

## Nigerian foodstuffs with tumour chemosuppressive polyphenols

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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. 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. In this context, one of the reveilles that have gone increasing the attention in cancer therapy has been the ionic radicals. In cancer, the over expression and the properties of the radicals of K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> Cl<sup>-</sup> play a crucial role in the growing/proliferation, migration and/or invasion of the cancerous cells. The presence of blocking bioactive activities of (SAAAB)-*Vernonia amygdalina* opens new perspectives in the treatment of this disease by means of the use of blocking or specific modulators of these radicals. Extracts of the plant have been used in various folk medicines as remedies against helminthic, protozoal and bacterial infections with scientific support for these claims. Phytochemicals such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthenes, anthraquinones, edotides and sesquiterpenes have been extracted and isolated from *Vernonia amygdalina*. These compounds elicit various biological effects including cancer chemoprevention. The chemopreventive properties of *Vernonia amygdalina* has been attributed to its abilities to scavenge free radicals, induce detoxification, inhibit stress response proteins and interfere with DNA binding activities of some transcription factors.

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.

### I. INTRODUCTION

Bitter leaf botanically called *Vernonia amygdalina*, 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<sup>1</sup>. Moreover, *Vernonia amygdalina* has been used in traditional medicine as an anthelmintic, 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<sup>2</sup>.

These observations stimulated research on the chemical principals of *Vernonia amygdalina*. Several stigmastane-type saponins such as vernoniosides A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub><sup>3</sup>, B<sub>2</sub>, B<sub>3</sub>, A<sub>4</sub><sup>4</sup>, and C<sup>5</sup> 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<sub>1</sub> 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<sup>1</sup>. 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<sup>6</sup>.

The current search for potential anti oxidative principles to replace suspected tumour-causing synthetic analogues such as BHT<sup>7</sup> necessitated this investigation. Antioxidative principles have been implicated as parts of anticancer formulations and patents<sup>8</sup>. Although luteoline has been reported to be a strong antioxidant<sup>9</sup>, 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 *Vernonia amygdalina* leaves and describes their antioxidant activities. This study is therefore designed to determine the clinical activities of (SAAAB) - *Vernonia amygdalina*, on different tumors in the body

### II. LITERATURE REVIEW

*Vernonia amygdalina* is a small tree that grows throughout tropical Africa. In some parts of West Africa, e.g. Nigeria, the plant has been domesticated and is locally known as "bitter leaf", while the Yoruba tribe call it

ewuro. 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.

Phytochemical screening of the leaves revealed the presence of tannins, phlobatannins, flavonoids, steroids, terpenoids, saponins and cardiac glycosides, which are the most important bioactive constituents of medicinal plants

These bioactive constituents of *vernonia amygdalina* have also curative active principles such as lovastatin antilipemia, Pleurotin antibiotics and beta-glucan polysaccharides with heavy molecular weight and immunomodulator, immunostimulant properties. Polysaccharides stimulate the immunological system using three mechanisms: interferon production, excitation of complement chains and the activation of macrophages, inducing organism's defense. This bitter leaf when consumed daily, prevent from oncogenesis and metastasis in cancer cases; therefore, it's used as a coadjuvant therapy in chemotherapy treatments.

In summary, *Vernonia amygdalina* extracts may help suppress, delay, or kill cancerous cell in many ways, such as: Induction of apoptosis as determined in cell culture and animal studies, Enhanced Chemotherapy Sensitivity - *V. amygdalina* extracts may render cancerous cells to be more sensitive to chemotherapy, Inhibition of the growth or growth signals of cancerous cells, Suppression of metastasis of cancerous cells in the body by the inhibition of NF- $\kappa$ B is an anti-apoptotic transcription factors as demonstrated in animal studies, Reduction of estrogen level in the body by the suppression of Aromatase activity. The Involvement of blood estrogen level in the etiology of estrogen receptor (ER) positive breast cancer has been widely reported. Additional source of estrogen production in humans besides the ovary and adrenal gland is the conversion of testosterone to estrogen in a reaction catalyzed by Aromatase. Many studies have shown positive correlations between blood estrogen levels and breast cancer risks. Therefore, compounds that inhibit Aromatase activity are used for the treatment of breast cancer, Antioxidants - *V. amygdalina* may provide anti-oxidant benefits, Enhancement of the immune system - Many studies have shown that *V. amygdalina* extracts may strengthen the immune system through many cytokines (including NF- $\kappa$ B, pro inflammatory molecule) regulation.

