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Anti-breast cancer activities of *Zingiber officinale* and *Allium sativum* ethanol extracts in 7,12-dimethylbenz[a]anthracene induced female Wister rats

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Abstract

Breast cancer belongs to a group of diseases classified under a generic name – cancer. It is the most frequently diagnosed cancer in women worldwide with more than two million new cases in 2020. Breast cancer caused 685,000 deaths in 2020 and its mortality rate has increased over the last three decades due to the change in risk factor profiles, better breast cancer registration, and breast cancer detection. This study evaluated the antibreast cancer potentials of *Zingiber officinale* and *Allium sativum* ethanol extracts administered separately and combined in 7,12-dimethylbenz[a]anthracene (DMBA) induced female Wister rats. After extraction, acute toxicity and phytochemical analysis of *Zingiber officinale* and *Allium sativum* ethanol extracts were performed separately. Evaluation of the antibreast cancer potency was done by inducing cancer in female albino Wister rats; measuring tumor sizes and tumor volumes on the 91st day post-treatment and testing for breast cancer marker notably CA-15. *Zingiber officinale* and *Allium sativum* are rich in phytochemicals and have good safety profile. All the groups treated with *Zingiber officinale* and *Allium sativum* in different proportions exhibited antibreast cancer effects by decreasing the mean tumor size, mean tumor volume and mean CA-15 level significantly ($p < 0.05$) when compared with the untreated group 2 and most remarkable in group 8. In conclusion, the herbs *Zingiber officinale* and *Allium sativum* ethanol extracts exhibited the best antibreast cancer activities when given in a combination of ZO:AS = 6:4 (318:212 mg/kg body weight) in a manner similar to that of the standard drug doxorubicin.

Keywords: *Allium* sativum; Anti-breast cancer; Doxorubicin; *Zingiber officinale*

1. Introduction

Breast cancer belongs to a group of diseases classified under a generic name – cancer – and according to a certain study, it is the most frequently diagnosed cancer in women worldwide with more than 2 million new cases in 2020 [1]. The study also reported that breast cancer caused 685,000 deaths in 2020 and its mortality rate has increased over the last three decades due to the change in risk factor profiles, better breast cancer registration, and breast cancer detection [1]. The amount of risk factors of breast cancer is significant and includes both modifiable factors and non-modifiable factors. Presently, about 80% of patients with breast cancer are individuals aged 50 years and above. Survival depends on both stage and molecular sub-type. Invasive breast cancers comprise wide-spectrum tumors that show a variation concerning their clinical presentation, behavior, and morphology [1]. Based on mRNA gene expression levels, breast cancer can be divided into molecular subtypes (Luminal A, Luminal B, HER2-enriched, and basal-like) [2]. The molecular

