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PHYTOCHEMICAL SCREENING OF ACTIVITY DIRECTED EXTRACTS OF *VERNONIA AMYGDALINA* LEAVES

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ABSTRACT

Fractionation of the crude ethanolic extract of *vernonia amygdalina* using organic solvents of increasing polarities yielded activity directed fractions of benzene, chloroform, ethyl acetate, butanol, methanol and residue E. Phytochemical screening of the fractions show that there were significant differences ($p \leq 0.05$) qualitatively in the levels of tannins, flavonoids, cardiac glycosides, terpenoids, saponins and alkaloids in the various fractions. While the benzene extract had very low levels of tannins, saponins and alkaloids, the ethyl acetate fraction had low levels of tannins and terpenoids as well as very low levels of flavonoids, cardiac glycosides, and alkaloids. The butanol fraction had a high level of tannins, low level of flavonoids and saponins while the methanol fraction had high levels of tannins and flavonoids as well as low levels of cardiac glycosides, saponins and alkaloids with a very low level of terpenoids. The water soluble residue E had very high levels of tannins, a high level of saponins, low levels of alkaloids and very low levels of flavonoids, cardiac glycosides and terpenoids. Phytochemical content of the various fractions increased with increasing polarities of the organic solvent used. The relatively high Phytochemical content of the butanol, methanol and residue E fractions may be exploited for medicinal purposes.

INTRODUCTION

Vernonia amygdalina is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDS (Ohigashi, *et al,*1991). These leaves are exported from several African countries. The roots of *v. amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity (Jisaka *et al,* 1992). *V. galamensis* is used as an oilseed in East Africa. *Vernonia* species are used as food plants by the larvae of some Lepidoptera species including *Coleophora vernoniaeella* (which feeds exclusively on the genus) and *Schinia regia* (which feeds exclusively on *V. texana*).

Vernonia amygdalina (compositae) is a small shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf due to its bitter taste. The macerated leaves of the plant are used in making soup while the water extract serves as a tonic drink. In the local community the leaves are used as antihelmint, a laxative and an antimalarial because they contain quinine substitute. It is noted that the leaves contain stigmastane-type saponins such as vernoniosides A1, B1, A2, A3, B2, D2, A4 and C which have been identified in the leaves (Ohigashi *et al* 1991; Jisaka *et al,* 1992; Kamperdick *et al,* 1992). It was also noted by Philipson *et al,* 1993, that the antiplasmodial effects of some sesquisterpene and steroidal constituents of *Vernonia amygdalina* are also effective against *plasmodium falciparium in vitro.* Earlier investigation on *Vernonia amygdalina* showed that purified chloroform fractions identified as vernodaline, vernolide and vernomygdine elicited cytotoxic effects in human carcinoma involving narsopharynx cells with IC_{50} values of 1.8, 2.0 and 1.5 µg/ml respectively (Mayerson and Inzucchi, 2002). It was concluded that the activities were dependent on their possession of the (alpha-methyl-gamma-lactone group) as their structures (Jisaka *et al,* 1992).

For years now since the discovery of *Vernonia amygdalina* there has been a store of information on the clinical value of this plant. However less than 10% of the world's flora has been studied chemically in detail to determine their active constituents (phytochemcial components). Yang and Health, 2002; reported that medicinal plants contain different therapeutic agents like saponins, alkaloids, glycosides and tannins. The following agents are found in *Vernonia amygdalina.*

Saponins: one of the most active compounds in *Vernonia amygdalina,* are complex molecules in which the glycans are triterpeniods or steroidal (Ross *et al,* 2000). Saponins are starch glycosides with complex sugar components attached to 3 hydroxyl groups (Tedder, 2003). It is a white or light brown amorphous powder with a sweet taste, which later has a bitter taste and even acid burning taste. Saponins are believed to be too irritating to the gastrointestinal tract, to be used internally. (Martindale, 1978). The stigmastene type steroidal saponins are vernoniosides and all cause the characteristic bitter taste in *Vernonia amygdalina* (Jisaka, 1998 and Kamperdick, 1998). Also noted were the dominant stigmastene type saponin, vernonioside D which makes up 35% of the saponins responsible for the characteristics of *Vernonia amygdalina* when it was washed. Experimentally, steroidal saponins in *Vernonia amygdalina* seems to be higher than that found in other plants. (Igile *et al,* 1999).