**Tumour**, also spelled tumor, also called neoplasm, a mass of abnormal tissue that arises without obvious cause from preexisting body cells, has no purposeful function, and is characterized by a tendency to independent and unrestrained growth. Tumours are quite different from inflammatory or other swellings because the cells in tumours are abnormal in appearance and other characteristics. Abnormal cells—the kind that generally make up tumours—differ from normal cells in having undergone one or more of the following alterations: (1) hypertrophy, or an increase in the size of individual cells; this feature is occasionally encountered in tumours but occurs commonly in other conditions; (2) hyperplasia, or an increase in the number of cells within a given zone; in some instances it may constitute the only criterion of tumour formation; (3) anaplasia, or a regression of the physical characteristics of a cell toward a more primitive or undifferentiated type; this is an almost constant feature of malignant tumours, though it occurs in other instances both in health and in disease. In some instances the cells of a tumour are normal in appearance; the differences between them and normal body cells can be discerned only with some difficulty. Such tumours are more often benign than not. Other tumours are composed of cells that appear different from normal adult types in size, shape, and structure; they usually belong to tumours that are malignant. Such cells may be bizarre in form or may be arranged in a distorted manner. In more extreme cases, the cells of malignant tumours are described as primitive, or undifferentiated, because they have lost the appearance and functions of the particular type of (normal) specialized cell that was their predecessor. As a rule, the less differentiated a malignant tumour's cells are, the more quickly the tumour may be expected to grow.

Malignancy refers to the ability of a tumour ultimately to cause death. Any tumour, either benign or malignant in type, may produce death by local effects if it is appropriately situated. The common and more specific definition of malignancy implies an inherent tendency of the tumour's cells to metastasize (invade the body widely and become disseminated by subtle means) and eventually to kill the patient unless all the malignant cells can be eradicated.

**Metastasis** is thus the outstanding characteristic of malignancy. Metastasis is the tendency of tumour cells to be carried from their site of origin by way of the circulatory system and other channels, which may eventually establish these cells in almost every tissue and organ of the body. In contrast, the cells of a benign tumour invariably remain in contact with each other in one solid mass centred on the site of origin. Because of the physical continuity of benign tumour cells, they may be removed completely by surgery if the location is suitable. But the dissemination of malignant cells, each one individually possessing (through cell division) the ability to give rise to new tumours in new and distant sites, requires complete eradication by a single surgical procedure in all but the earliest period of growth.

A mass of tumour cells usually constitutes a definite localized swelling that, if it occurs on or near the surface of the body, can be felt as a lump. Deeply placed tumours, however, may not be palpable. Some

tumours, and particularly malignant ones, may appear as ulcers, hardened cracks or fissures, wartlike projections, or a diffuse, ill-defined infiltration of what appears to be an otherwise normal organ or tissue.

Pain is a variable symptom with tumours. It most commonly results from the growing tumour pressing on adjacent nerve tracts. In their early stages all tumours tend to be painless, and those that grow to a large size without interfering with local functions may remain painless. Eventually, however, most malignant tumours cause pain by the direct invasion of nerves or the destruction of bone.

All benign tumours tend to remain localized at the site of origin. Many benign tumours are enclosed by a capsule consisting of connective tissue derived from the structures immediately surrounding the tumour. Well-encapsulated tumours are not anchored to their surrounding tissues. These benign tumours enlarge by a gradual buildup, pushing aside the adjacent tissues without involving them intimately. Malignant tumours, by contrast, do not usually possess a capsule; they invade the surrounding tissues, making surgical removal more difficult or risky.