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subtypes provide insights into new treatment strategies and patient stratifications that impact the management of breast cancer patients. Breast cancer arises from the conversion of normal cells into tumor cells in a multi-stage process that generally progresses from a pre-cancerous lesion to a malignant tumor [2]. These changes are the result of the interaction between a person's genetic factors and three categories of external agents, including physical carcinogens, such as ultraviolet and ionizing radiation; chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant), and arsenic (a drinking water contaminant); and biological carcinogens, such as infections from certain viruses, bacteria, or parasites [2]. Generally, the burden of cancer incidence and mortality is rapidly growing worldwide; this reflects both the aging and growth of the population as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development [2]. In an earlier study, breast cancer was referred to as a worldwide public health dilemma and is currently the most common tumor in the globe [3]. Awareness of breast cancer, public attentiveness, and advancement in breast imaging have made a positive impact on the recognition and screening of breast cancer [3]. The researchers reiterated that breast cancer is a life-threatening disease in females and the leading cause of mortality among women population [3]. For the preceding two decades, studies related to the breast cancer have guided us to an amazing advancement in our understanding of breast cancer, resulting in further proficient treatments [3]. Amongst all the malignant diseases, breast cancer is considered one of the leading causes of death in post-menopausal women accounting for 23% of all cancer deaths [3]. In Nigeria, an estimated 72,000 cancer deaths occur annually, and 102,000 new cases are diagnosed from its population of approximately 200 million people [4]. Breast cancer accounted for the highest mortality, followed by prostate cancer [4]. In a study done in Delta state of Nigeria which was aimed at analyzing the age, gender, and topography of cancer in Delta State, the researchers concluded that breast cancers was one of the most common cancers [5]. Breast cancer among adolescents and young adult (AYA) females aged 15-39 years is associated with different patterns of aggressiveness, as well as psychosocial and economic issues [5]. At present, the problem of breast cancer among this age group is unknown in Nigeria and this is hindering the development of breast cancer care programs appropriate for this age group [5]. A certain study that highlighted the burden of breast cancer with an emphasis on AYAs in Nigeria reported that among AYA females in Nigeria, breast cancer was by far the most common cancer, constituting 50% of all cancers and 51% (2798 of 5469) of all breast cancer cases [6]. The high proportion of AYA with breast cancer is an important feature suggesting that urgent actions are required to ensure early detection and improve breast cancer care among this age group [6]. Many conventional antibreast cancer drugs are in use now but they have limitations [7]. Treatment of breast cancer is complex and involves a combination of different modalities including surgery, radiotherapy, chemotherapy, hormonal therapy, or biological therapies delivered in diverse sequences [7]. Despite the facts that improvements in breast cancer treatment have resulted in decreased breast cancer related mortality, there is increasing recognition that many breast cancer patients or survivors can develop cardiovascular diseases, either due to the breast cancer itself or as a result of antibreast cancer therapy [7]. According to the study, cardiovascular disease (CVD) was the leading cause of mortality among women diagnosed with breast cancer, as cancer survivorship is largely influenced by the latent effects of CVD caused by cancer treatment [7]. There was a study that quantified the out-of-pocket (OOP) cost of breast cancer management and the associated rate of catastrophic healthcare expenditure (CHE) at a public tertiary care facility in Ile-Ife, Nigeria. In the study, it was discovered that the mean cost of diagnosis and treatment of breast cancer was $2,049 (SD $1,854) [8]. The mean cost of radiotherapy was $462 (SD $223) and the mean cost of trastuzumab was $6,568 (SD $2,766) [8]. The researchers concluded that the out-of-pocket cost of breast cancer care in Nigeria was significant [8]. The current status of breast cancer care in Nigeria is such that there is no availability of an up-to-date National Drug Formulary in Nigeria, hence no practical guide on drug administration and use; most of the hospitals do not have enough drugs in their pharmacy stores, thereby worsening access to oncology drugs by patients; there are challenges of affordability, quality assurance and access to oncology drug as most pharmaceutical companies have their industries outside Nigeria [9]. There is a significant potential for improving outcomes by promoting early diagnosis and facilitating access to multi-modality treatment [9]. This study is therefore aimed at evaluating the anti-breast cancer potentials of combined administration of *Zingiber officinale* and *Allium sativum* in 7, 12-dimethylbenz [a] anthracene induced female Wister rats.

2. Material and methods

2.1. Animals

Female Swiss virgin Albino rats aged 7 – 8 weeks old were used for this study. To reduce individual susceptibility to chemical-induced cancer as well as variation in drug response, the animals were bred in the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology under ideal conditions of temperature, humidity, and light. The animals were fed with pelletized feed (Vital Feeds, Nigeria) and had access to filtered water *ad libitum*. The animals can live an average of 3 years, starting its reproductive function at 50 to 60 days of age which lasts for about 1 year. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (National Institute of Health (NIH) (2011) Pub No: 85-23). Ethical

approval was obtained from the Animal Care and Ethics Committee of Enugu State University of Science and Technology (ESUT) with the approval number ESUT/AEC/0431/AP397. Ethical approval was also obtained from the Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria with approval number (NAU/AREC/2024/0021).

2.2. Plant materials

The plants used in this study were *Zingiber officinale* rhizome and *Allium sativum* bulb*.* These plants were procured in a market in Enugu state of Nigeria.

