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## **PRELIMINARY PHYTOCHEMICAL SCREENING OF VERNONIA SCHIMPERI**

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### **ABSTRACT**

In the present study the dried material of *Vernonia schimperi* (Syn. *Vernonia abyssinica* Sch. Bip. ex Walp.) was successively extracted with acetone and 96% ethanol. These extracts were further fractionated with different solvents and were analyzed for their phytoconstituents using standard protocols. From the study it was found that alkaloids and terpenoids are totally absent while the test for phenols found positive in all the fractions. Hexane fractions as well as acetonitrile fraction of one minute acetone extract showed the presence of glycosides. The sterols are only present in hexane fraction of one minute acetone extract. Most of the constituents are present in polar solvent fractions. Over all plant is rich in pharmaceutically important phytochemicals.

**Keywords:** *Vernonia schimperi*, Extract, Phytochemical screening.

### **INTRODUCTION**

The search for medicinal plants has been an integral part of the human society to cure the disorders associated with the human beings since the earliest time recorded. It is estimated that there are 250,000 to 500,000 species of plants on Earth [1]. A relatively small percentage (1 to 10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes [2].

The use of medicinal herbs and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries [3]. Nowadays, these plant based drugs have their existence as herbal drugs and symbolize safety in contrast to synthetic drugs that are considered as unsafe for human and environment.

Unlike modern drugs that invariably comprise a single active species, herbal extracts contain multiple active constituents. Interestingly, natural compounds contained in these "herbal cocktails" can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side effects [4].

Amongst the medicinal plants used in these

herbal preparations for their therapeutic potential, Some have been investigated methodically and some of are still left so there is need to explored such plants.

Standardization of plant materials or plant based drugs is the need of the day. In many pharmacopoeias the monographs of plant materials have been described only the physicochemical parameters. Hence for the proper standardization of herbal drugs and its formulations should be assayed to the modern methods describing the identification and quantification of active constituents in the plant material [5].

Of the various classes of plants secondary metabolites, phenolic compounds are much concerned and have been recognized to possess wide range of biological properties including antiallergenic, antimicrobial, antiartherogenic, antithrombotic, anti inflammatory, vasodilatory, cardio protective effects and antioxidant activity [6-8].

*Vernonia* (Family: Asteraceae) under the tribe of Vernoniae is the largest genus having about 1000 species [9]. It is found mostly in tropical regions and have wide range of habitats of broad ecological diversity and climatic conditions included: tropical forests, marshes and wet

areas, dry planes, tropical savannahs desert xeric or dry sites or even frosty regions of eastern North America [9-10].

Ethnopharmacological survey of vernonia revealed that the species of this genus used in variety of ailment by folk healers as anti infective agents, wound healing, anthelmintic, rheumatic pain, antidiabetic, leprosy and scabies, hepatitis, diuretic, hysteria, epilepsy, gastric and intestinal ulcers, respiratory tract disorder, cancer, immune enhancing etc [11].

The genus vernonia has been reported to contain phytoconstituents like triterpene, steroids and lignoids but the more frequent compounds are sesquiterpenoid lactones and flavonoids, [12] besides this coumarins and sucrose ester are also reported as bio active compound from the genus [11].

A total of 109 vernonia species were identified in the literature to have medicinal properties. One hundred and five plants were linked to the treatment or management of 44 human diseases or health conditions. Plants of the genus also feature in ethno veterinary and zoopharmacognostic practices. A total of twelve *vernonia* species were identified to be used in ethno veterinary medicine while two species are used self medication practices by chimpanzees and gorillas [11].

In view of the above medical properties, *vernonia schimperi* was taken for phytochemical work in order to get new pharmaceutically active compound(s). *V. schimperi* is a less known species and widely distributed in the Arab region. The species is not explored for their phytoconstituents. In the present study efforts have been made for the preliminary phytochemical screening of the different extracts of the plant. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the discovery of new phytomolecules of pharmaceutical interest.

