



Contents lists available at [Bio-Research](#)
Journal of Biological Research and Biotechnology

journal homepage: <https://www.bio-research.com.ng> and
<https://www.ajol.info/index.php/br/about>



Therapeutic Symphony: *Annona muricata* Extract Orchestrates Metabolic Resilience in Mammary Carcinogenesis.

Abraham E. Ubhenin¹ · Fatimah Anura¹ · Silvanus O. Innih² · Ramatu Iya Idris¹

¹ Department of Biochemistry, Faculty of Sciences, Federal University Lafia, Nasarawa State, Nigeria

² Department of Anatomy, Faculty of Medicine, University of Benin, PMB 1154, Benin City, Nigeria

Received: 27 June 2025 / Accepted: 1st July 2025

© The Author(s), under exclusive license to Journal of Biological Science., part of Bio-Research 2025

DOI: <https://dx.doi.org/10.4314/br.v23i2.1>

Abstract

Breast cancer is still a major global health concern and is caused by a combination of hormonal, environmental, and hereditary factors. 7,12-Dimethylbenz(a) anthracene (DMBA) is a major contributor to the development of mammary cancer. This study demonstrated that the *Annona muricata* orchestrates metabolic adaptability in mammary carcinogenesis. The study focuses on specific enzymes pivotal to cancer metabolic pathways to provide an understanding of breast carcinogenesis. The implicit remedial benefits of *Annona muricata* are studied by carefully examining the following enzymes: Glucose-6-Phosphate Dehydrogenase, Glutamate Dehydrogenase, Glutaminase, Hexokinase 2, Pyruvate Kinase M2, Lactate Dehydrogenase A, Matrix Metalloproteinases, Superoxide Dismutase, Glutathione Peroxidase, and Catalase. The safety of the bioactive substances present in the *Annona muricata* was evaluated by oral acute toxicity testing. Mature albino Wistar rats were separated into experimental groups and were given DMBA injections before being them with *Annona muricata*. The effect of the extract on particular enzymes central to cancer metabolism and antioxidant enzyme activity was estimated. The results showed a substantial increase in these enzyme levels after DMBA induction, which could indicate metabolic changes connected to cancer. The *Annona muricata* treated groups reached nearly normal enzyme levels when given at 100 and 250 mg/ kg body weight, suggesting a remedial benefit. Histopathological study revealed that the *Annona muricata* effectively treated tumor-related issues and averted tumor incursion. The study concludes that *Annona muricata* has the implicit to impact the biochemical pathways linked to cancer and slow the growth of breast tumors. The need for further mechanistic exploration is one of the limitations of the study; therefore, additional studies should estimate the safety and effectiveness of this treatment in human subjects while concentration on the possibility of synergizing it with traditional cancer treatments.

Keywords: Breast cancer, Mammary carcinogenesis, 7,12-Dimethylbenz[a]anthracene (DMBA), *Annona muricata* Extract, Enzyme activities, Antioxidative markers, Histopathological examination.

Introduction

Breast cancer is one of the most prevalent and dangerous foes in the field of oncology, it continues to pose a

significant global health burden. Breast cancer, characterized by the unchecked growth of cancerous cells within the breast tissue, is caused by a complex interplay of hormonal, environmental, and genetic factors (Luo et al.,

2015). In 2020 alone, 2.3 million new cases and 685,000 deaths.

worldwide were attributed to breast cancer, highlighting the disease's impact on public health (Nolan et al., 2023). A polycyclic aromatic hydrocarbon known as 7,12-Dimethylbenz[a]anthracene (DMBA) is noteworthy among the substances known to contribute to the onset and spread of breast cancer due to its proven ability to cause mammary carcinogenesis through mutagenicity, genotoxicity, and DNA adduct formation (Ubhenin et al., 2024).

The signs of breast cancer can vary, from painless lumps to abnormal nipple fluid, skin abnormalities, and changes in the breast's size, shape, or appearance (Vandebroek & Picking, 2020). Interestingly, around half of breast cancers happen to women who have no known risk factors, highlighting the disease's complexity and multifaceted nature (Chu et al., 2020). The following are risk factors for breast cancer: age, gender (female gender is the biggest risk factor), obesity, alcohol consumption, radiation exposure, family history, reproductive history, tobacco use, and postmenopausal hormone therapy (Shetty, 2021). Crucially, the risk of breast cancer is greatly increased by hereditary mutations in BRCA1, BRCA2, and PALB-2 (Hu et al., 2020).

Breast cancer, like in many other nations, is a serious health issue in Nigeria. The prevalence of breast cancer in Nigeria is a growing concern, as the disease's incidence has been increasing over time (Azubuike et al., 2018). Genetic predisposition, environmental factors, and socioeconomic determinants interact intricately to make breast cancer a common disease in Nigeria. Issues with early detection, awareness, and healthcare infrastructure exacerbate the impact of breast cancer in Nigeria. With improvements in molecular characterization, diagnostic algorithms, and treatment modalities, efforts to prevent breast cancer have undergone a substantial transformation. Breast cancer subtypes, including hormone receptor status (ER, PR, and HER2), have changed treatment strategies. Along with targeted medicines, neoadjuvant combination therapy has become the standard of care, particularly for HER2-positive and triple-negative breast cancer. This has an impact on post-neoadjuvant tactics and surgical de-escalation (Miglietta et al., 2021; Mackenbach, 2015). There is a bright side to the advancements gained in recent decades, despite the difficulties presented by breast cancer. From the 1980s to 2020, age-standardized mortality decreased by 40% in high-income nations, demonstrating the benefits of improvements in early identification, treatment approaches, and all-encompassing management (Attiq et al., 2017).

Nonetheless, the differences in results highlight the need for creative and approachable therapeutic strategies, especially in environments with low resources like Nigeria.