2.3. Methods

2.3.1. Handling & disposal of carcinogenic agent

All carcinogenic substances were lock-stored except when in immediate use. Access to the store was restricted to named, authorized staff. Carcinogens were stored in appropriate, closed, clean, and clearly labelled containers. Over-stocking was avoided. Cupboards and refrigerators containing carcinogens were labelled with a "carcinogen" sign. Carcinogenic waste products were clearly labelled and stored safely until disposed of in the manner designated by a written procedure. Adequate control of exposure was ensured. The process and handling systems were totally enclosed unless this was not reasonably practicable. Generation of spills, leaks, fumes and vapors of carcinogens was minimized. The quantity of carcinogens in the workplace was limited. The number of persons who might be exposed to a carcinogen was minimum. None essential personnel were excluded. Eating, drinking, smoking in areas that may be contaminated by carcinogens, the use of snuff, the application of cosmetics and finger-mouth contact were prohibited. Hygiene measures including adequate washing facilities were provided. All users was mandated to wash their hands in lukewarm (rather than hot) water before leaving the laboratory or work area. There was regular cleaning of walls and surfaces. Suitable and sufficient warning signs were used in designated areas. The safe storage, handling and disposal of carcinogens, and the use of closed and clearly labelled containers were of utmost important. All workers put on protective clothing. Known or suspected carcinogens were disposed of safely: this was done by incineration or by disposal to a specialist contractor. These agents were not washed down the drains or placed in the general waste bins. Waste litter from the cages of animals exposed to known or suspected carcinogens was emptied into plastic bags and incinerated unopened [10].

2.3.2. Selection of herbs

The selection of the herbs was based on the types of cancer they can prevent/treat and their availability. *Zingiber officinale* and *Allium sativum* were selected because several studies confirmed their effectiveness against breast cancer. Also, the herbs are readily available.

2.3.3. How we ensured that there was no remnant of the solvent (ethanol) in the extract

To ensure that no ethanol remained in the extracts, we used a rotary evaporator for the final stage of the drying process [11]. The rotary evaporator was connected to a vacuum pump which enabled the rotary evaporator to use the vacuum to lower the boiling point of ethanol, resulting in quicker evaporation [11]. The resulting extract was then completely free of ethanol. Rotary evaporators work on the principle that solvents have a range of boiling points, which decrease under reduced pressure [11]. The evaporation flask rotates at a specified speed forcing the materials to form large area of thin film on the inner wall of the flask [11]. The flask was heated evenly and the material with the lowest boiling point rapidly evaporated [11].

2.3.4. Extraction of the active components

The plant sources, fresh *Zingiber officinale* rhizome and *Allium sativum* bulb, after being purchased from the market were washed and dried. After drying they were pulverized separately. 200 g of each pulverized plant parts was macerated in one liter of ethanol for 48 hours. The filtrates was collected by sieving through a muslin cloth. The filtrates were concentrated in a water bath at 50 °C and stored in the refrigerator until used.

2.4. Phytochemical analysis of *Zingiber officinale* and *Allium sativum* separately

The qualitative phytochemical analysis of the extracts and fractions were carried out using standard methods described by Odoh *et al*., [12].

2.5. Test for alkaloids

The plant extracts and fractions (0.2 g) were heated in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 min. The resulting solutions were allowed to cool and then filtered then 5 mL of the filtrates used for the following tests: [11]

Dragendorff's test: To each labeled test tube, 5 mL of the sample was added, followed by 1 mL of Dragendorff's reagent. Formation of orange or red precipitates indicates the presence of alkaloids [11].

Hager's test: The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate indicated the presence of alkaloids [11].

Wagner's test: The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. A reddish-brown precipitate indicated the presence of alkaloids [11].

Mayer's test: A quantity of 5 mL of each of the samples was placed in labeled test tubes and a few drops of Mayer's reagent (potassium mercuric iodide solution) were added. Formation of cream color precipitate indicated the presence of alkaloids [11].

2.6. Test for glycosides

The samples were extract with 1% H_2SO_4 solution in hot water bath for about 2 minutes. The resulting solution was filtered and made distinctly alkaline by adding 4 drops of 20% KOH (confirmed with litmus paper). One milliliter of Fehling's solution (equal volume of A and B) was added to the filtrates and heat on hot water bath for 2 minutes. Brick red precipitate indicates the presence of glycosides [11].

2.7. Test for saponins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the following test: [11].

Frothing test: The samples (5 mL) were placed in labeled test tubes and 5 mL of distilled water was added and the mixtures and shaken vigorously. The test tubes were observed for the presence of persistent froth [11].

2.8. Test for tannins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the test. To 3 mL of each of the samples, a few drops of 1% Ferric chloride were added and observed for brownish green or a blue-black coloration [11].

2.8.1. Test for flavonoids

Using methanol, 0.2 g of the plant extracts and fractions were dissolved individually and resulting solutions were used for the following test: [11].

Ammonium hydroxide test: A quantity of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicated the presence of flavonoids [11].

Sodium hydroxide solution test: A quantity of 10 mL of 10% sodium hydroxide solution was\ added to a portion of each of the samples and observed for color changes in the lower alkaline layer. Yellow color (flavones), Blue to violet color (anthocyanins), yellow to orange color (flavonones) [11].