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Tannins: Tannins have a characteristic that is marked with acidity and relatively present in most groups in the Kingdom *Plantae.* Tannins form colloidal solids and hence do not crystallize. Tannins are known to precipitate alkaloids and proteins; hence are resistant to proteolytic enzymes. These characteristics outline its basis for therapeutic uses. Tannins are further classified into two main groups or types; Syrogallol and pyrocatechol tannin. In medicine, tannins are useful as astringents (Jisaka, 1998).

Glycosides: Glycosides are compounds that contain a saccharide unit which is attached to a non-carbohydrate moiety usually called a glycone (Igile *et al,* 1999). They are classified based on their sugar units, the nature of aglycone-suguar linkage and sometimes according to their specific properties (Trease *et al,* 1994).

Alkaloids: Alkaloids are compounds that their nitrogen atom forms an integral part of a cyclic system and are white crystalline solids. Most of the alkaloids are well defined crystalline substances, which interact with acids to form salts. Some alkaloids are colourless; some are liquid and have a bitter taste. Mostly alkaloids are used as stimulants, analgesics, and muscle relaxants and even in the treatment of psychiatric disordesr (Tedder and Philip, 1972). Alkaloids occur in plant tissues at points of intense cell activity in leaves, roots and seeds and they are generally found in living tissue at a point of intense cell activity rather than in dead tissues.

Flavonoids (flavones): *Vernonia amygdalina* leaves also contain flavones that are antioxidants and have the medicinal value of combating carcinogens as well as the ageing process. (Huang and Fararo, 1992). Most flavonoids are derivatives of two phenylchroman (flava). They are representatives of heterocyclic compounds containing in their molecule the functional groups C=O, C=H, as well as a prominent fragment of a quinol ring. It is the presence of these groups in their molecules and some sugar components that gives these components their common physical and chemical properties. Flavones occurring in *Vernonia amygdalina* leaves have luteolin 7-O-β-glucoronoside, luteolin 7-O-β-glucosides and leutolin. The antioxidant activities of these flavonoid compounds isolated from the leaves have been reported using coupled oxidation of β-carotene and linoleic acid. Luteolin 7-O-β-glucoronoside which is the most abundant of the flavones is the more potent antioxidant (Igile *et al,* 1999). Flavones exert their effects on certain organs of the body like the smooth muscle, the internal organ vessels and bile ducts.

This research was therefore carried out to ascertain the relative presence of the various phytochemicals in the respective fractions of *vernonia amygdalina* as a measure of the ability of each organic solvent to extract to extract them.

MATERIALS AND METHODS

Fresh leaves of *Vernonia amygdalina* were harvested from the endocrine research farm in the University of Calabar and identified in the Department of Botany. They were dried under shade, crushed and soaked in 98% ethanol for 48 hours; then filtered and allowed to evaporate at room temperature to obtain the crude extract.

The crude extract was subjected to fractionation using organic solvents of varying polarities. It was first soaked in benzene in a separating funnel, shaken and allowed to separate into two fractions. The benzene soluble fraction was obtained and allowed to dry under room temperature to obtain the benzene extract. The resulting residue (residue A) was dried, and then fractionated using chloroform. The chloroform soluble part was dried at room temperature to obtain the chloroform extract while the resulting residue (residue B) was dried and subsequently fractionated using ethyl acetate. The ethyl acetate soluble fraction was then dried to obtain the ethyl acetate extract and an insoluble residue, (residue C). The insoluble residue obtained was again resuspended in butanol, shaken in a separating funnel, and allowed to separate into two distinct layers. The butanol soluble fraction was dried at room, temperature, to obtain the butanol extract, while the resulting residue (residue D) was dried and further fractionated using methanol. The methanol soluble fraction was dried to obtain the methanol extract, while the insoluble portion was dried to obtain an insoluble residue (residue E) which was soluble in water. Dried fractions obtained from the various organic solvents were redissolved in ethanol and dried under shade to blow off residue of the various organic solvents.

Qualitative tests were carried out on the various activity directed extracts using standard procedures to identify its phytochemcial constituents as described by the methods of Sofowara, 1993.

1. Test for Alkaloids

One gram of each sample was treated with 5ml of one percent (w/v) hydrochloric acid. The filtrate was treated with a few drops of Mayers reagent and Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extracts.

2. Test for Tannins

The samples were properly dried, 0.5g of the dried sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

3. Test for Saponins

Two grams of the dried sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

4. Test for cardiac glycosides

Five milliliters of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout the thin layer indicating the presence of cardiac glycosides.

5. Test for flavonoids

In the method used to determine the presence of flavonoids in the plant sample, 5ml of dilute ammonia solution were added to a portion of each plant extract followed by addition of concentrated sulphuric acid. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

6. Test for terpenoids

Four milliliters of each extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration formed at the interface was taken as a positive result for the presence of terpenoids.

