMBA (P < 0.05)

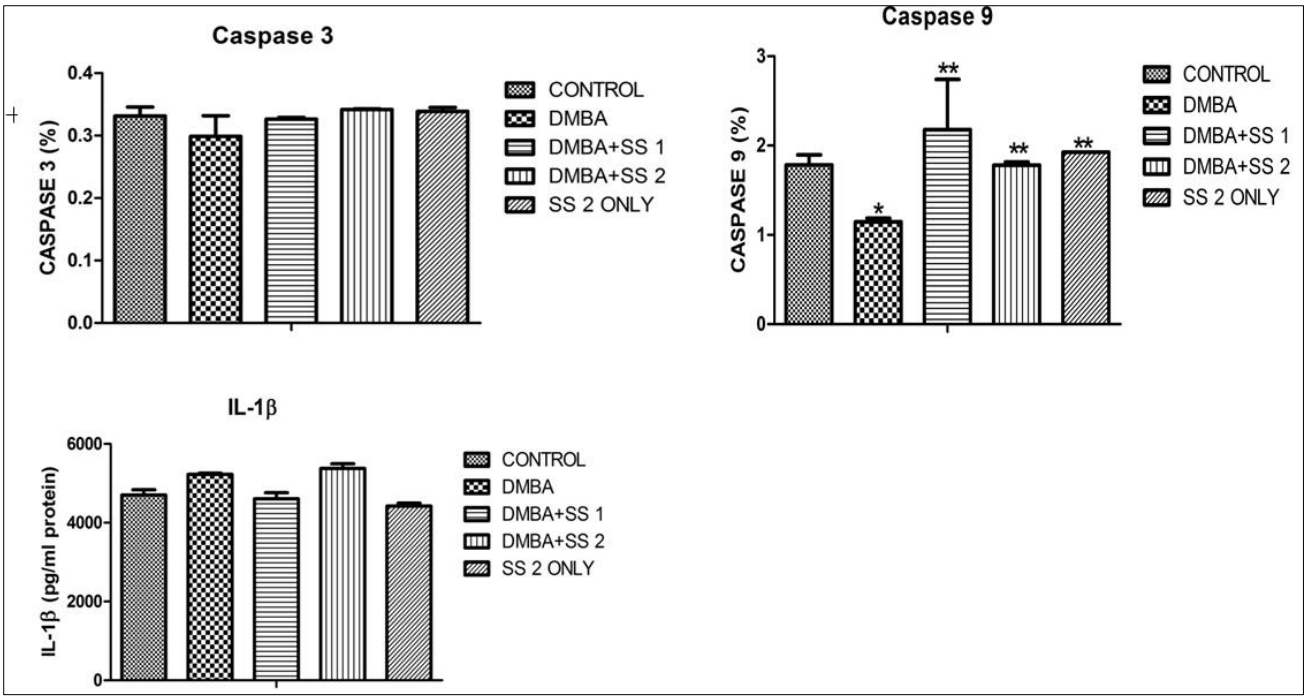


Figure 7: Effects of Sour sop (HFAM) on the levels of caspases-3, -9 and IL-1β in rats treated with DMBA

HFAM = hexane fraction of Annona muricata, *= significantly different from control (P < 0.05), ** = significantly different from DMBA (P < 0.05)

3.4 Gas chromatography-mass spectrometry analysis of *Annona muricata*

Adaramoye *et al.,* (2018) shows that n-hexane seed extract of *Annona muricata* contained seven main compounds (Table 3); 9,19-cyclolanostan-3- ol,24-methlene-(3β), Tirucallol, 9,19-cyclolanost-24-en-3-ol, (3β), Lupeol, β-Amyrin, Lanost-7-en-3-one,(9β, 13α, 14β, 17α), 2,8-Dimethyl-2-(3E,7E)-4,8,12-Trimethyltrideca, and 3,7,11- trien-1-yl)chroman-6-ol. Out of the compounds identified, compound 2 (Tirucallol) was the most abundant while 9,19-cyclolanostan-3- ol,24-methlene-,(3β) is the least compound identified by GC-MS (Table 3).

