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Phytochemical and Biological Study on Saabfat 7 with a Focus on Vernonia Amygdalina

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ABSTRACT: *Cancer is considered at the moment one of the main causes of death worldwide. The current tendency in the treatment of cancer pursues to obtain a more successful treatment that do not increases alone its effectiveness but rather it diminishes its adverse effects .One of such that provides a better alternative is naturaceuticals, tagged as natural products. They are able to scavenge free radicals, induce detoxification, inhibit stress response proteins and interfere with DNA binding activities of some transcription factors. In these new therapeutic slopes the treatments are included that modify the biological answer, starting from emergent pharmacological agents able to modulate the transduction of signs inducing a selective death of the tumoral cells. The presence of blocking bioactive activities of SAABFAT 7 with Vernonia amygdalina as it active ingredient opens new perspectives in the treatment of this disease by means of the use of blocking or specific modulators of these radicals.*

The affectation in the expression of these radicals and other important markers in tumoral cells of epithelial origin confirms the antitumoral effect and they reaffirm to this cocktail of natural antitumoral products as a novel and attractive alternative in the treatment of cancer.

Keywords*: cancer, SAABFAT 7, naturaceuticals, tumoral, free radicals*

I. INTRODUCTION

The use of herbal medicine dates back to thousands of years. Although it originated in India and China, it is widely practiced in Africa. Herbal therapy which started as folk medicine in most developed countries is becoming increasingly more popular with patients seeking alternative treatment options.

Eisenberg et al., (1998) reported that, in developed countries the number of visits to the alternative medicine practitioners is growing rapidly with the number of visits in US was estimated to be 629 million in 1997; it was believed to have exceeded the number of visits to all primary care physicians.
It is a known fact that, a large proportion of African population uses some form of alternative medicine and many do not inform their physician about it.

Most patients seek alternative medicine because of lack of basic health facilities, when conventional therapy has failed or they feel the products have no side effects because they are of natural origin.
Herbal therapy has also been used extensively in Nigeria. Although more than 80 percent of the people in both the underdeveloped and the developed countries depend on herbal medicines for their medical needs
The major problems with herbal medicines in such countries still remain their poor and sometimes unhealthy presentation.

Being faced with the panorama of resistivity and the attendant side effects associated with orthodox drugs, the focus is now being channeled to naturaceuticals also known as natural products.

Bitter leaf botanically called Vernonia amygdalina, is one of such products. Bitter leaf is a medicinal plant, which grows in the humid tropical secondary forests of Africa. Bitter leaf is among several natural products used by traditional healers in Western Nigeria to treat a number of bacterial infections. The leaves are used as a leafy vegetable for preparing the popular bitter-leaf soup and the juice or extract serves as a tonic drink. It contains 18% protein, 8.5% fiber in a dry matter, and a good composition of macroelements[1.] Moreover, Vernonia amygdalina has been used in traditional medicine as an antihelminth, an antimalarial, and a laxative herb. It was observed that an apparently sick wild chimpanzee chewed this plant to extract bitter juice and after a while it seemed to return to its normal activity[2].

These observations stimulated research on the chemical principals of Vernonia amygdalina. Several stigmastane-type saponins such as vernoniosides A_1, A2, A_3[3], B_2, B_3, A_4[4], and C[5] have been identified in the leaves. It was shown that the A series of these saponins were bitter, mixtures of saponins as well as vernonioside A_1 were shown to affect body and liver weights, urine and fecal output, and plasma and liver cholesterol concentrations in mice fed diets amended with these compounds[1]. The antiplasmodial activity of some sesquiterpene and steroidal constituents of Vernonia amygdalina was tested, and some were proved to be active against *Plasmodium falciparum* in vitro[6].

The current search for potential anti oxidative principles to replace suspected tumour-causing synthetic analogues such as BHT[7] necessitated this investigation. Antioxidative principles have been implicated as parts of anticancer formulations and patents[8]. Although luteoline has been reported to be a strong antioxidant[9], no report has so far been given on the antioxidative potentials of its tannin, alkaloid, anthroquinone, anthracyanosidic and glycosidic derivatives.

The present paper characterizes flavonoids, tannin, alkaloid, anthroquinone, anthracyanosidic and glycosidic derivatives of SAABFAT 7 with Vernonia amygdalina leaves as one of its active ingredients. This study is therefore designed to determine the clinical activities of (SAABFAT 7) with Vernonia amygdalina as one of its active ingredients, on animals infused with carcinogen.