Annona muricata, also known as graviola or soursop, is a promising treatment option in the search for new approaches to breast cancer treatment. Its diverse pharmacological profile, which includes bioactive components like phenolic compounds, alkaloids, and acetogenins, has attracted much attention. According to the study, these components allow *Annona muricata* to reduce the effects of carcinogens, trigger apoptosis, and prevent the development of cancer cells (Chu et al., 2020). The tropical fruit tree, *Annona muricata*, is a member of the Annonaceae family and has large, glossy, dark-green foliage and heart-shaped, edible green fruits. Native to the rainforests of Africa, South America, and Southeast Asia, this botanical treasure is used for its culinary properties in addition to its many traditional medicinal uses (Qazi et al., 2018).

Historically, a variety of plant parts, such as leaves, pericarp, fruits, seeds, and roots, have been used to treat a wide range of illnesses, including diabetes, coughs, skin conditions, cancers, arthritis, and malaria (Al Kazman et al., 2022). The foundation for current research, especially in the field of cancer treatment, is laid by the extensive traditional knowledge about *Annona muricata*.

A thorough phytochemical analysis of *Annona muricata* has revealed a complex composition with acetogenins, alkaloids, flavonoids, terpenoids, and saponins as the main constituents (Ilango et al., 2022). The plant's pharmacological actions, with a particular emphasis on its anticancer potential, are based on this complex profile. *Annona muricata* has cytotoxic activity against a variety of cancer types, such as hematological malignancies, liver cancer, cervical cancer, head and neck squamous cell carcinoma, breast cancer, and colon carcinoma (Kaur et al., 2018). Research shows *Annona muricata* has a broad-spectrum anticancer effect on cell lines such as MCF-7, HCT-116, HSC-3, Raji, HepG2, and HeLa (Foster et al., 2020). IC50 values, cell cycle modification, apoptotic pathway influence, and synergistic actions with chemotherapeutic medicines, as demonstrated by docetaxel, are noteworthy outcomes (Zeweil et al., 2019).

The investigation of *Annona muricata's* anticancer potential goes beyond in vitro evaluations and includes in vivo research and clinical trials. Graviola extract exhibits notable chemopreventive and chemotherapeutic activity in Zeweil models, especially against DMBA-induced breast carcinogenesis (Alshaeri, 2018). An ethanol-soluble fraction of the aqueous extract from *Annona muricata*

leaves exhibits cytotoxicity against colorectal cancer cells in clinical trials; further research shows that the leaves also have anti-inflammatory and immune-modulating properties in patients with colorectal cancer (Spence et al., 2020).

A clinical case report also demonstrates stability and improvement in a patient with metastatic breast cancer who took capecitabine and a decoction of *Annona muricata* leaves (Spence et al., 2020). These results together provide a strong basis for the manuscript's investigation of how the extract from *Annona muricata* coordinates metabolic resilience in the development of breast cancer.

Clarifying the relationship between *Annona muricata* and 7,12-Dimethylbenz[a]anthracene's carcinogenic effects on mammary tissue was the goal of this manuscript. To close the gap between conventional botanical knowledge and contemporary scientific investigation, our research carefully examines the mechanisms underlying the pharmacological effects of *Annona muricata*. Through the diligent use of scientific methodologies, our goal is to improve our understanding of *Annona muricata*'s potential therapeutic benefits for breast cancer and provide important insights for future research and treatment approaches.

The comprehensive analysis of this work focuses on assaying for a panel of specific enzymes that are crucial to cancer metabolic pathways. Combined, these enzymes provide a comprehensive picture of the metabolic dynamics involved in 7,12-Dimethylbenz[a]anthracene-induced breast carcinogenesis in female albino rats. By looking for these enzymes, we want to uncover the intricate web of molecular mechanisms that underlie the emergence of cancer and shed light on the potential impacts of *Annona muricata*.

* **Abraham E. Ubhenin**

ehibram@yahoo.co.uk Tel: +2348133893080

Published online:

Our objective is to improve our understanding of *Annona muricata*'s potential therapeutic benefits for breast cancer and provide important insights for future research and treatment approaches.

This study's comprehensive analysis focuses on assaying for a panel of specific enzymes crucial to cancer metabolic pathways. Together, these enzymes provide a comprehensive understanding of the metabolic dynamics at work in the setting of 7,12-Dimethylbenz[a]anthracene-induced breast carcinogenesis in female albino rats. By

testing these enzymes, we intend to uncover the intricate web of molecular mechanisms that underlie the emergence of cancer and offer details regarding the potential impacts of *Annona muricata*.

Materials and Methods

Materials

Ozi. *Annona muricata* samples were collected from a ranch in Keffi, Nasarawa State, Nigeria. These plant samples were verified and authenticated by the Department of Botany at the Federal University of Lafia. Afterward, the collected material underwent a thorough purification process, which included washing under a continuous stream of water to remove dirt and debris. The cleaned leaves were then air-dried at room temperature for two weeks. Once dried, the leaves were ground into a fine powder using mechanical crushing. The powdered particles were carefully stored in a deep container and kept in a controlled environment with low temperature and minimal humidity.

Preparation of Extract

The powdered botanical material was macerated with methanol for an extended period of roughly 72 hours; all conducted under medium room temperature conditions. Latterly, the performing admixture was subordinated to filtration, employing Whatman No. 1 sludge paper, and conducted into a conical beaker. The filtrate was dried to remove water content via evaporation, carried out at a controlled temperature of 40 °C and low pressure, employing a Rotary evaporator outfit. The concentrated waterless extract was also precisely stored within an air-barred vessel, conserving its integrity. The storehouse terrain was constantly maintained at a temperature of 4 °C, ensuring optimal preservation until its intended operation.

Experimental Rats

An aggregate of forty-nine mature male albino Wistar rats within a weight range of 100- 180 grams was carried from the Animal House Department of Biochemistry at the University of Nigeria, Nsukka, Nigeria. The rats were housed in normal rat coops and given two weeks to get used to their new environment before the start of the study. They were provided with ad libitum access to standard broiler feed and drinking water. The exploration was conducted in strict adherence to ethical protocols, and ethical approval was attained from Ahmadu Bello University, Zaria (A.B.U.C.A.U.C./ 2018/029). Throughout the study,

established procedures for the ethical use of experimental animals were strictly observed.

