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EVALUATION OF ANTICANCER ACTIVITY OF *STEGANOTAENIA ARALIACEA* (CARROT TREE) BARK EXTRACT IN CANCER INDUCED MAMMARY GLANDS OF FEMALE SPRAGUE DAWLEY RATS

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ABSTRACT

Ethnopharmacological relevance: Steganotaeniaaraliacea is a common small, aromatic, deciduous tree which is widely distributed in the rocky parts of Zimbabwe. It is used by the local population for different medicinal purposes which include; treatment of stomach aches, snake bites, the common cold, and cancer. A significant number, 40%, of registered traditional practitioners in Zimbabwe use the plant to treat cancer*Aim:* The aim of the study was to test for the anti-tumor activity of *Steganotaeniaaraliacea* in 7,12-Dimethylbenz (a) anthracene (DMBA)-induced breast cancer in Sprague Dawley rats. *Methodology:* The anticancer activity of methanolic bark extract of *S. araliacea* was evaluated in-female Sprague Dawley rats with DMBA-induced breast cancer. Cancer was induced by subcutaneous injection of 20mg DMBA in virgin female Sprague Dawley rats. The plant crude extract was administered by gastric gavage on a daily basis for a period of 20 days. The doses administered were 0.22g/ml, in one test group, and 0.45g/ml, in the second test group. The study animals used had an average weight of 281g. Tumor size was determined before and after administration of the crude plant extract and compared against the antitumor effect of a standard drug, cyclophosphamide. After the study, the animals were sacrificed. *Results:S.araliacea* crude extract showed a significant decrease in DMBA induced mammary tumor size in the rats. Phytochemical screening of the crude extract revealed the presence of flavonoids, tannins, saponins, and alkaloids. *Conclusion:* The results showed that the methanolic extract of *S.araliacea* bark has the effect of reducing tumor size, however, is less efficacious compared to the standard drug, cyclophosphamide.

KEYWORDS: *S.araliacea*, 7,12-Dimethylbenz (a) Anthracene, Mammary Tumor, Breast Cancer & Cyclophosphamide

1. INTRODUCTION

Herbal medicine, also known as herbalism, is a medical practice based on the use of plants or plant extracts that may be taken orally or applied to the skin. Since ancient times, herbal medicine has been used by many different cultures throughout the world to treat illness and to assist bodily functions. Nearly all cultures from ancient times to the present day have used plants as a source of medicines. As a result, different remedies tended to develop in different parts of the world.

Throughout humankind evolution, the importance of natural products for medicine and health has been enormous. Earliest ancestors chewed certain herbs to relieve pain, or wrapped leaves around wounds to improve healing. Natural products were the sole means to treat diseases and injuries. During the past decades, after the advent of molecular biology

and combinatorial chemistry made possible the rational design of chemical compounds to target specific molecules, natural products have taken a secondary role in drug discovery and drug development. The past few years have witnessed a renewed interest in the use of natural compounds and, more importantly, their role as a basis for drug development. The modern tools of chemistry and biology have currently allowed scientists to detail the exact nature of the biological effects of natural compounds on the human body, as well as to uncover possible synergy, which holds much promise for the development of new therapies against many devastating diseases, including dementia and cancer [Hong-Fang *et al*, 2009]. Modern chemistry has ushered in a new era for the study and use of natural products. Analytical and structural chemistry has provided the tools to purify various compounds and to determine their structures, which, in turn, has given insights into their action on the human body. In 1805, a German pharmacist Friedrich Wilhelm Sertürner (1783–1841) isolated morphine from opium, and it became both the first pure naturally derived medicine and the first to be commercialized, by Merck in 1826 [Hong-Fang *et al*, 2009]. Medicinal plants, either as extracts, pure compounds or as derivatives, offer unlimited opportunities for the discovery of new drugs.

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. It is one of the leading causes of morbidity and mortality in the world [Kanavos, 2006] Cancer, of all non-communicable diseases, is the second leading cause of death, only after cardiovascular diseases [WHO, 2005; Mathers and Loncar, 2006; Lopez *et al*, 2006; Hoyert *et al*, 2006]. Furthermore, the number of cancer deaths is projected to rise from 7.1 million in 2002 to 11.5 million in 2030 [Mathers and Loncar, 2006]. Common sites for the development of malignant tumors are the skin, lungs, prostate, breasts, and large intestine (colon).