Concentrated sulphuric acid test: A portion of each of the samples was mixed gently with conc. Sulphuric acid and observed for color change, yellowish orange color (anthocyanins), yellow to orange color (flavones), and orange to crimson (flavonones) [11].

2.8.2. Test for steroids and terpenoids

Salkowski test: The plant extracts and fractions were dissolved in methanol individually and the resulting solutions were used for the test. A 5 mL of each of the samples was mixed in 2 mL of chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration at the interface indicates a positive test [11].

Liebermann-Burchard test: Acetic anhydride (2 mL) was added to 0.5 g of each of the fractions and methanol extracts. Concentrated H_2SO_4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green [11].

Acute toxicity studies (LD_{50}) of *Zingiber officinale* and *Allium sativum* ethanol extracts

The actual median lethal dose (LD_{50}) estimation of the *Zingiber officinale* and *Allium sativum* ethanol extracts was conducted with the method described by Lorke, (1983) [13] with modifications according to the description by Ihekwereme *et al*., [14].

2.9. Anticancer potency study

2.9.1. Experimental design

The rats were grouped into 10 (10 animals per group). Group 1: Un-induced control (Naïve; received 5 ml/kg body weight of distilled water). Group 2: DMBA control (5 ml/kg body weight of distilled water). Group 3: Ginger treatment 530 mg/kg body weight. Group 4: Garlic treatment 530 mg/kg body weight. Group 5: Ginger and Garlic combination 2:8 (106: 424 mg/kg body weight). Group 6: Ginger and Garlic combination 4:6 (212: 318 mg/kg body weight). Group 7: Ginger and Garlic combination 5:5 (265: 265 mg/kg body weight). Group 8: Ginger and Garlic combination 6:4 (318: 212 mg/kg body weight). Group 9: Ginger and Garlic combination 8:2 (424: 106 mg/kg body weight). Group 10: Reference standard 5 mg/kg doxorubicin.

2.9.2. Breast cancer induction

Breast cancer was induced by using chemical-induction method using 7, 12-Dimethyl Benz (A) Anthracene (DMBA). DMBA was purchased from Sigma Aldrich Chemicals, USA. A single dose of 20 mg of DMBA diluted in soy oil (1 mL) was given intragastrically by gavage. Physical examination of the animals was done weekly and daily from 12^{th} weeks post-induction. The first tumor appearance occurred on the 14 week while an additional two weeks was allowed for tumor development in more animals. Double the number of animals required per group was induced to account for death and resistance to tumor induction. Treatment commenced on the 16^{th} week after the animals have been randomized into groups ensuring non-significant differences in tumor volume across groups.

Measurement of tumor volume and growth

The tumor mass was measured horizontally and vertically with Vernier Calipers weekly [15].

Tumor volume (V) (cm^3) = $(L \times B^2)/2$ [15].

Where L = large diameter and B = small diameter.

The percentage of tumor growth inhibition in each week was calculated using this formula:

$\frac{(T1-T0)-(t1-t0)}{T1-T0} \times 100$ [15].

Where T is the tumor volume of the DMBA control group (initial tumor volume will be considered as T0 and in subsequent weeks it will be considered as T1); and t is the tumor volume of treated groups [15].

2.10. Serum preparation

At the end of the study (20^{th} week), blood samples were collected through retro-orbital plexus into a plain covered test tube. After collection of the whole blood, the blood samples were allowed to clot by leaving it undisturbed at room temperature for 30 minutes. The clots were removed by centrifuging at 2,000 x g for 10 minutes in a refrigerated centrifuge. The resulting supernatant (serum) was immediately transferred into a clean polypropylene tube using a Pasteur pipette. The samples were maintained at 2–8 °C while handling and apportioned into 0.5 ml aliquots, stored,

and transported at –20 °C or lower. Freeze-thaw cycles were avoided due to possible detrimental effect to many serum components.