Acute Toxicity Studies

The acute oral toxicity of the *Annona muricata* methanol extract was assessed in Albino mice, following the system delineated by Lorke (1983). The experimental rats were divided into six groups, each conforming to three individualities. Groups 1- 3 were subjected to late fasting before giving a single oral dose of *Annona muricata* methanol extract at 1, 1.5, and 2 grams per kilogram of body weight, independently. Meanwhile, Groups 4- 6 were given a single oral dose of *Annona muricata* extract at 3, 4, and 6 grams per kilogram of body weight, independently. Subsequently, the rats were monitored for 12 hours to assess acute toxins and, for another 12 hours to check for mortality. The observation for mortality was extended to 14 days to establish the sub-chronic toxicity of *Annona muricata*. The median lethal dose (LD50), was calculated from the geometric means. The LD50 value was determined to be less than 6000 milligrams per kilogram of body weight; hence, the extract dosage listed for administration was 100 and 250 milligrams per kilogram of body weight.

Drug Preparation and Tumor Induction

Mammary gland tumor induction in rats was initiated through intraperitoneal injection of a single dose of 90 milligrams per kilogram of body weight of 7,12-dimethylbenz (a) anthracene (DMBA) dissolved in olive oil. To assess tumor development, the rats underwent daily palpation. Contemporaneously, the *Annona muricata* extract was orally administered to the rats at a concentration of 100 and 250 milligrams per kilogram of body weight.

Experimental Design

The study encompassed a group of 25 female albino rats, divided into four distinct experimental groups, each consisting of five individual rats. The groups were designated as follows:

1. Group A (Normal Control): This group served as the reference, with each rat giving a controlled oral administration of 0.5 milliliters of distilled water over five months.
2. Group B (DMBA- Induced Carcinogenesis): Rats in this group administered a single intraperitoneal injection of 90 milligrams per kilogram of body weight of 7,12- Dimethylbenz(a) anthracene (DMBA). Subsequently, they received a daily 0.5 milliliter oral

administration of distilled water for 28 days, commencing five months after DMBA injection.

3. Group C (DMBA-Induced Carcinogenesis plus Cyclophosphamide Treatment): Rats in this group also underwent DMBA intraperitoneal injection. Following carcinogen formation, they were treated with cyclophosphamide at a therapeutic dose of 100 milligrams per kilogram of body weight for 28 days, commencing five months after the original DMBA administration.
4. Group D (DMBA-Induced Carcinogenesis plus *Annona muricata* Extract Treatment): Rats in this group underwent DMBA via intraperitoneal injection, and subsequently, they were treated with the *Annona muricata* aqueous extract at a concentration of 100 milligrams per kilogram of body weight per day for 28 days.
5. Group E (DMBA- Induced Carcinogenesis *Annona muricata* Extract Treatment): In this group, rats entered a single intraperitoneal injection of DMBA at a dose of 90 milligrams per kilogram of body weight, leading to carcinogenesis. They were also subordinated to treatment with the *Annona muricata* aqueous extract at a dose of 250 milligrams per kilogram of body weight per day, commencing four months after the original DMBA treatment, and continuing for a 28-day duration.

This strictly designed experimental frame provides a structured approach to probe the impacts of 7,12-dimethylbenz (a) anthracene and the remedial prospect of *Annona muricata* extract in a controlled and methodical manner.

The administration of the extract was done using an orogastric tube over 28 successive days. Following an overnight deprivation of food, the animals were euthanized, sacrificed, and subjected to abdominal lacerations. Blood samples were collected via cardiac perforation and placed in plain sample bottles, and serum was attained through a 2-hour incubation at room temperature, followed by centrifugation at 2000 revolutions per minute. The collected serum was employed for the assessment of different biochemical parameters. The mammary glands and excrescence (tumor) were isolated and instantly saved in formal saline for posterior histopathological analysis.

Antioxidant Enzyme Quantification

The quantification of Glutamate Dehydrogenase (GLUD), Glutaminase (GLS), and Hexokinase 2(HK2) activities in serum employed marketable kits, following the protocols

established by (Csibi et al., 2013; Curthoys & Lowry, 1973; Ciscato et al., 2020; Rajala et al., 2016), respectively.

A coupled enzyme assay was used to measure pyruvate kinase M2 (PKM2) enzyme activity, following the method described by Wróblewski & Ladue (1955). Lactate Dehydrogenase A (LDHA) activity in serum was determined using a well-established spectrophotometry method, as outlined by Beutler et al. (1966). The assessment of the Glucose-6-Phosphate Dehydrogenase (G6PD) level employed a spectrophotometric assay. This system gauged the reduction of NADP to NADPH while G6PD converted glucose-6-phosphate to 6-phosphogluconolactone (Kupai et al., 2010). Matrix metalloproteinase (MMP) level in serum was measured through an enzyme activity assay, employing substrates designed to mimic their natural targets (Oberley & Spitz, 1984). Superoxide Dismutase (SOD) activity in serum was determined through an enzyme activity assay, assessing its capability to dismutate superoxide radicals (Kayanoki et al., 1996). The Glutathione Peroxidase (GPx) and Catalase, both antioxidant enzymes, were measured using enzyme activity assays in serum. These assays furnished insight into the oxidative stress response (Nishikawa et al., 2002; Crespi & Grossi, 1992).

Histopathological Analysis

Histopathological examination was conducted on both the mammary gland and excrescence (tumor) detached from the different groups of the rats. The samples were saved in a 10% formalin, subjected to dehumidification in an arranged ethanol series (70 to 100), cleared in xylene, and later

Table 1: Effect of serum levels of *Annona muricata* Extract on specific enzymes vital to cancer-associated metabolic pathways after the inauguration of tumorigenesis via DMBA injection.