A benign tumour may undergo malignant transformation, but the cause of such change is unknown. It is also possible for a malignant tumour to remain quiescent, mimicking a benign one clinically, for a long time. The regression of a malignant tumour to benign is unknown.

Among the major types of benign tumours are the following: lipomas, which are composed of fat cells; angiomas, which are composed of blood or lymphatic vessels; osteomas, which arise from bone; chondromas, which arise from cartilage; and adenomas, which arise from glands.

### III. MATERIALS AND METHODS

#### 3.1 MATERIALS

##### Equipments and Reagents

Conical flask, Glass pipettes, Measuring cylinders, Cotton wool, Masking tape, Filter paper (whatman no 1), Dried *Vernonia amygdalina* leaves, Distilled water, Absolute alcohol, Silver plate, Hot air oven, Blender, pH meter. Hydrochloric acid (HCl), Dranggendoff's reagent, Ferric chloride solution, Ammonia solution, Sodium hydroxide, Concentrated sulphuric acid(H<sub>2</sub>SO<sub>4</sub>)  
Chloroform, Glacial acetic acid, Acetic anhydride.

#### 3.2 METHODS

##### 3.2.1 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. 150g of the ground dried leaves of *Vernonia amygdalina* was extracted with 1 litre of hot water and another 150g with 1 litre of 70% alcohol respectively.

##### (A) 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 1 hour, and then filtered to obtain the coloured extract. The filtrate obtained was oven-dried to get rid of the residual water. 14g of the extract was obtained.

##### (B) 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 48 hours. The extract was then filtered to obtain coloured extract. The filtrate was oven-dried to get rid of the residual water 10.24g of the dry extract was obtained.

##### 3.2.2 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 steam 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
<b>Alkaloid</b> <b>(i) 1ml of extract + few drops of Drangendoff's reagent</b>	<b>Yellowish brown colour</b>	<b>Alkaloid inferred</b>
<b>(ii) 1ml of extract + few drops of Mayers reagent</b>	<b>Yellowish colour seen</b>	<b>Alkaloid inferred</b>
<b>(iii) 1ml of extract + Wagner's reagent</b>	<b>Dark turbid brown</b>	<b>Alkaloid inferred</b>

### 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
<b>(i) 1ml of extract + 1ml ferric chloride</b>	<b>Blue black precipitate</b>	<b>Catecol tannin present</b>
<b>(ii) of extract + bromine water</b>	<b>Brownish red turbid</b>	<b>Condensing tannins</b>

### Flavonoid

(i) Lead Acetate Test: 0.2ml of the extract was added to 0.2ml of 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
<b>(i) Lead acetate on extract</b>	<b>Dirty brownish precipitate</b>	<b>Flavonoid present</b>
<b>(ii) ferric chloride test</b>	<b>Wooly brownish colour</b>	<b>Flvonoid present</b>
<b>(iii) Sodium hydroxide test</b>	<b>Golden yellow precipitate obtained</b>	<b>Flavonoid present</b>

### 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 (0.5) was dissolved in 2ml of acetic anhydride and cooled well in ice., concentrated sulphuric acid was carefully added.

(iii) Salkowski'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
<b>(i) Kedde's Test</b>	<b>Brownish precipitate</b>	<b>Canclenolide</b>
<b>(ii) Lieberman's Test</b>	<b>Deep greenish blue</b>	<b>Steroid nuclei inferred</b>
<b>(iii) Salowski's Test</b>	<b>Reddish brown colour at interface</b>	<b>Deoxysugar, characteristics of cardenolide</b>

### 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. A **tumor** (or **tumour**) is commonly used as a synonym for a neoplasm<sup>[1]</sup> (a solid or fluid-filled [cystic] lesion that may or may not be formed by an abnormal growth of *neoplastic* cells) that appears enlarged in size. *Tumor* is not synonymous with cancer. While cancer is by definition malignant, a tumor can be benign, pre-malignant, or malignant, or can represent a lesion without any cancerous potential whatsoever.