2.11. Serum tumor marker measurements

Serum rat CA 15-3 activity was estimated by quantitative sandwich enzyme immunoassay (ELISA) technique as described by Choi *et al*., [16] using rat specific CA 15-3 assay kit (Elabscience Biotechnology Co. Ltd., China). All reagents, working standards, and samples were brought to room temperature without additional heating and mixed thoroughly by gentle swirling before pipetting as described in the kit manual. One hundred microliters of the serum samples or blank and standards were placed in duplicates to their designated wells. The wells were covered with a sealer and incubated at 37 °C for 90 minutes. After incubation, the fluids in the wells were aspirated (without washing) followed immediately by the addition of 100 µl of dilute 1x biotinylated detection antibody. The wells were covered again with a sealer, and the contents were gently mixed and incubated for 60 minutes at 37 °C. After incubation, the contents of the wells were emptied by aspiration and then the wells washed by adding 350 µl of dilute wash buffer to each well, this was allowed to soak for 2 minutes and then the solution was decanted and the plate pat dry with a clean absorbent paper. The washing was repeated three times. Then 100 µl of dilute horseradish peroxidase (HRP) conjugate working solution was added to each well, the plate/wells covered with a plate sealer and incubated at 37 °C for 30 minutes. After incubation, the contents of the wells were decanted and the plate pat dry on absorbent paper. The wash process was repeated five times this time with 350 µl of dilute wash buffer which was allowed to soak for one minute during each wash. The plate was also emptied and pat dry during each wash (five times). After washing, 90 µl of substrate reagent was added to each well and the plate covered with a new plate sealer. The plate was incubated for 15 minutes at 37 °C with the plate/wells well protected from light. After incubation, 50 µl of stop solution was added to each well and the absorbance read immediately at 450 nm using the CA 15-3 programme of the Diatek Microplate/ELISA Reader (Wuxi Hiwell Diatek Insstruments Co Ltd, Wuxi, China). Serum concentration of CA 15-3 was determined using a regression equation of absorbance vs concentration graph of the standard.

3. Results

3.1. Results of phytochemical analysis of *Zingiber officinale* and *Allium sativum* ethanol leaf extracts

Phytocompounds in *Zingiber officinale* were: Alkaloids, Tannins, Flavonoids, Steroids and terpenoids while those in *Allium sativum* were Alkaloids, Saponins, Flavonoids, and Glycosides (Ihekwereme *et al*., [14].

3.2. Results of acute toxicity studies

The actual lethal doses of *Zingiber officinale, Allium sativum,* and a combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively (Ihekwereme *et al*., [14].

3.3. Results of anti-breast cancer studies

Table 1 Results of the effects of the various treatments on tumor volume (TV) after 20 weeks

Groups	Treatments (/kg body weight)	Mean TV ± S.E.M (%)	P-value
1	Naïve (5 ml DW)	0 ± 0	-
2	DMBA control (5 ml DW)	13.86 ± 0.36	-
3	*Zingiber officinale* (ZO) 530 mg	5.04 ± 0.33	9.17×10^{-8}
4	*Allium sativum* (AS) 530 mg	7.12 ± 0.12	1.06×10^{-6}
5	ZO:AS (2:8) 106:424 mg	7.32 ± 0.26	4.60×10^{-7}
6	ZO:AS (4:6) 212:318 mg	5.36 ± 0.17	2.48×10^{-8}
7	ZO:AS (5:5) 265:265 mg	5.04 ± 0.08	9.72×10^{-9}
8	ZO:AS (6:4) 318:212 mg	2.94 ± 0.32	1.45×10^{-8}
9	ZO:AS (8:2) 424:106 mg	4.96 ± 0.19	2.09×10^{-8}
10	Doxorubicin 5 mg	1.96 ± 0.12	1.14×10^{-9}

Table 2 Results of the tumor weight (TW) after week 20 of treatment

Groups	Treatments/kg body weight	Mean TW ± S.E.M (g)	P-value
1	Naïve (5 ml DW)	0 ± 0	-
2	DMBA control (5 ml DW)	7.82 ± 0.42	-
3	*Zingiber officinale* (ZO) 530 mg	3.80 ± 0.24	3.25×10^{-5}
4	*Allium sativum* (AS) 530 mg	5.18 ± 0.26	0.000725
5	ZO:AS (2:8) 106:424 mg	5.46 ± 0.38	0.003226
6	ZO:AS (4:6) 212:318 mg	3.98 ± 0.19	3.39×10^{-5}
7	ZO:AS (5:5) 265:265 mg	3.30 ± 0.93	6.02×10^{-6}
8	ZO:AS (6:4) 318:212 mg	1.68 ± 0.24	1.39×10^{-6}
9	ZO:AS (8:2) 424:106 mg	3.04 ± 0.15	5.25×10^{-6}
10	Doxorubicin 5 mg	0.92 ± 0.08	2.28×10^{-8}