II. METHODOLOGY

EXPERIMENTAL ANIMALS

Nine rabbits with an average weight of 1.0 kg each were used for this experiment. The rabbits were kept in the animal house of the Ben Amodu farm and research centre, Abuja. They were acclimatized to the environment for 2 weeks. They were fed with commercial feeds pellet and clean water ad-libitum. The cages were cleaned daily

SOURCES OF VERNONIAL AMYGDALINA

The leaves of Vernonia amygdalina were harvested fresh from Ben Amodu's farm in kogi state, Nigeria; the required parts of these plants were collected carefully from the farm at the appropriate time. These parts were dried in the sun for one (1) day while it was subjected to seven (7) days air drying under a shade. Care was taken not to allow contamination of any sort. The plant was botanically identified by Dr. U.U Usman of the Botany Department, University of Agriculture Makurdi, Benue State. 150g of the ground dried leaves of *Vernonia amygdalina* was extracted with 1 litre of hot water and another 150g with 1litre of 70% alcohol respectively.

SOURCES OF CARCINOGEN

Metronidazole is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals. The metronidazole was obtained from H.J. Harkins company, Inc. USA.

Hot Water Extraction

A measured quantity (150g) of the ground *Vernonia amygdalina* leaves was dissolved in hot water, mixed by shaking vigorously. This was allowed standing for 1hour, and then filtered to obtain the extract. The filtrate obtained was oven-dried to get rid of the residual water. 14g of the extract was obtained.

Using Alcohol as Solvent

150g of ground *Vernonia amygdalina* leaves was dissolved in one liter (1L) of 70% alcohol (ethanol). This was allowed standing for 48hours and then filtered to obtain the extract. The filtrate was oven-dried to get rid of the residual water; 10.24g of the dry extract was obtained.

III. PRELIMINARY PHYTOCHEMICAL SCREENING

The extract of the *Vernonia amygdalina* leaves was screened to determine the presence of the following metabolites through the preliminary phytochemical screening. The following active constituents and metabolites were tested for:

(i) Alkaloid
(ii) Flavonoid
(iii) Tannin
(iv) Cardiac glycosides
(v) Anthroquinone
(vi) Saponin
(vii) Anthracyanosides

ALKALOIDS

The extract (0.5g) was stirred with 5ml of dilute HCl on a stream bath; 1ml of the filtrate was treated with few drops of Mayers and a second 1ml portion was treated similarly with dragendoff's reagent and finally another 1ml portion with wagner's reagent.

Test	Observation	Inference
Alkaloid (i) 1ml of extract + few drops of Drangendoff's reagent	**Yellowish brown colour**	**Alkaloid inferred**
(ii) 1ml of extract + few drops of Mayers reagent	**Yellowish colour seen**	**Alkaloid inferred**
(iii)1ml of extract + Wagner's reagent	**Dark turbid brown**	**Alkaloid inferred**

TANNINS

Each portion of alcohol and water extract (5g) was stirred with 10ml of alcohol and distilled water respectively. They were filtered and ferric chloride reagent was added to filtrates and 1ml portion of the extract was treated with bromine water.

Test	Observation	Inference
(i) 1ml of extract + 1ml ferric chloride	**Blue black precipitate**	**Catecol tannin present**
(ii) 1ml of extract + bromine water	**Brownish red turbid**	**Condensing tannins**

FLAVONOID

(i) Lead Acetate Test: 0.2g of the extract was added to 0.2ml 0f 10% Lead acetate, the mixture was gently shaken to avoid emulsion.
(ii) Ferric Chloride Test: 0.2ml of 10% ferric chloride was added to the extract. The mixture was shaken together to observe colour.
(iii) Sodium Hydroxide Test: 0.2ml of dilute NaOH was added to 0.2ml of the extract shaken gently.

Test	Observation	Inference
(i) Lead acetate on extract	**Dirty brownish precipitate**	**Flavonoid present**
(ii) ferric chloride test	**Wooly brownish colour**	**Flavonoid present**
(iii) Sodium hydroxide test	**Golden yellow precipitate obtained**	**Flavonoid present**

CARDIAC GLYCOSIDE

(i) Kedde's Test: 1ml of 8% solution of the extract was mixed with 1ml of 2% solution of 3,5 dinitrobenzoic acid in methanol and 1ml of 5.7% aqueous sodium hydroxide

(ii) Liebermann-burchard Test: The *Vernonia amygdalina* extract (0.5g) was dissolved in 2ml of acetic anhydride and cooled well in ice., concentrated sulphuric acid was carefully added.