Groups	GLUD (U/L protein)	GLS (nmol/min/ mgprotein)	HK2 (pg/ml)	PKM2 (U/L)	LDHA (U/L)
Group A	9.3±0.5 ^b	0.5±0.2 ^b	88.5±10.4 ^b	8.4±0.6 ^b	170±10.4 ^b
Group B	24.7± 1.7 ^a	1.5±0.7 ^a	180.5±23.8 ^a	32.8±3.7 ^a	300±20.1 ^a
Group C	12.6±0.7 ^{ab}	0.8±0.3 ^b	104±12.7 ^{ab}	12.8 ±0.9 ^{ab}	200±12.9 ^{ab}
Group D	14.8±0.4 ^{ab}	0.7±0.4 ^b	96.6±9.8 ^b	11.8±0.4 ^b	197±16.9 ^{ab}
Group E	10±0.6 ^b	0.6±0.3 ^b	91.3±11.9 ^b	9.7±0.3 ^b	190±11.9 ^b

The data are represented as mean ± S.D.; n = 5. Values in the same column with different alphabets (a, b) are significantly different (p<0.05). Group A - Normal Control Rats; Group B - Negative control (DMBA-Induced and untreated); Group C DMBA-Induced Carcinogenesis + Cyclophosphamide Treatment; Group D DMBA-Induced Carcinogenesis + *Annona muricata* Extract (100mg/kg wt.) Group E DMBA-Induced Carcinogenesis + *Annona muricata* Extract (250mg/kg b.wt.).

The results from Table 2 reveal that the induction of tumorigenesis by the administration of DMB led to a statistically significant (p ≤ 0.05) decrease in the serum levels of some specific enzymes central to metabolic pathways intricately linked with cancer. These enzymes

bedded in paraffin. Following these procedures, all the rat sections underwent examination under a light microscope after staining with hematoxylin(H) and eosin(E) (32).

Data analysis

The data were presented as the mean ± standard deviation (SD) of the six replicates in a group. Statistical analysis was performed using one-way analysis of friction (ANOVA) with SPSS version 10.0. Post hoc individual comparisons were conducted using the Duncan multiple range test (DMRT). A significant level of P<0.05 was employed to determine statistically significant differences between groups.

Results

The findings presented in Table 1 reveal a noteworthy upsurge (p ≤ 0.05) in the serum levels of specific enzymes vital to cancer-associated metabolic pathways after the induction of tumorigenesis via DMBA injection. specially, the enzymatic levels of GLUD, GLS, HK2, PKM2, and LDH displayed a statistically significant elevation.

The intervention with *Annona muricata* Extract, administered at 100 and 250 mg per kilogram of body weight, displayed a remarkable remedial effect. *Annona muricata* caused statistically significant (p ≤ 0.05) variations in the levels of these enzymes, effectively restoring them to concentrations that align with what's considered physiologically normal.

encompass G6PD, MMPs, as well as crucial antioxidant enzymes that include SOD, GPX, and CATALASE.

Remarkably, treatment with *Annona muricata* Extract at 100 and 250 mg per kilogram of body weight demonstrated a pronounced remedial effect. This intervention led to

statistically significant ($p \leq 0.05$) reversals of these enzymes, restoring them to levels nearly approaching the physiologically normal range.

Table 2: The effect of *Annona muricata* on G6PD, MMPs, as well as crucial antioxidant indicators (SOD, GPX, and catalase) in DMBA-caused mammary tumorigenesis.

Groups	G6PD (U/g hemo)	MMPs (ng/ml)	SOD (U/ml)	GPx (U/L)	CATALASE (MU/I)
Group A	11.9±1.9 ^b	150±20.7 ^b	1.8±0.3 ^b	208±31.7 ^b	140±21.7 ^b
Group B	22.1± 3.6 ^a	280±46.8 ^a	0.5±0.08 ^a	108±16.3 ^a	70±13.3 ^a
Group C	15.4±2.3 ^{ab}	170±17.9 ^b	1.1±0.6 ^b	180 ±40.6 ^b	106±26.4 ^b
Group D	12.7±2.1 ^b	180±22.4 ^b	1.2±0.5 ^b	175±28.9 ^b	128±31.9 ^b
Group E	13.7±17 ^b	160±25.6	1.3±0.9 ^b	213±36.2 ^b	133±27.4 ^b

The data are represented as mean ± S.D.; n = 5. Values in the same column with different alphabets (a, b) are significantly different ($p < 0.05$). Group A - Normal Control Rats; Group B – Negative control (DMBA-Induced and untreated); Group C DMBA-Induced Carcinogenesis + Cyclophosphamide Treatment; Group D DMBA-Induced Carcinogenesis + *Annona muricata* Extract (100mg/kg b.wt.) Group E DMBA-Induced Carcinogenesis + *Annona muricata* Extract (250mg/kg b.wt.).

Histological Examination Micrographs

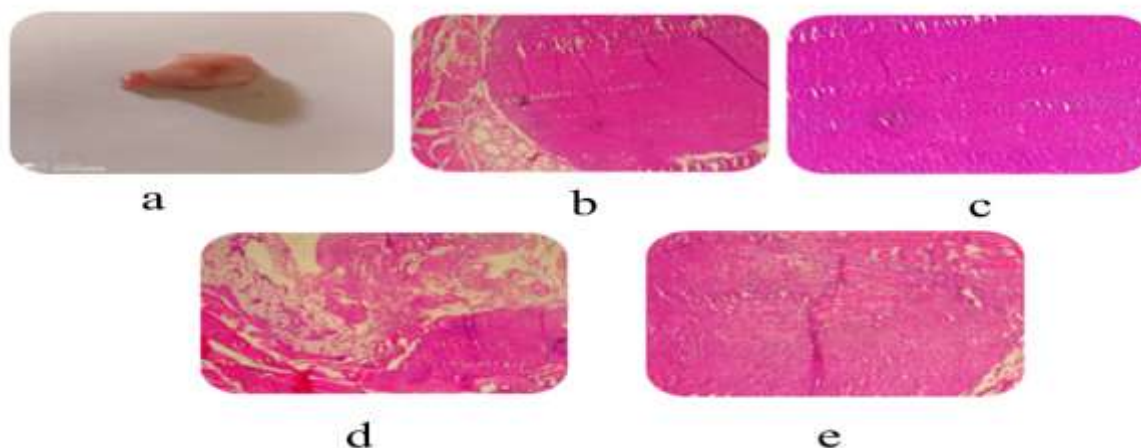


Figure 1: Provides a detailed visual examination of solid tumors obtained from Group B rats treated with DMBA only. Subfigures (b- e) offer an overall macroscopic view of solid tumors. Subfigures (b) and (c) present micrographs of tumors at x 40 and x100 magnifications, revealing intricate cellular structures and morphological features of Group B rat tumors. Additionally, Subfigures (d) and (e) show micrographs of tumors obtained from Group C rats, illustrating the impacts of cyclophosphamide in DMBA-induced carcinogenesis. Subfigure (d) is captured at x 40 magnification, while Subfigure (e) provides a closer examination at x 100 magnification. This relative analysis enables discernment into tumor development and treatment efficacy within the experimental groups.