The terms "mass" and "nodule" are often used synonymously with "tumor". Generally speaking, however, the term "tumor" is used generically, without reference to the physical size of the lesion. More specifically, the term "mass" is often used when the lesion has a maximal diameter of at least 20 millimeters (mm) in greatest direction, while the term "nodule" is usually used when the size of the lesion is less than 20 mm in its greatest dimension (25.4 mm = 1 inch).

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### Etymology

The term tumour/tumor is derived from the Latin word for "swelling" – *tumor*. It is similar to the Old French *tumour* (contemporary French: *tumeur*). In the Commonwealth the spelling "tumour" is commonly used, whereas in the U.S. it is usually spelled "tumor".

In its medical sense it has traditionally meant an abnormal swelling of the flesh. The Roman medical encyclopedist Celsus (ca 30 BC–38 AD) described the four cardinal signs of acute inflammation as *tumor*, *dolor*, *calor*, and *rubor* (swelling, pain, increased heat, and redness). His treatise, De Medicina, was the first medical book printed in 1478 following the invention of the movable-type printing press.

In contemporary English, the word tumor is often used as a synonym for a cystic (liquid-filled) growth or solid neoplasm (cancerous or non-cancerous),<sup>[2]</sup> with other forms of swelling often referred to as swellings.<sup>[3]</sup>

Related terms are common in the medical literature, where the nouns tumefaction and tumescence (derived from the adjective tumefied), are current medical terms for non-neoplastic swelling. This type of swelling is most often caused by inflammation caused by trauma, infection, and other factors.

Tumors may be caused by conditions other than an overgrowth of neoplastic cells, however. Cysts (such as sebaceous cysts) are also referred to as tumors, even though they have no neoplastic cells. This is standard in medical billing terminology (especially when billing for a growth whose pathology has yet to be determined).

### Causes



Neoplastic tumor of the cheek skin, here a benign neoplasm of the sweat glands called hidradenoma, which is not solid but is fluid-filled

A neoplasm can be caused by an abnormal proliferation of tissues, which can be caused by genetic mutations. Not all types of neoplasms cause a tumorous overgrowth of tissue, however (such as leukemia or carcinoma in situ).

Recently, tumor growth has been studied using mathematics and continuum mechanics. Vascular tumors are thus looked at as being amalgams of a solid skeleton formed by sticky cells and an organic liquid filling the spaces in which cells can grow.<sup>[4]</sup> Under this type of model, mechanical stresses and strains can be dealt with and their influence on the growth of the tumor and the surrounding tissue and vasculature elucidated. Recent findings from experiments that use this model show that active growth of the tumor is restricted to the outer edges of the tumor, and that stiffening of the underlying normal tissue inhibits tumor growth as well.<sup>[5]</sup> Benign conditions that are *not* associated with an abnormal proliferation of tissue (such as sebaceous cysts) can also present as tumors, however, but have no malignant potential. Breast cysts (as occur commonly during pregnancy and at other times) are another example, as are other encapsulated glandular swellings (thyroid, adrenal gland, pancreas).

Encapsulated hematomas, encapsulated necrotic tissue (from an insect bite, foreign body, or other noxious mechanism), keloids (discrete overgrowths of scar tissue) and granulomas may also present as tumors. Discrete localized enlargements of normal structures (ureters, blood vessels, intrahepatic or extrahepatic biliary ducts, pulmonary inclusions, or gastrointestinal duplications) due to outflow obstructions or narrowings, or abnormal connections, may also present as a tumor. Examples are arteriovenous fistulae or aneurysms (with or without thrombosis), biliary fistulae or aneurysms, sclerosing cholangitis, cysticercosis or hydatid cysts, intestinal duplications, and pulmonary inclusions as seen with cystic fibrosis. It can be dangerous to biopsy a number of types of tumor in which the leakage of their contents would potentially be catastrophic. When such types of tumors are encountered, diagnostic modalities such as ultrasound, CT scans, MRI, angiograms, and nuclear medicine scans are employed prior to (or during) biopsy and/or surgical exploration/excision in an attempt to avoid such severe complications.

The nature of a tumor is determined by imaging, by surgical exploration, and/or by a pathologist after examination of the tissue from a biopsy or a surgical specimen.

#### See also

- History of medicine

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