(iii) Salkwoski's Test: 0.5g of the *Vernonia amygdalina* extract was dissolved in 2ml of chloroform, concentrated sulphuric acid was carefully added to form lower layer.

(iv) Keller Killiani's Test: The extract of the *Vernonia amygdalina* (0.5g) was dissolved in 2ml of glacial acetic acid containing one drop (1 drop) of ferric chloride solution. This was under-layered with concentrated sulphuric acid.

Test	**Observation**	**Inference**
(i) Kedde's Test	**Brownish precipitate**	**Canclenolide**
(ii) Lieberman's Test	**Deep greenish blue**	**Steroid nuclei inferred**
(iii) Salowiski's Test	**Reddish brown colour at interface**	**Deoxysugar,characteristics of cardenolide**

Anthraquinone Glycosides

These occurred in both free and bound form

(i) Free Anthraquinone: The extract 0.1g was dissolved with 10ml hot water for aqueous extract and 10ml of alcohol extract, both were put in water bath to steam for 5 minutes, the solution were filtered hot, the filtrate were extracted with chloroform layer was taken off. This layer was washed with 5ml of water and was shaken with 5ml ammonia solution.

(ii) Bound Anthraquinone

A second set of the mixture was prepared with 0.1g of the extract with 10ml of ferric chloride solution and 5ml hydrochloric acid. The sample was hydrolyzed by heating on water bath for 10 minutes, filtered hot and treated as with free anthraquinone.

Test	**Observation**	**Inference**
Free anthraquinone Bontrager's test	**Presence of red colour in ammonia upper phase.**	**Free anthraquinone present**

SAPONINS

The ability of saponin to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening.

(i) Frothing Test: A little portion of the extract was shaken with water in a test tube.

(ii) Haemolysed Test: Exactly 0.2g Vernonia amygdalina extract was dissolved in 10ml of warm water and filtered, remaining the filtrate. 2ml of 1.8% sodium chloride (NaCl) solution was put into two test tubes. To one of these 2ml distilled water was added. The concentration of sodium chloride in each test tube was isotonic with blood serum. Five drops of blood were added to each tube and the tubes were inverted gently to mix the contents

EXPERIMENTAL DESIGN

The rabbits were randomly divided equally into 3 groups, each group with three animals each.

TABLE: 1 Administration of the extract and the carcinogen

Groups	Dose (mg/kg) orally	Duration in days
(A) SAABFAT 7	100	21
(C) Control (distilled water)	-	21

TABLE 2: Administration of the carcinogen alone

Groups	Mg/Kg/Day	Duration in Days
(B) Metronidazole	500	21
(C) Control (distilled water)	-	21