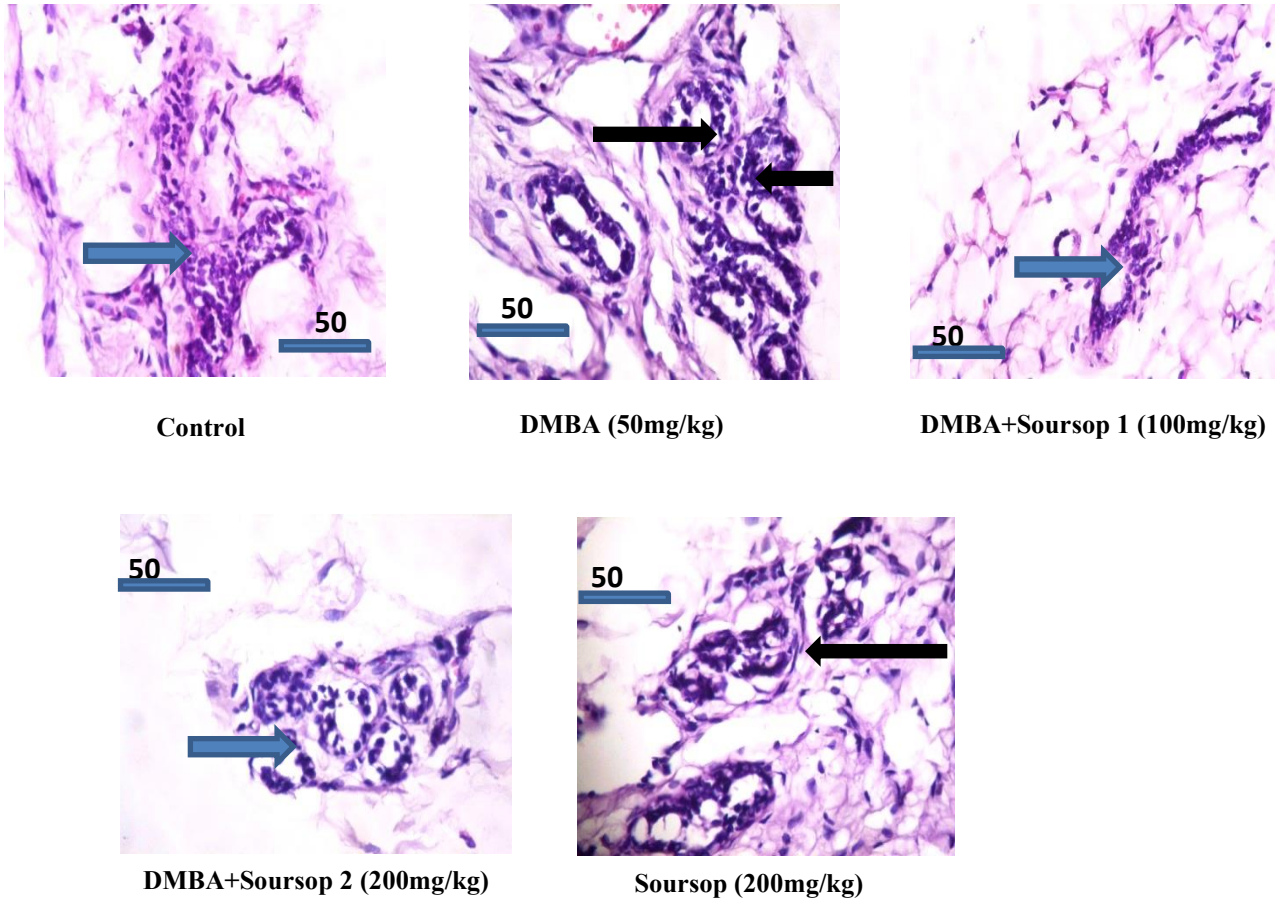
Table 3: Putatively identified compounds from n-hexane extract of *Annona muricata* seed