Breast cancer is the most frequent malignancy among women worldwide [WHO, 2014]. It is a highly heterogeneous disease represented by tumors that have a diverse natural history, complex histology, and a variable response to therapy.

Medicinal plant drug discovery continues to provide new and important leads to different kinds of diseases including cancer. Current strategies to overcome the global problem of cancer and other diseases include research in finding new and innovative chemicals from plants. *Steganotaeniaaraliacea* is an example of such plants. The medicinal plant, *Steganotaeniaaraliaceae*, was identified on the basis of its ethnomedicinal claims established from a survey (part of this study) on plants used to treat cancer by traditional practitioners in Zimbabwe.

1.1 Problem Statement

A large proportion of cancer patients are estimated to be using herbal medicines [Cassileth and Deng, 2004]. However, only a relatively small number of plant species has been studied for possible anticancer properties. Data on efficacy and safety are available for an even smaller number of plants, their extracts, and active constituents and preparations containing them. There is little or no information regarding the phytoconstituents and the pharmacological effects of these plant medicines on animal models [Mir, 2006]. Thus, there is a great need for the screening for phytochemicals and testing for the efficacy of such medicinal plants that are widely or commonly used to treat various ailments in communities.

1.2 Significance of Study

Cancer is among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer-related deaths in 2012 [WHO, 2014]. The number of new cases is expected to rise by about

70% over the next 2 decades. According to the Zimbabwe national cancer registry report for 2012, breast cancer contributes 7% of cancer incidence in Zimbabwe and it is fourth on the top 10 list. In terms of mortality, breast cancer causes 4% of all cancer deaths. A total of 1 556 cancer deaths comprising (49.4%) males and (50.6%) females were recorded in Harare in 2012 [Chokunonga*et al*, 2014]. With such statistics, cancer is a serious cause for concern.

Many cancer patients have turned to natural medicines, as complementary and/or alternative medicine (CAM), like herbs and probiotics, with the hope of finding a cure to their illnesses as well as to make them feel better. Surveys on the use of CAM by cancer patients have been reported to be as high as 64% and as low as 7% in some cases [Ernst and Cassileth, 1998]. As the use of CAM by cancer patients increases, the concern for its efficacy and safety in cancer patients has also increased [Balneaves *et al*, 1999]. In spite of the mass use of natural medicine as a form of CAM therapy, very little is known of the efficacy and safety of many of the CAM therapies that cancer patients use. With these statistics, there is a need to enhance knowledge on the efficacy of these plant medicines.

1.3 Aim of Study

The aim of the study was to test for possible anticancer activity of *Steganotaeniaaraliacea* bark extract against DMBA induced mammary gland cancer in Sprague Dawley rats.

2. RESEARCH METHODOLOGY

The research was conducted in accordance with the internationally accepted principles of laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC)

2.1 Ethnomedicinal Survey

Interviews were done with identified and registered traditional practitioners in all the 10 provinces in Zimbabwe. The registered practitioners were identified with the assistance of the Traditional Medicine Practice Control of Zimbabwe (TMPCZ). A questionnaire was used as the tool in the interviews. Consent forms were given to and signed by the participants in the ethnomedicinal study.

2.2 Extraction of Active Constituents in *SteganotaeniaAraliacea*

The plant material (bark) was collected from the rocky areas between Norton and Chegutuin Zimbabwe. Identification of the plant was done by taxonomists from the local botanical gardens.

The *Steganotaeniaaraliacea* bark was peeled, cut into small pieces and weighed. The bark was left to dry out in direct sunlight. It was weighed every 3 days until a constant mass was obtained.

Figure 1: Peeled Bark of Steganotaeniaaraliacea

The dry bark was then ground into a powder using pestle and mortar and weighed.

400g of the powder was weighed and put into a 2Lcontainer. Using a measuring cylinder, 1L of 99.5% methanol was measured and poured into the container with the powder. The mixture was vigorously agitated for approximately 5minutes, on a daily basis, for 5 days. The mixture was then filtered and the filtrate was placed in a rotary evaporator where the solvent was driven off at a temperature of 71°C.