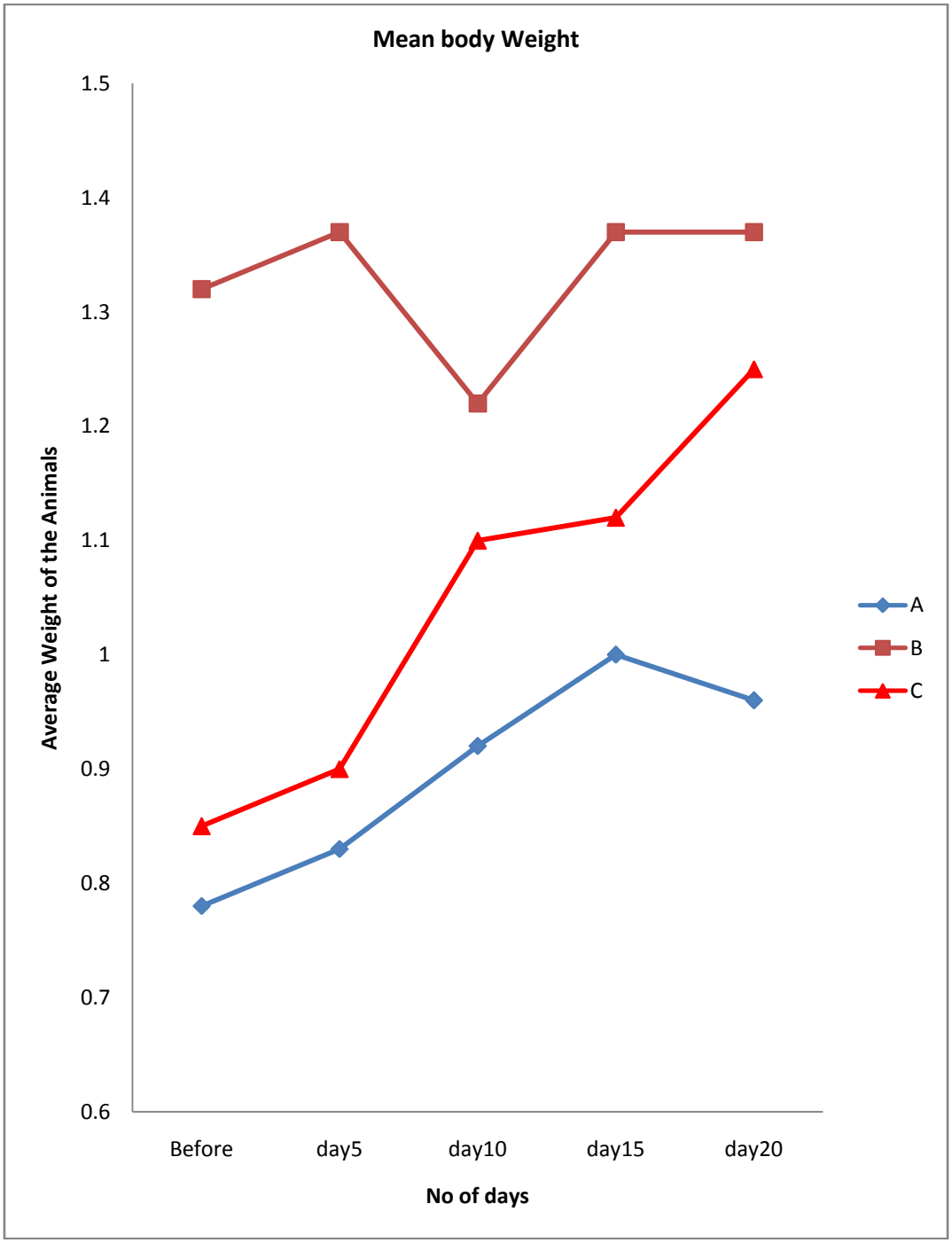
The administration of the extracts lasted for 21 days. The extracts and the carcinogen were mixed with small quantity of the animals' feed for those in group **A** before they were given while the carcinogen with the small quantity of the animals' feed was given separately for those in group **B**. Group **A** animals were given 100mg/kg/day of the extract and the carcinogen, Group **B** animals were given 500mg/kg/day of the carcinogen and the animals' feed and Group **C** animals were given distilled water. The animals were watched closely and vital signs taken for the period the experiment lasted and were euthanatized at the end of the 21 day. Cotton wool was dipped into chloroform and the animals were allowed to inhale the chloroform in a desiccator. They were opened up and the liver and kidney were harvested. The selected pieces of tissue were processed into paraffin wax, sectioned at 5µm and stained by the **H** and **E** method, and **Perl's Prussian blue** method. The stained slides were examined under the microscope and photomicrographs needed were taken.

IV. BODY WEIGHT

The body weight for both the experimental and control animals were taken before and during administration of the extract at interval of 5 days .The mean body weight of each group were calculated and represented on the graph as shown bellow.

TABLE 1: Mean body weight for each group

Groups	Day 0	Day 5	Day 10	Day 15	Day 20
(A) *Extract With Carcinogen* (100mg/kg)	0.78	0.83	0.92	1.25	0.96
(B) *Carcinogen alone* (500mg/kg)	1.32	1.37	1.22	1.37	1.37
(C) Control (D/ w)	0.85	0.9	1.10	1.12	1.25



Graph illustrating the mean body weight

RECTAL TEMPERATURE AND VITAL SIGNS

The rectal temperature taken was to ensure the animals were not sick. This was also done during administration days. The temperature falls in the normal range of 37-39 degree Celsius for rabbits.