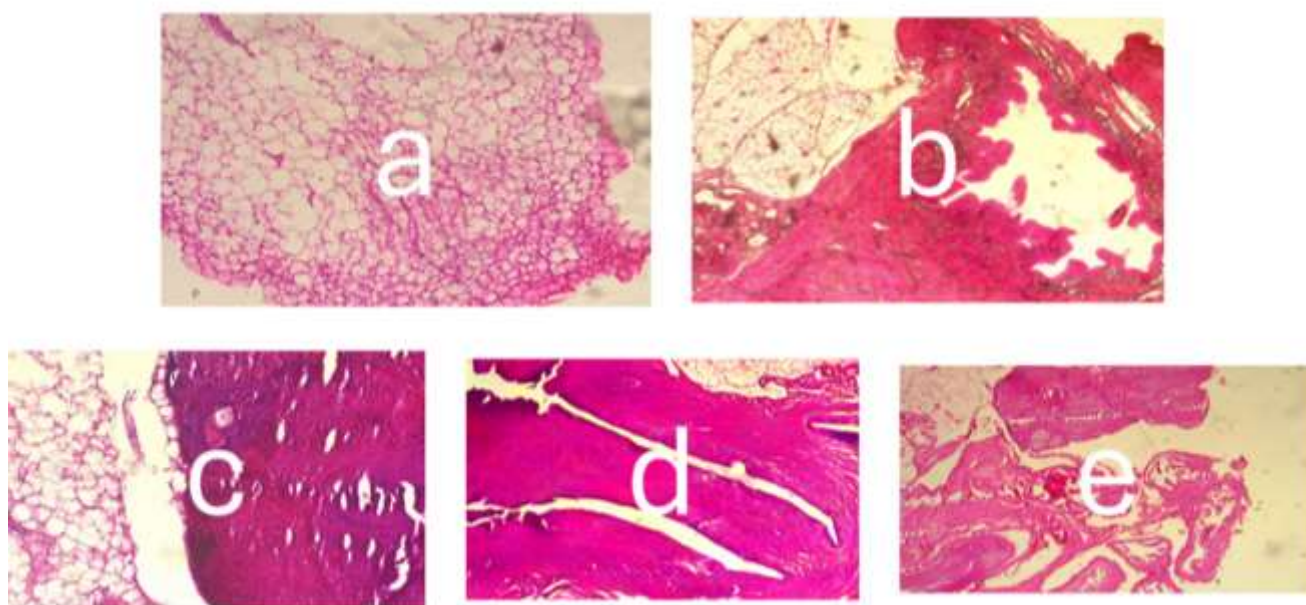


Figure 2: Presents a series of micrographs illustrating histological changes in tissues obtained from the animal's breast from distinct experimental groups. Subfigures(a) to (e) correspond to breast tissue samples from Groups A (Normal Control), B (DMBA-Induced Carcinogenesis), C (DMBA- Induced Carcinogenesis Cyclophosphamide Treatment), D(DMBA-Induced Carcinogenesis plus *Annona muricata* Extract Treatment 250 mg/kg), and E (DMBA-Induced Carcinogenesis plus *Annona muricata* Extract Treatment 500 mg/kg), independently.

The micrographs visually capture variations in cell structure and morphology across experimental conditions. Subfigure(a) serves as a reference, representing normal tissue obtained from a group A animal's breast. Subfigures (b) to (e) depict the impact of DMBA treatment in carcinogenesis and different treatments.

Discussion

The observed results, as detailed in Table 1 of this study, are reflective of a compelling response within the biochemical terrain of the mammary tumor-induced rats, highlighting the profound impact of *Annona muricata* Extract administration. The crucial enzymes studied are GLUD, GLS, HK2, PKM2, and LDH. These play integral functions in the intricate metabolic pathways associated with cancer progression. The significant upsurge ($p \leq 0.05$) in the activities of these enzymes following DMBA-caused tumorigenesis reflects the marked changes in the cellular metabolism associated with cancer. These enzymes, including GLUD (Glutamate

Dehydrogenase), GLS (Glutaminase), HK2 (Hexokinase 2), PKM2 (Pyruvate Kinase M2), and LDH (Lactate Dehydrogenase), are known to be crucial players in cancer metabolism (Islam et al., 2017; Bott et al., 2019). GLUD and GLS are involved in amino acid metabolism, contributing to the biosynthesis of macromolecules necessary for cancer cell growth (Kooshki et al., 2022). HK2 and PKM2 are pivotal in the glycolytic pathway, easing increased glucose usage for energy and anabolic processes in cancer cells (San-Millán & Brooks, 2017). LDH, a vital player in the Warburg effect, promotes lactate production, which sustains the glycolytic phenotype in cancer cells (Abdul Wahab et al., 2018). The remarkable remedial impacts observed upon treatment with *Annona muricata* Extract at therapeutic concentrations of 100 and 250 mg per kilogram of body weight can be related to different mechanisms. *Annona muricata*, known for its rich bioactive composites that may include acetogenins, alkaloids, and flavonoids, possesses antioxidant and anti-inflammatory compounds (Torres et al., 2012). These bioactive composites may contribute to the downregulation of cancer-associated

metabolic pathways, causing a reduction in enzymatic functions (Cassé, 2018).