## MATERIALS AND METHODS

### Collection of the plant material

The plant material was collected in February, 2013 from the Jabal Shada Al-aala hills Saudi Arabia. The collected plant material was identified by taxonomist Dr. M. Yusuf, and the voucher specimen of plant (# 16012) was deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

### Extraction Process

The aerial part was dried under shade and grinded. The material (938.2g) was extracted at room temperature in acetone (8 L) for only one minute. Then the plant material was further extracted at room temperature with acetone (10 L x 3) and ethanol 96% (10 L x 3) respectively. All the extracts were evaporated to dryness at 40 °C under reduced pressure using Buchi Rotavapour yielded green syrupy mass 4.7, 16.2 and 26.9 G

respectively. Both the acetone extracts (4.5 and 16.0 g) were separately partitioned between hexane and acetonitrile (presaturated with each other). The fractions obtained were evaporated to dryness at 40 °C under reduce pressure and the weight of hexane fractions were 363.3 mg (VS1) and 2.9 g (VS3) while the acetonitrile fractions gave 4.1 g (VS2) and 11.9 g (VS4) respectively. The methanol soluble portion (26.0 gm) of 96% ethanol extract was dried, in which 25 gram was treated with ethyl acetate which gave ethyl acetate soluble fraction 14.5 g (VS5) and insoluble fraction 10.4 g (VS6). All the fractions were kept at 4 °C in refrigerator for further use.

### Qualitative phytoconstituents analysis

The phytochemical screening of different fractions of the extracts was done using standard procedures [13-15].

#### 1. Test for Tannins

A Small amount of each sample was put in a test tube and 4 ml of distilled water was added and heated to boiling. The mixture was then filtered and 5 % of FeCl<sub>3</sub> was added to the filtrate and observations made. A brownish green color or a blue black color indicates the presence of tannins.

#### 2. Test for Saponins

The small amount of each sample was mixed with 5 ml of water and vigorously shaken. It gave the formation of stable foams which indicates the presence of saponins in the tested material.

#### 3. Test for Flavonoids

In a small amount of each sample few fragments of magnesium turnings (prewashed with dilute hydrochloric acid and water) and few drops of concentrated hydrochloric acid were added. Appearance of magenta red color after few minutes indicates the presence of flavonoids.

#### 4. Test for Terpenoids

In a small amount of each extract 2 ml of chloroform was added and vigorously shaken, then evaporated to dryness. To this, 2 ml of concentrated sulphuric acid was added and heated for about 2 minutes. No greyish color was observed which indicated the absence of terpenoids in the tested material.

#### 5. Test for Glycosides

**Salkowski's test:** To the small portion of each fraction was mixed with 2 ml of chloroform and carefully added 2 ml of concentrated sulphuric acid and shaken gently. Appearance of red brown color, which indicate the presence of steroidal ring (glycone portion of glycoside).

#### 6. Test for Cardiac glycoside

**Keller Kiliani test:** A Small amount of each fraction was mixed with 2 ml of glacial acetic acid and added 1-2 drops of 5% solution of  $\text{FeCl}_3$ , and then the mixture was slowly added into a test tube containing 2 ml of concentrated sulphuric acid. Formations of a brown ring at the interface of the two solutions indicate the presence of cardiac glycoside.

#### 7. Test for Sterols (Liebermann-Burchard test)

Small amount of each sample were treated with few drops of chloroform, acetic anhydride and concentrated sulphuric acid and observation was made. Formation of the dark pink or red color shows the presence of sterols.

#### 8. Test for Alkaloids (Dragon Droff Test)

Small amount of the each sample was treated with Dragon Droff reagent, no formation of turbidity was observed which indicates the absence of alkaloids.

#### 9. Test for Phenol

The sample extracts was put in a test tube and treated with a few drops of 5% of  $\text{FeCl}_3$ , blue green or black coloration indicate the presence of phenols.