FIVE DAYS AFTER THE INITIAL ADMINISTRATION OF THE EXTRACTS AND THE CARCINOGEN

The group A that was administered the extracts and the carcinogen were moving freely without any sign of sickness while in group B that was administered the carcinogen without the extract were seen with swellings on the eyes, breast and scrotum. There was palpable loss of hairs, nose bleeding and weight loss.

V. DISCUSSION

There was reduction in the body weight of group **A** (100mg/kg) animals at about day 15 of the administration. This effect of decrease in body weight manifest at around day 5 in group **B** (500mg/kg),but they started increasing in weight again around day 10.The reduction in weight was as a result of the extract ,because group **C**(control) does not show any reduction in body weight at all. Statistical analysis done shows that the reduction in the body weight is not significant statistically with none of the group having a p-value less than 0.05.

The rectal temperature taken shows that the extract does not cause pyrexia in the experimental animals and along with control group all are having temperature that falls between the normal range of 37-39 degree Celsius.

Five days after the administration of the extract and carcinogen respectively, the group **A** that was administered the extracts and the carcinogen were moving freely without any sign of sickness while in group **B** that was administered the carcinogen without the extract were seen with swellings on the eyes, breast and scrotum, there was a palpable loss of hairs, nose bleeding and weight loss.

The histological section demonstrated by Perls Prussian blue reaction shows no iron deposition in the kidney section of both control and experimental animals. Liver section from group **A** (100mg/kg) show deposition of iron which were in the same quantity with group **C** (control) and are limited within the hepatocytes but group **B** (500mg/kg) shows more of iron deposition that extended into the portal region.

The kidney section demonstrated using **H** and **E** in group **A** shows normal histology of the kidney, but in group **B** renal tubules contain cellular debris, lobular disarray and a lot of pyknotic cells suggestive of acute tubular necrosis which may be due to the effect of the carcinogen while the section from group **C** animals also appears normal.

he liver section from all the groups shows normal hepatocytes, but there was periportal necrosis in one of the animals in group **A** (100mg/kg).In group **B** (500mg/kg) canaliculi were dilated, filled with bile and central vein with blood and cellular debris, lobular disarray and a lot of pyknotic cells which may be as a result of the carcinogen.

VI. CONCLUSION

SAABFAT 7 with Vernonia amygdalina as it active ingredient had been used in traditional medicine for the treatment of various ailments (Igile *et al*.,2004),it has been used to treat gastrointestinal disorders, haematoma, malaria, inflammation, cancer etc. The methanolic extract of the leaves was investigated on the histology of the liver and kidney. The kidney section demonstrated by Perls Prussian blue reaction shows no iron deposition. The liver sections show more of iron deposition with the carcinogen. There were no significant histological deviation observed in the kidney and liver section of experimental animals compared with control demonstrated by Haematoxylin and Eosin staining technique.

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APPENDIX

Descriptives

WEIGHT

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
con b4 adm	3	.8500	.26458	.15275	.1928	1.5072	.65	1.15
cont dy1	3	.9000	.26458	.15275	.2428	1.5572	.70	1.20
cont dy2	3	1.1000	.20000	.11547	.6032	1.5968	.90	1.30
cont dy3	3	1.1167	.22546	.13017	.5566	1.6767	.90	1.35
cont dy4	3	1.2333	.35119	.20276	.3609	2.1057	.90	1.60
A b4 adm	3	.7833	.05774	.03333	.6399	.9268	.75	.85
A dy1	3	.8333	.05774	.03333	.6899	.9768	.80	.90
A dy2	3	.9167	.02887	.01667	.8450	.9884	.90	.95
A dy3	3	1.0000	.05000	.02887	.8758	1.1242	.95	1.05
A dy4	3	.9667	.07638	.04410	.7769	1.1564	.90	1.05
B b4	3	1.3000	.10000	.05774	1.0516	1.5484	1.20	1.40
B dy1	3	1.3667	.05774	.03333	1.2232	1.5101	1.30	1.40
B dy2	3	1.2167	.17559	.10138	.7805	1.6529	1.05	1.40
B dy3	3	1.3667	.11547	.06667	1.0798	1.6535	1.30	1.50
B dy4	3	1.3667	.11547	.06667	1.0798	1.6535	1.30	1.50
Total	45	1.0878	.24935	.03717	1.0129	1.1627	.65	1.60

ANOVA

WEIGHT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.854	14	.132	4.506	.000
Within Groups	.882	30	.029		
Total	2.736	44			