Furthermore, *Annona muricata* Extract may modulate apoptotic and anti-proliferative pathways in cancer cells, affecting the expression of these enzymes (Cassim et al., 2020). It might also promote the restoration of normal cellular metabolism by impeding the Warburg effect, thereby shifting cancer cells toward an oxidative metabolism, which is less aggressive (Yang et al., 2019).

The significant variations in the enzymatic concentrations observed in this study after *Annona muricata* extract treatment can be related to its multifaceted impact on the intricate biochemical processes in cancer cells.

These findings suggest the implicit remedial efficacy of *Annona muricata* extract in attenuating the aberrant metabolic pathways that characterize cancer progression. Additional examinations into the specific mechanisms underpinning these impacts are warranted to interpret the full scope of its remedial usage in cancer treatments.

The results presented in Table 2 of this study give helpful intuitions into the biochemical changes associated with mammary tumorigenesis and the successive beneficial properties of *Annona muricata* extract. The study concentrated on specific enzymes and antioxidative indicators integral to the intricate metabolic pathways associated with cancer. The significant increase ($p \leq 0.05$) in the serum level of G6PD, MMPs, and associated by a drop ($p \leq 0.05$) in the serum level of the antioxidative pointers, including SOD, GPX, and catalase, following DMBA- caused tumorigenesis is reflective of the profound metabolic changes that ensue in response to cancer development. The elevated level of G6PD in response to tumorigenesis is likely associated with the increased demand for NADPH (a reducing agent) in cancer cells. NADPH is essential for maintaining redox balance, lipid production, and ribonucleotide reductase function. Elevated action of G6PD supports these processes, enabling cancer cells to gain and repel oxidative stress (Winkler et al., 2020).

MMPs are critical to the breakdown of the extracellular matrix and tissue transformations, and pivotal for tumor incursion and metastasis (Duffy et al., 2000). The increased level of MMPs is harmonious with the invasive nature of cancer and the breakdown of the cell walls (Coria-Téllez et al., 2018).

The observed significant drop ($p \leq 0.05$) in antioxidative indicators, including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT),

is likely a response to the increased oxidative stress associated with cancer. These enzymes are part of the cellular defense system against reactive oxygen species (ROS). The decrease in antioxidative indicators suggests oxidative damage caused by cancer.

Remarkably, the administration of *Annona muricata* extract at concentrations of 100 and 250 mg per kilogram of body weight produced a pronounced remedial effect. The observed statistically significant ($p \leq 0.05$) reversals in enzymatic oxidative indicators may be attributed to several biochemical mechanisms.

Annona muricata is known for its rich content of phytochemicals with antioxidants, like flavonoids and acetogenins. These composites can scavenge ROS and alleviate oxidative stress, increasing antioxidative enzyme activities (Kubczak et al., 2021).

Inflammation is a hallmark of cancer, and *Annona muricata* extract is known to be anti-inflammatory. Reducing inflammation may ultimately lower MMP levels, limiting the invasive tendency of cancer cells (Ojo et al., 2022). *Annona muricata* extract may affect cellular signaling pathways involved in the regulation of G6PD and antioxidative enzymes. This could help restore normal enzymatic functions in response to cancer.

The micrographs of breast sections obtained from normal rats, and DMBA-treated group plus *Annona muricata* displayed micrographs characterized by normal features and adipose tissue with no significant diversions. Again, the group treated with DMBA alone presented histological micrographs characterized by, the features that included a well-defined but locally infiltrative lesion comprising stringy (spindle/ stretched) cells arranged in fascicles, displaying fairly invariant nuclear characteristics. These histological characteristics are reflective of a fibrous tumor, suggesting the presence of either fibrosarcoma or fibrous tumors with aggressive attributes.

The histopathological findings support the implicit anticancer effect of *Annona muricata* extract. Stressing the significant discrepancy between the normal cell features in treated groups and aggressive fibrous tumors in the DMBA-exposed group, therefore emphasizing the *Annona muricata's* remedial potential in attenuating mammary tumor progression. Additional examinations are essential to delineate the specific mechanisms underpinning this effect.

Histopathological examination of (tumor) cells detached from the DMBA-induced

group revealed apparent excrescence extending into skeletal muscle filaments. In the tissues examined, tumor cells were observed penetrating and separating skeletal muscle filaments, causing a significant atrophic change characterized by individual, large, atrophic cells. Likewise, the micrographs displayed the presence of inflammatory cells, generally lymphocytes, localized at the center of the image, indicating an active immune response elicited by the excrescence penetration.

Annona muricata treated group in stark discrepancy, no excrescence (tumor) cells were detected in the samples from the *Annona muricata*-treated groups. This observation suggests the implicit efficacy of *Annona muricata* treatment in precluding excrescence cell proliferation. The DMBA-induced excrescences, which were pre-existing, displayed a notable response to *Annona muricata* treatment. Specifically, these excrescences began forming external abscesses. Over the course of *Annona muricata* treatment, these abscesses demonstrated a significant retrogression, eventually shrinking in size. It's essential to admit some limitations of this study. The specific mechanisms by which *Annona muricata* exert its impacts on the metabolic pathways and histopathological changes need a deeper disquisition. Also, the study primarily concentrated on a rat model, relating these findings to human clinical scenarios necessitates careful consideration.

Conclusion

This study underscores the implicit remedial efficacy of *Annona muricata* extract in alleviating mammary excrescence (tumor) progression and its significant impact on the intricate biochemical processes within cancer cells. The observed changes in enzymatic and antioxidative activities following *Annona muricata* treatment give a marked comprehension of its mechanisms of action. Also, the histopathological findings demonstrate *Annona muricata's* capability to inhibit tumor penetration into muscle filaments and its probable function in resolving tumor-related complications, like the progression and regression of external abscesses. The promise of *Annona muricata* extract as a potential option for advanced research and development in cancer care is supported by these findings. These histopathological findings raise interesting questions regarding the underpinning mechanisms responsible for the

successive retrogression of these abscesses. Likewise, the study provides the implicit remedial potentials of *Annona muricata* from the perspective of cancer and its related complications.