2.3 Qualitative Phytochemical Tests

The extract was tested for the presence of tannins, flavonoids, alkaloids, saponins and athocyanins using Ferric chloride, sulphuric acid, Mayer's, foam and sodium hydroxide tests, respectively.

2.4 Formulation of DMBA Emulsion For Injection

550mg of DMBA powder was dissolved in 11mls of sunflower oil by placing the mixture in a sonicator. After the DMBA had dissolved, 11mls of saline was added gradually to the oil mixture. 22mls of the emulsion was formed (25mg of DMBA per 1ml).

2.5 Induction of Cancer

25 mg of DMBA (1ml of the emulsion) was administered to each study animal from groups II to V.

2.6 Grouping of Animals

25 virgin female Sprague Dawley rats, 8-10weeks old, were divided into five groups. Group I animals (negative control) were not induced with cancer but provided with normal feed and water. All the study animals in groups II to V received DMBA for cancer induction. The doses administered to the test groups were adopted from the dose range results from another study done by Agunu*et al* on the same plant [Agunu*et al*, 2004]. Group II animals (Control), did not receive any treatment but normal feed and normal saline. Group III animals (positive control group), received a standard drug, cyclophosphamide, at a dose of 10 mg/kg via the intra-peritoneal route according to Samy et al, 2006 [Samy *et al*, 2006]. Group IV animals(test group 1), received the methanolic extract of *Steganotaeniaaraliacea* at a dose of 0.22g/ml. Group V animals (test group 2) were administered the methanolic extract of *Steganotaeniaaraliacea*at a dose of 0.45g/ml. 1ml of DMBA emulsion was injected subcutaneously beneath the mammary gland of rats in group II, III, IV, and V. The rats in all the groups were kept in their cages for 13weeks, receiving water and food. After 13 weeks, the animals were examined for

breast tumors and the tumors were measured using Vernier calipers. The average tumor sizes per group were calculated.

2.7 Treatment

The rats were weighed and their weights ranged from 253g to 301g. The average weight was 281g. Tumor volumes were calculated before dosing using the following formula:

$$\text{Tumor volume}=\frac{1}{2}(\boldsymbol{length} \times \boldsymbol{width^2})$$

All treatments were administered to the study animals at consistent times for a period of 20 days. Group III animals, the positive control, received the standard drug, Cyclophosphamide, at a dose of 10mg/kg per oral, that is, 2.81mg of cyclophosphamide per rat. Group IV animals, (test group 1), were administered 1ml (0.22g/ml) of the pure methanolic extract of *S. araliacea*.2.75g of the extract was dissolved in 12mls of 75% Dimethyl sulfoxide. Each rat in this group was given 1ml (0.22g/ml) of the preparation by intra-gastric gavage. Group V animals,(test group 2) were administered 1ml (0.45g/ml)of the methanolic extract of *S.araliacea*.2.75g of the extract was dissolved in 6mls of 75% Dimethyl sulfoxide. Each rat in this group received 1ml (0.45g/ml) of the preparation by intra-gastric gavage.

2.8 Determination of the Percent Decrease in Tumor Size

After 20 days of treatment, the tumors were measured once again and the average tumor volumes calculated.

The percent change in tumor size was calculated as per the formula is given below:

$$\frac{\text{Size of the tumor before administration of plant extract - percent size of the tumor after administration of plant extract X (100)}}{\text{Size of the tumor before administration of plant extract}}$$

3. RESULTS

3.1 Ethnomedicinal Survey

A total of 20 traditional practitioners were interviewed. 8 practitioners cited that they use the carrot tree bark to manage cancer.

3.2 Phytochemical Tests Results

The crude bark extract was found to contain alkaloids, saponins, anthocyanins, flavonoids, and tannins.