GC Peak No	Compounds	Retention Time	Quantity %
1.	9,19-cyclolanostan-3- ol,24-methlene-,(3β)-	39.30	1.66
2.	Tirucallol	41.70	35.10
3.	9,19-cyclolanost-24-en-3-ol, (3β)	42.31	23.75
4.	Lupeol	42.56	7.44
5.	β-Amyrin	42.73	7.67
6.	Lanost-7-en-3-one,(9β, 13α, 14β, 17α)	42.19	3.30
7.	2,8-Dimethyl-2-(3E,7E)-4,8,12-Trimethyltrideca-	44.08	21.18

Source: Adaramoye *et al.,* 2018

3.5 Effects of HFAM on the histology of mammary glands of DMBA-treated rats

Figure 8 depicts the effects of HFAM on the cyto-architecture of mammary glands of rats administered DMBA. In the control slides, the stroma and adipose tissue in the mammary area had normal appearance with very slight necrosis, but in the DMBA-treated rats, the periductal fibrous tissues and inflammatory cells were markedly enhanced. However, the periductal fibrous tissues and stroma of HFAM-administered group (100 and 200 mg/kg) were normal and did not contain any inflammatory cells.



Control **DMBA (50mg/kg)** **DMBA+Soursop 1 (100mg/kg)**

DMBA+Soursop 2 (200mg/kg) **Soursop (200mg/kg)**

Figure 8: Effect of sour sop (HFAM) on the cyto-architecture of the mammary gland of rats treated with DMBA (7,12-dimethylbenzanthracene)

4. DISCUSSION

Although there have been advancements in diagnosis and treatment for breast cancer, it remains a significant health issue for women (Barrios, 2022). In recent years, researchers have become increasingly interested in plant phenolic compounds due to their health benefits. People who consume a diet high in phytochemicals, particularly phenolic compounds, have a lower chance of developing several diseases, including cancer. The phenolic compounds in herbal extracts have demonstrated strong pharmacological activity as a safe anticancer treatment due to their broad range of target properties and low harmful side effects (Tueche *et al*, 2018). The present study examines the *in vitro* and *in vivo* protective effects of HFAM. The anti-proliferative effect of HFAM was evaluated in the MCF-7 cell line using the MTT assay. Consistent with earlier findings (Eka *et al*, 2012; Kim *et al*, 2018; Ilango *et al*, 2022), HFAM exhibited anti-proliferative activity since it inhibited the proliferation of the breast cancer cell line. This result reveals that HFAM inhibited cell viability in a concentration-dependent manner within 72 h of drug incubation, which elicited a similar anti-proliferative effect as vincristine, the standard drug. *In vivo*, our findings showed that the overall weight-gain significantly decreased in animals administered DMBA while the body weight-gain was attenuated upon co-treatment with HFAM, suggesting that the plant extract may likely enhance appetite and promote energy metabolism in these animals.

Natural products, like chemotherapeutic drugs, can promote apoptosis. Cancer chemotherapy has been shown to trigger apoptosis, which is beneficial for disease prevention and treatment (Badrooh *et al.,* 2022). Apoptosis is mediated by a variety of signaling pathways that are linked to a large number of regulatory molecules. Apoptosis is caused by both extrinsic and internal routes that use the tumor suppressor gene p53. Depending on the mechanism utilized, apoptosis can be caused either intrinsically through mitochondria or extrinsically via death receptors (Almutairi *et al.,* 2023). Intrinsic apoptosis is caused by a variety of unusual situations, including DNA damage, excess ROS, growth factor withdrawal, endoplasmic reticulum stress, replication stress, and mitotic abnormalities (Pieme *et al.,* 2013; Hadisaputri *et al.,* 2021). Many tumors have been shown to express high levels of Bcl-2 and low levels of Bax. Bcl-2, an anti-apoptotic protein, interacts with the pro-apoptotic Bax protein, preventing Bax and Bad dimerization and thereby inhibiting apoptosis (Choumessi *et al.,* 2012; Hadisaputri *et al.,* 2021; Adefisan-Adeoye *et al.,* 2025). Overall, the coordination of these molecules is critical to controlling cell growth

and death. Caspase family proteins also play a key part in the molecular events that occur during apoptosis. Cancer cells could be driven to undergo apoptosis by enhancing the status of pro-apoptotic proteins, which is a well-known approach for inducing apoptosis in cancer cells (Hadisaputri *et al.,*2021). This study found that HFAM triggered the apoptotic pathway in both in vitro and in vivo experiments by increasing BAX, caspases-3, and -9 levels. These findings suggest that HFAM's pro-apoptotic potential stems from the activation of the intrinsic apoptosis pathway. This conclusion is consistent with the findings of Pieme *et al.,* (2013) and Kim *et al.,* (2018), who related plant extracts' anticancer properties to the induction of apoptosis and cell cycle arrest at the Go/G1 phase.