Authors contribution

EC conceptualized the work, contributed to data curation, formal analysis, funding acquisition, investigation, methodology, and writing of the original draft. PJ supervised the work, validated it, and reviewed and edited it. All authors read and approved of the final manuscript.

Recommendation

Further surveys that will include molecular/DNA characterizations of the microalgae is highly recommended for this lake; this will reveal some species we are not able to record. We sent out our sample for metagenomics analysis but the feedback we got do not align with our expectations; so, we had to discard the results.

Funding

This study received a support grant from Tetfund; with Tetfund grant No.: TETF/DR&D/CE/UNI/NSUKKA/BR/2020/VOL.I, which covered good aspects of the costs for this study.

Data availability

This article has no associated data.

Ethics approval and Informed Consent

The study was conducted in accordance with the regulations and ethical approval of the Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka with the approval number UNN/FBS/EC/1219. The nuts were authenticated by Mr. Alfred Ozioko of the Bioresource Development and Conservation Program (BDCP), Research Centre Nsukka.

Informed consent. With the informed consent of the donors, the research was done in accordance with the Declaration of Helsinki and Tokyo for humans.

Consent For Publication

Not applicable

Competing interests

The authors declare no conflict of interest.

Acknowledgment

This study received a support grant from Tetfund, with the Tetfund grant

References

- Abdul Wahab, S. M., Jantan, I., Haque, M. A., & Arshad, L. (2018). Exploring the leaves of *Annona muricata* L. as a source of potential anti-inflammatory and anticancer agents. *Frontiers in pharmacology*, *9*, 661.
- Al Kazman, Bassam SM, Joanna E. Harnett, and Jane R. Hanrahan. "Traditional uses, phytochemistry and pharmacological activities of annonaceae." *Molecules* 27.11 (2022): 3462.
- Alshaeri, H. K. (2018). *Anticancer Activity of Annona Muricata Extract on Triple Negative Breast Cancer Cells*. Massachusetts College of Pharmacy and Health Sciences.
- Attiq, A., Jilil, J., & Husain, K. (2017). Annonaceae: breaking the wall of inflammation. *Frontiers in pharmacology*, *8*, 752.
- Azubuikwe, S. O., Muirhead, C., Hayes, L., & McNally, R. (2018). Rising global burden of breast cancer: the case of sub-Saharan Africa (with emphasis on Nigeria) and implications for regional development: a review. *World journal of surgical oncology*, *16*(1), 1-13.
- Beutler E. A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. *Blood* 1966 October;28(4):553-62.
- Bott, A. J., Maimouni, S., & Zong, W. X. (2019). The pleiotropic effects of glutamine metabolism in cancer. *Cancers*, *11*(6), 770.
- Cassé, C. (2018). Molecular mechanisms of *Annona muricata* anti-proliferative/anti-cancer properties. *Review Article Biomedical Genetics and Genomics*, *4*(1), 1-4.
- Cassim, S., Vučetić, M., Ždraljević, M., & Pouyssegur, J. (2020). Warburg and beyond: the power of mitochondrial metabolism to collaborate or replace fermentative glycolysis in cancer. *Cancers*, *12*(5), 1119.
- Chu, Y., Zhang, W., Kanimozhi, G., Brindha, G. R., & Tian, D. (2020). Ginsenoside Rg1 induces apoptotic cell death in triple-negative breast cancer cell lines and prevents carcinogen-induced breast tumorigenesis in sprague dawley rats. *Evidence-Based Complementary and Alternative Medicine*, 2020.
- Chu, Y., Zhang, W., Kanimozhi, G., Brindha, G. R., & Tian, D. (2020). Ginsenoside Rg1 induces apoptotic cell death in triple-negative breast cancer cell lines and prevents carcinogen-induced breast tumorigenesis in sprague dawley rats. *Evidence-Based Complementary and Alternative Medicine*, 2020.
- Ciscato, F., Filadi, R., Masgras, I., Pizzi, M., Marin, O., Damiano, N., ... & Rasola, A. (2020). Hexokinase 2 displacement from mitochondria-associated membranes prompts Ca²⁺-dependent death of cancer cells. *EMBO reports*, *21*(7), e49117.
- Coria-Téllez, A. V., Montalvo-González, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of chemistry*, *11*(5), 662-691.
- Crespi, R., & Grossi, S. G. (1992). A method for histological examination of undecalcified teeth. *Biotechnic & histochemistry*, *67*(4), 202-206.
- Csibi, A., Fendt, S. M., Li, C., Poulgiannis, G., Choo, A. Y., Chapski, D. J., ... & Blenis, J. (2013). The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. *Cell*, *153*(4), 840-854
- Curthoys, N. P., & Lowry, O. H. (1973). The distribution of glutaminase isoenzymes in the various structures of the nephron in normal, acidotic, and alkalotic rat kidney. *Journal of Biological Chemistry*, *248*(1), 162-168.
- Duffy, M. J., Maguire, T. M., Hill, A., McDermott, E., & O'Higgins, N. (2000). Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast cancer research*, *2*(4), 1-6.
- Foster, K., Oyenih, O., Rademan, S., Erhabor, J., Matsabisa, M., Barker, J., ... & Delgoda, R. (2020). Selective cytotoxic and anti-metastatic activity in DU-145 prostate cancer cells induced by *Annona muricata* L. bark extract and phytochemical, annonacin. *BMC Complementary Medicine and Therapies*, *20*, 1-15.
- Hu, C., Polley, E. C., Yadav, S., Lilyquist, J., Shimelis, H., Na, J., ... & Couch, F. J. (2020). The contribution of germline predisposition gene mutations to clinical subtypes of invasive breast cancer from a clinical genetic testing cohort. *JNCI: Journal of the National Cancer Institute*, *112*(12), 1231-1241.
- Ilango, S., Sahoo, D. K., Paital, B., Kathirvel, K., Gabriel, J. I., Subramaniam, K., ... & Nirmaladevi, R. (2022). A review on *annona muricata* and its anticancer activity. *Cancers*, *14*(18), 4539.
- Islam, R. A., Hossain, S., & Chowdhury, E. H. (2017). Potential therapeutic targets in energy metabolism pathways of breast cancer. *Current Cancer Drug Targets*, *17*(8), 707-721.
- Kaur, V., Kumar, M., Kumar, A., Kaur, K., Dhillon, V. S., & Kaur, S. (2018). Pharmacotherapeutic potential of phytochemicals: Implications in cancer chemoprevention and future perspectives. *Biomedicine & Pharmacotherapy*, *97*, 564-586.
- Kayanoki, Y., Fujii, J., Islam, K. N., Suzuki, K., Kawata, S., Matsuzawa, Y., & Taniguchi, N. (1996). The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. *The journal of biochemistry*, *119*(4), 817-822..