3.3 Tumor Volume

Figure 2: Sprague Dawley Rats with Breast Tumors after DMBA Administration

Table 1: Results for Tumor Volume at the Start of Treatment (Data Presented as the Mean of 5 rat Tumors)

Group	Average Length	Average Width (cm)	Average Tumor Volume (cm^3)
I: Negative control	0.00	0.00	0.00
II: DMBA (control)	2.1	1.85	3.59
III: Positive control	3.4	2.18	8.08
IV: Test group 1	1.68	1.52	1.94
V: Test group 2	1.85	1.35	1.69

Table 2: Results for Tumor Volume After Treatment (Data is Presented as the Mean of 5 Tumors)

Group	Average Length	Average Width (cm)	Average Tumor Volume (cm^3)
I: Negative control	0.00	0.00	0.00
II: DMBA (control)	2.40	2.15	5.46
III: Positive control	2.80	1.98	5.49
IV: Test group 1	2.11	1.59	2.67
V: Test group 2	1.81	1.28	1.48

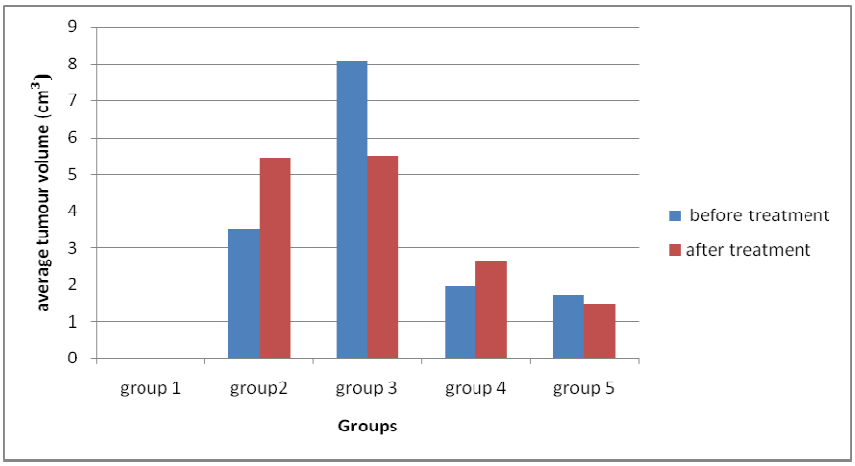

Figure 3: Comparison of Tumor Volumes Before and After Treatment

Table 3: Percentage Change in Tumor Size After Intervention

Group	Percentage change (%)
I: Negative control	No change
II: DMBA (control)	Increased by 54%
III: Positive control	Decreased by 32%
IV: Test group 1	Increased by 36%
V: Test group 2	Decreased by 12%

4. DISCUSSIONS

The ethnomedicinal survey identified *Steganotaeniaaraliacea*(carrot tree)as one of the medicinal plants commonly used by traditional practitioners in Zimbabwe in the management of cancer; out of the interviewed 20 practitioners, 8 cited the carrot tree. The practitioners commonly use the bark of the plant. The *Steganotaeniaaraliacea* plant's bark extract was found to contain flavonoids, alkaloids, anthocyanins, tannins, and saponins. Saponins are glycosides consisting of sugar units which are linked to a steroid aglycone or a triterpene. They have various medicinal properties that include anti-inflammatory activity as well as cytotoxic effects on malignant tumor cells [Mahasneh, 2002].

Saponins have been shown to enhance targeted toxins/therapies in the treatment of cancer [Panghal*et al*, 2011, Doughari, 2009]. It has been demonstrated that saponins and chemotherapeutic agents can be combined to enhance membrane transportation [Konate*et al*, 2011]. This combination may result in the reduction of adverse effects of the conventional chemotherapeutic medicines [Doughari, 2009].

Flavonoids are water-soluble phytochemicals that have a wide spectrum of activity including antimicrobial, anti-tumor and anti-inflammatory activities. In addition, they are powerful antioxidants [Govindappa*et al*, 2011, Brunetti *et al*, 2013]. They have the ability to protect the body from various cancerous diseases by neutralizing Reactive Oxygen Species (ROS) [Taabodi*et al*, 2017]

Other phytochemicals identified in the bark extract include tannins and anthocyanins. Tannins are naturally occurring polyphenolic compounds with high molecular weight [Byarugaba, 2009]. They are known for their antimicrobial and antioxidant activity, making them useful in the treatment of a number of different ailments. Studies have suggested that anthocyanins may play important roles in helping reduce the risk of cardiovascular disease (CVD), cognitive decline, and cancer. The role of anthocyanins in the prevention of these diseases has been linked to their antioxidant properties, but some research now suggests that anthocyanins health benefits are likely to be from unidentified chemical properties beyond their antioxidant capacity [Tsuda, 2012].