RESULTS

Results of the phytochemical screening of activity directed extracts of *vernonia amygdalina* leaves obtained by stepwise fractionation of the whole extract using solvents of increasing polarities is presented in Table 1. The results show that there were significant differences ($p<0.05$) qualitatively in the levels of tannins, flavonoids, cardiac glycosides, terpenoids, saponins and alkaloids in the various fractions. The fractions were those of benzene, chloroform, ethyl acetate, butanol, methanol, and residue E. The benzene extract had very low levels of tannins and alkaloids. The chloroform fraction had very low levels of tannins and alkaloids as well as low levels of flavonoids and cardiac glycosides. Ethyl acetate fraction had low levels of tannins and terpenoids, its level of flavonoids, cardiac glycosides, and alkaloids were very low. The butanol fraction had high levels of tannins and low level of flavonoids and saponins while the methanol fraction had high levels of tannins and flavonoids as well as low levels of cardiac glycosides, saponins and alkaloids with a very low level of terpenoids. The water soluble residue E had very high levels of tannins, a high level of saponins, low levels of alkaloids and very low levels of flavonoids, cardiac glycosides and terpenoids.

Table 1: Phytochemical screening of activity directed fractions *Vernonia amygdalina* leaves

	tannins	**flavonoids**	**Cardiac glycosides**	**Terpenoids**	**saponins**	**alkaloids**
Benzene	+	-	-	-	+	+
Chloroform	+	++	++	-	-	+
Ethyl acetate	++	+	+	++	-	+
Butanol	+++	++	-	-	++	-
Methanol	+++	+++	++	+	++	++
Residue E	++++	+	+	+	+++	++

Key

_	=	Not present
+	=	Present; very low level
++	=	Present; low level
+++	=	present; high level
++++	=	present; very high level

DISCUSSION

Plant foods contain in addition to the traditional macronutrients, a wide range of substances known as phytochemicals that are specific chemicals produced to perform metabolic functions (Chang, 1996). These substances include; alkaloids, flavonoids, polyphenols, cardiac glycosides, tannins and saponins (Grube *et al,* 2001). Phytochemicals are reported to possess biological effects in a range of health conditions such as cancer, acquired immune deficiency syndrome (AIDS), hypercholesterolemia, hormonal disorder etc (Wong and Chenng, 2001). Results of the phytochemcial screening of activity directed extracts of *vernonia amygdalina* obtained by stepwise fractionation of the whole extracts using solvents of increasing polarities is presented in table 1. The results show that while the benzene fraction had very low levels of tannins, saponins and alkaloids, the chloroform fraction had very low levels of tannins and alkaloids as well as low levels of flavonoids and cardiac glycosides, while the ethyl acetate fraction had low levels of tannins and terpenoids with very low in levels of flavonoids, cardiac glycosides, and alkaloids. The butanol fraction had high levels of tannins and low level of flavonoids and saponins while the methanol fraction had high levels of tannins and flavonoids, low levels of cardiac glycosides, saponins and alkaloids with a very low level of terpenoids. Saponins are known to inhibit the proliferation of cancer cells and lower cholesterol in blood by binding in the digestive tract (Rao and Sung, 1995). Cardiac glycosides constitute a group of steroidal compounds that can increase cardiac output and alter the electrical functions of the heart. They are known to contain the alpha and beta unsaturated gamma lactone ring. In addition to inhibiting Na^+-K^+ ATPase in cell membranes of cardiac muscles, they also influence smooth muscle and other tissues (Gannong, 1999).

Furthermore, flavonoids are known to function as antioxidants. They are perhaps known for their ability to enhance the effect of ascorbic acid. Many of the vitamins function of vitamin C also appear to require the presence of flavonoids (Gannong, 1999). They have also been shown to possess anti-inflammatory and antibiotic activities (Middleton and Kandaswani, 1992). Phytochemical content of the various fractions generally increased with increase in the polarities of the organic solvents used In fractionation The higher content of saponins, tannins and flavonoids in the methanol, butanol and residue E fractions of *vernonia amygdalina*

could be responsible for the higher antihyperglycemic activity of these fractions (Ekam, 2008).

CONCLUSION

Fractionation of the crude extract of *vernonia amygdalina* yielded fractions of benzene, chloroform, ethyl acetate, methanol, butanol and residue E. Phytochemical screening of each of the fractions showed varying qualitative levels of saponins, tannins, cardiac glycosides, terpenoids, alkaloids and flavonoids in the various fractions. The levels of phytochemicals were higher at higher polarities of organic solvents used for fractionation.

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