- Kooshki, L., Mahdavi, P., Fakhri, S., Akkol, E. K., & Khan, H. (2022). Targeting lactate metabolism and glycolytic pathways in the tumor microenvironment by natural products: A promising strategy in combating cancer. *Biofactors*, 48(2), 359-383.
- Kubczak, M., Szustka, A., & Rogalińska, M. (2021). Molecular targets of natural compounds with anti-cancer properties. *International Journal of Molecular Sciences*, 22(24), 13659.
- Kupai, K., Szucs, G., Cseh, S., Hajdu, I., Csonka, C., Csont, T., & Ferdinandy, P. (2010). Matrix metalloproteinase activity assays: Importance of zymography. *Journal of pharmacological and toxicological methods*, 61(2), 205-209.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, 54, 275-287.
- Luo, H., Tu, G., Liu, Z., & Liu, M. (2015). Cancer-associated fibroblasts: a multifaceted driver of breast cancer progression. *Cancer letters*, 361(2), 155-163.
- Mackenbach, J. P. (2015). Socioeconomic inequalities in health in high-income countries: the facts and the options. *Oxford textbook of global public health*, 1, 106-126.
- Miglietta, F., Dieci, M. V., Griguolo, G., & Guarneri, V. (2021). Neoadjuvant approach as a platform for treatment personalization: focus on HER2-positive and triple-negative breast cancer. *Cancer Treatment Reviews*, 98, 102222.
- Nishikawa M, Tamada A, Kumai H, Yamashita F, Hashida M. Inhibition of experimental pulmonary metastasis by controlling biodistribution of catalase in mice. *Int J Cancer*. 2002;99:474-479
- Nolan, E., Lindeman, G. J., & Visvader, J. E. (2023). Deciphering breast cancer: from biology to the clinic. *Cell*.
- Oberley, L. W., & Spitz, D. R. (1984). Assay of superoxide dismutase activity in tumor tissue. In *Methods in enzymology* (Vol. 105, pp. 457-464). Academic Press
- Ojo, O. A., Grant, S., Amanze, J. C., Oni, A. I., Ojo, A. B., Elebiyo, T. C., ... & Ogunlakin, A. D. (2022). *Annona muricata* L. peel extract inhibits carbohydrate metabolizing enzymes and reduces pancreatic β -cells, inflammation, and apoptosis via upregulation of PI3K/AKT genes. *Plos one*, 17(10), e0276984
- Qazi, A. K., Siddiqui, J. A., Jahan, R., Chaudhary, S., Walker, L. A., Sayed, Z., ... & Macha, M. A. (2018). Emerging therapeutic potential of graviola and its constituents in cancers. *Carcinogenesis*, 39(4), 522-533.
- Rajala, R. V., Rajala, A., Kooker, C., Wang, Y., & Anderson, R. E. (2016). The Warburg effect mediator pyruvate kinase M2 expression and regulation in the retina. *Scientific reports*, 6(1), 37727.
- San-Millán, I., & Brooks, G. A. (2017). Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis*, 38(2), 119-133.
- Shetty, M. K. (2021). Imaging of the Symptomatic Breast. In *Breast & Gynecological Diseases: Role of Imaging in the Management* (pp. 27-79). Cham: Springer International Publishing.
- Spence, D., Brown, K., Smith, S., Grant, D., & Picking, D. (2022). Jamaican Cultural and Spiritual Guidance in Caring for Cancer Patients, Jamaica. *Global Perspectives in Cancer Care: Religion, Spirituality, and Cultural Diversity in Health and Healing*, 454.
- Torres, M. P., Rachagani, S., Purohit, V., Pandey, P., Joshi, S., Moore, E. D., ... & Batra, S. K. (2012). Graviola: a novel promising natural-derived drug that inhibits tumorigenicity and metastasis of pancreatic cancer cells in vitro and in vivo through altering cell metabolism. *Cancer letters*, 323(1), 29-40.
- Ubhenin, A. E., Adefolalu, A. A., Oriakhi, K., Adamude, F. A., Dingwoke, E. J., Ikeburo, J. O., ... & Omege, K. (2024). *Caesalpinia pulcherrima* lowered serum carcinoembryonic antigen and antigen 125 in 7, 12-Dimethylbenz [a] anthracene-induced Mammary Carcinogenesis in Female Albino Rats. *Heliyon*, 10(1).
- Vandebroek, I., & Picking, D. (2020). *Popular medicinal plants in Portland and Kingston, Jamaica* (pp. 89-94). Springer.
- Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J., & Werb, Z. (2020). Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nature communications*, 11(1), 5120.
- Wróblewski, F., & Ladue, J. S. (1955). Lactic dehydrogenase activity in blood. *Proceedings of the society for experimental biology and medicine*, 90(1), 210-213.
- Yang, H. C., Wu, Y. H., Yen, W. C., Liu, H. Y., Hwang, T. L., Stern, A., & Chiu, D. T. Y. (2019). The redox role of G6PD in cell growth, cell death, and cancer. *Cells*, 8(9), 1055.
- Zeweil, M. M., Sadek, K. M., Taha, N. M., El-Sayed, Y., & Menshaw, S. (2019). Graviola attenuates DMBA-induced breast cancer possibly through augmenting apoptosis and antioxidant pathway and downregulating estrogen receptors. *Environmental Science and Pollution Research*, 26, 15209-15217.

Publisher's Note: Bio-Research remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Bio-Research or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.