These results from a phytochemical assay of *Steganotaeniaaraliacea* bark extract are related to those found in another research on the same plant by Omolo et al in 2014 [Omolo*et al*, 2014].

At week 13, after administration of DMBA, all the study animals were showing visible tumors. This is supported by another study done by Carlos et al in 2004, that is; at 13 weeks, 100% of the study animals had at least 1 tumor [Barros *et al*, 2004]. At the start of the treatments, as shown in table 1, the tumor volumes ranged from an average of 1.69cm³ to 8.08cm³. The standard positive control had the highest tumor volume and the test group 2 had the lowest tumor volume. The tumor volumes were presented as the mean of five rat tumors. The negative group did not have any tumor growth at the start of treatment.

From Table 2, the negative control group animals still did not have any visible tumors at the time treatment was stopped in the other groups. Group II, the DMBA control group, showed an increase in tumor volume from an average of 3.59cm³ to 5.46cm³ i.e. a 54% increase in tumor volume. This was because there was no intervention administered to the study animals in this group. Group III study animals showed a decrease in tumor volume from an average of 8.08cm³ to 5.49cm³ i.e. a 32% decrease in tumor size. This percentage decrease in tumor volume, though significant, does not tally with results from another study done in India in 2015 which showed a decrease in tumor volume of 72% after administration of cyclophosphamide (10mg/kg) to Sprague Dawley rats for 20 days [Nair *et al*, 2015]. The difference could have been due to the fact that in the above-mentioned publication, the rats used were 45 days old (6 weeks) while the rats used in this study were of varying weeks, from 8 to 10 weeks leading to differences in response and also dietary differences could have contributed to the difference in response.

Group IV animals were given 0.22g/ml of the crude extract. This group showed an increase in tumor volume from 1.94cm³ to 2.67cm³, that is, a 36% increase in tumor size. The increase in tumor size suggests that the dose of 0.22g/ml had minimal anti-tumor activity. The dose of the extract was way too low to be effective in preventing tumor growth and at the same time reducing the tumor sizes. Nevertheless, the extent of tumor volume increase was significantly reduced from 54%

to 36%, an indication that the extract had some antitumor activity.

Group V animals received 0.45g/ml of the crude extract. This group exhibited a decrease in tumor volume from 1.69cm³ to 1.48cm³, that is, a 12% decrease in tumor size. Such a decrease shows that the bark extract of *Steganotaeniaaraliacea*at an increased dose can exhibit substantial anticancer activity albeit less than that of cyclophosphamide at the dose used in this case. Figure 3summarizes the comparison of tumor volumes before and after treatment.

The reduction in tumor size as a result of treatment with the plant extract shows that the plant extract can be used as an adjunct or alternative to standard conventional treatment. Therefore, tests for possible interaction of the extract with these standard drugs should be carried out to confirm if such combinations may not cause harm or any negative interaction with the drug.

From the study, it can be postulated that the anticancer property of *S.araliacea* may be attributed to some anti-cancer activity of some of the phytochemical. For further studies, whole plant extract could be used because, although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming increasingly recognized that the beneficial effects of plants are due to a complex interplay of the composite mixture of compounds present in the whole plant (additive/synergistic and/or antagonistic) rather than single constituent agents alone [Karna *et al*, 2012].

Another recommendation is that tests using higher doses that go beyond the LD50 of the extract could be explored to observe if the anticancer activity of the plant is enhanced or not. Strategies to remove the toxic elements of the extract could then be explored if doses beyond the LD50 were more effective. Also, the duration of treatment with various extract doses can be extended from 20 days in future studies on the *S. araliacea* plant.

5. CONCLUSIONS

The study demonstrated the anticancer properties of the *S.araliacea*bark extract. Treatment with the methanolic extract of *S.araliacea* bark of DMBA induced mammary gland tumors showed a decrease in tumor volume. The reduction in mammary tumor volume was dose-dependent, although more doses could be explored. However, the standard drug, cyclophosphamide, proved to be more effective compared to the extract. Further studies on higher extract doses as well as synergism between the extract and conventional drug(s) can be explored. Also, histopathological examinations of the induced tumors can be further studied.

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