The development and survival of breast cancer has been proposed to be influenced by inflammatory cytokines (Yasmin *et al.,* 2015; Abdul-Wahab *et al.,* 2018; Adefisan-Adeoye *et al.,* 2025). The anti-inflammatory properties of *Anonna muricata* have been studied by numerous researchers (Fisher *et al.,*2020; Cerato *et al.,* 2021). As demonstrated by the downregulation of key biomarkers, IL-1β, nitric oxide, and myeloperoxidase, both *in vitro* and *in vivo*, HFAM reduced inflammation in the MCF cell line and rats given DMBA. The outcomes support the findings of Laksmitawati *et al.,* (2016), who found that the leaf extract of *A. muricata* has anti-inflammatory properties and decreased nitric oxide, TNF-α, IL-1β, and IL-6, which are inflammatory mediators. These findings illustrate and corroborate *A. Muricata*'s potential as a plant for the treatment of inflammatory illnesses. Inflammatory damage induced by reactive oxygen species (ROS) such as lipid peroxidation (LPO) alters the structure of membranes and enzymes, resulting in their deactivation (Ayala *et al.,*2014). If a high amount of LPO is not removed by antioxidant defense mechanisms, it can have disastrous consequences for biological systems and organs, including mammary tissues (Adefisan *et al.,*2021; 2025). In the current investigation, oxidative damage was characterized by a large increase in malondialdehyde and a concurrent decrease in antioxidant status, which supports earlier findings (Ogbu *et al.,* 2020; Sanni *et al.,*2020; Adefisan-Adeoye *et al*, 2024). Antioxidants not only safeguard cellular integrity but also keep the host immune system in balance. The cellular fate of genomic integrity is determined by the redox condition of the cells, which balances pro-oxidants and antioxidants.

Exposure to DMBA has been associated with mammary gland accumulation of ROS, which may result in depletion of the mammary antioxidant defense system, a mechanism that may further enhance DMBA-induced mammary toxicity (Ayala *et al*, 2014; Chen *et al*, 2022). The activities of mammary antioxidant enzymes monitored during this study showed that DMBA administration significantly decreased the activities of catalase (CAT), glutathione-s-transferase (GST), glutayhione peroxidase (GPx) and superoxide dismutase (SOD). Decreased mammary CAT, SOD, GPx and GST activities reflect the susceptibility of mammary gland to DMBA-induced oxidative stress. This is further confirmed by the corresponding increase in mammary malondialdehyde levels, suggesting that oxidative stress ensued in DMBA rats; however, the activities of these enzymes were significantly elevated in DMBA rats that received HFAM. In the same manner, HFAM increases the activities of catalase and superoxide dismutase in MCF-7 cell lysates. These results suggest that HFAM confers protection against DMBA-induced toxicity in rats by augmenting the mammary antioxidant defense system. In photomicrographs of mammary glands of rats administered DMBA, peri-ductal fibrous tissue and chromatin were moderately increased compared to stromal and adipose tissue in the control group, with mild necrosis. Treatment of DMBA-administered rats with HFAM (100 mg/kg) significantly attenuated the presence of fibrous tissues and chromatins. The potent activity of HFAM may be due to the abundance of alkaloids, especially tirucallol, which is known with strong anti-inflammatory effect.

In conclusion, HFAM showed anti proliferative, antioxidative, anti-inflammatory and pro apoptosis activities in MCF-7 cells and DMBA-administered rats. The seed of *Annona muricata* may protect the mammary gland from the adverse effects of chemical agents that can initiate the process of carcinogenesis.

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