Table 3 Results of the CA 15-3 after week 20 of treatment

Groups	Treatments/kg body weight	Mean CA 15-3 ± S.E.M (U/ml)	P-value
1	Naïve (5 ml DW)	11.02 ± 0.58	-
2	DMBA control (5 ml DW)	36.84 ±1.90	-
3	*Zingiber officinale* (ZO) 530 mg	20.16 ± 1.33	0.000103
4	*Allium sativum* (AS) 530 mg	23.52 ± 1.29	0.000404
5	ZO:AS (2:8) 106:424 mg	23.12 ± 1.50	0.000476
6	ZO:AS (4:6) 212:318 mg	19.30 ± 1.17	4.99×10^{-5}
7	ZO:AS (5:5) 265:265 mg	18.32 ± 1.24	3.81×10^{-5}
8	ZO:AS (6:4) 318:212 mg	14.02 ± 1.01	5.47×10^{-6}
9	ZO:AS (8:2) 424:106 mg	16.22 ± 0.88	1.18×10^{-5}
10	Doxorubicin 5 mg	11.80 ± 0.57	1.46×10^{-6}

4. Discussion

From the results of the anticancer study, the tumor volume (TV), tumor weight (TW), and the mean concentrations of the cancer marker, cancer antigen 15-3 (CA 15-3) were recorded for groups 1-10 after 20 weeks of treatment. According to a certain study, cancer antigen 15-3 (CA 15-3) is a common tumor marker and its serum level is evaluated periodically during treatment in breast cancer patients [17]. In another study that considered thirteen categories of breast tumor markers, CA 15-3 was among the categories that showed evidence of clinical utility and it was recommended for use in practice: [17]. In this current research, it was shown that the naïve group 1 which was not induced, did not develop any breast cancer hence both TV and TW were recorded as zero (0) [18]. However, the naïve group had natural CA 15-3 serum concentration of 11.02 ± 0.58 µg/ml. Group 2 which was induced with DMBA yet not treated expectedly had the largest TV, TW, and mean serum concentration of CA 15-3 (13.86 ± 0.36%, 7.82 ± 0.42 g, and 36.84 ±1.90 µg/ml) respectively. Group 3 which was treated with *Zingiber officinale* (ZO) ethanol extract alone (530 mg/kg body weight) decreased both the TV, TW, and CA 15-3 levels significantly ($p < 0.05$) when compared with group 2. Group 4 which was treated with *Allium sativum* (AS) ethanol extract alone (530 mg/kg body weight) also reduced the three parameters significantly ($p < 0.05$) when compared with group 2. However, the reduction in group 3 was more than those in group 4 and indicated that ZO exhibited better anticancer effects than AS. The results also showed that all the tested combinations at all tested proportions of ZO and AS had remarkable anticancer activities which were very significant

when compared with untreated group 2. Interestingly, group 8 which was treated with the two herbs in a ratio of ZO:AS = 6:4 (318:212 mg/kg body weight) had the best and significant anticancer effects than the other combinations. Group 8 had their 20th week mean TV reduced to 2.94 ± 0.19% ($p = 1.45 \times 10^{-6}$) and their TW reduced to 1.68 ± 0.24 g ($p = 1.39 \times 10^{-6}$); as opposed to the untreated group 2 that had TV and TW values of 13.86 ± 0.36% and 7.82 ± 0.42 g respectively. As a confirmation to these activities, group 10 which was treated with a standard anticancer drug (doxorubicin 5 mg/kg body weight) reduced TV, TW, and CA 15-3 serum levels to 1.96 ± 0.12%, 0.92 ± 0.08 g, and 11.80 ±0.57 µg/ml respectively. These were highly significant when compared with the untreated group 2 with p-values of 1.14×10^{-9}, 2.28×10^{-7}, and 1.46×10^{-6} respectively. Doxorubicin is an antibiotic derived from the *Streptomyces peucetius* bacterium [18]. It has widespread use as a chemotherapeutic agent since the 1960s and it may be used to treat a number of cancer types including breast cancer [19].

5. Conclusion

The herbs *Zingiber officinale* and *Allium sativum* ethanol extracts exhibited remarkable anti-breast cancer activities. This was paramount when the two herbs were combined in a proportion of ZO:AS = 6;4; which implied 318:212 mg/kg body weight of the extracts. This combination had anti-breast cancer effects that were similar to that of the standard drug, doxorubicin.

Complaince with ethical standard

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directives 2010/63/EU for animal experiments. Guides for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes (Approval number is NAU/AREC/2023/00021).

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