### RESULTS AND DISCUSSION

Results obtained for qualitative analysis of phytoconstituents of the *Vernonia schimperi* is summarized in table 1. Nine phytochemical constituents (Alkaloids, Saponins, Tannins, Flavonoids, Cardiac Glycosides, Glycosides, Terpenoids, Sterols and Phenols) were screened for the different fractions (VS1-VS6). Out of these nine phytochemicals the alkaloids and terpenoids are not present in any one of the tested samples. Fraction VS6 is the only fraction showing the presence of tannins. Fractions VS2, VS4 and VS6 gave the positive test for flavonoids. Cardiac glycosides are present in acetonitrile fractions (VS2 and VS4) and ethyl acetate soluble and insoluble fractions (VS5 and VS6). Salkowski's test for glycoside is positive in VS1 to VS3. While the sterols are only present in VS1, the presence of sterols in hexane fractions corroborates the reports of Ugochukwu *et al.*, 2013 [16]. Test for the phenols is only test which shows positive results in all the fractions. The results of the study clearly indicate the plant is rich in pharmaceutically important phytochemicals.

Screening of the plants for the phytoconstituents exposed the presence and absence of constituents known for potential therapeutic and physiological activity [14].

The only high polar solvent fraction (VS6) revealed the presence of saponins which are known to have anti-inflammatory effects [17] and have property of precipitating and coagulating red blood cells [18]. Some

other characteristics of saponins includes formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [19-20]. Tannins, which are present in VS6 is also an important class of secondary plant metabolites and possess many biological properties. The most important property of the tannins is the astringent property which allows them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels [21]. Some tannins also being reported to inhibit the replication of HIV selectively in addition the use of diuretics [22].

Flavonoids (present in VS2, VS4 and VS6) are very important class of plant secondary metabolites. Pharmacological properties associated with flavonoids includes: antioxidant activity, vitamin C sparing activity and other activities of 5-lipoxygenase, cyclo-oxygenase, protein kinase C, tyrosine kinase and genetic toxicity. Free radical and antioxidant properties of the flavonoids are also reported for the anticancer and anti ageing activities [23-25].

This plant is also a good source of glycosides (VS1, VS2 and VS3) belongs to secondary metabolites, which are formed by the glycosidic linkage in which a sugar moiety and to a non-carbohydrate part. The cardiac glycosides (VS2 and VS4-VS6) are used especially for heart disorders, gives strength and allow the normal functioning of the heart [26]. The cardiac glycosides involved in enzymatic biochemical reactions and they bind to and inhibit  $\text{Na}^+/\text{K}^+$ -ATPase. Due to the inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase, the sodium level increases in the cardiac myocytes, which ultimately increase the calcium ions level and hence increase in cardiac contraction force [27]. The anticancer property of the cardiac glycosides also has created the great interest towards the plant secondary metabolite. The cardiac glycosides based drugs have taken lead to clinical trial in clinics [28].

Steroids, which are present in VS1, have also been reported for their antibacterial activity [29]. Besides this they are very important class of compounds in particular due to their relationship with compounds such as sex hormones [30].

The phenolic compounds, which are presents in every fractions (VS1-VS6) possess different biological activities such as anti apoptosis, antiaging, anticarcinogen, anti-inflammatory, antiatherosclerosis [31] and the more recent cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities has also been reported [32]. The antioxidant activity of the various plants rich in phenolics have also been described by various workers [33-34].

**Table 1. Phytochemical screening of different fractions of *Vernonia schimperi* extracts**

Sample Code	Test for phytoconstituents								
	Alkaloids	Saponins	Tannins	Flavonoids	Cardiac Glycosides	Glycosides	Terpenoids	Sterols	Phenols
VS 1	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
VS 2	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve
VS 3	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
VS 4	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
VS 5	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve
VS 6	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve

## CONCLUSION

The phytochemical screening of the tested fractions of the plant extracts suggests the presence of many pharmaceutically important molecules. Therefore the plant should be ascertained for their biological activities. Furthermore it should be chemically investigated for their individual chemical constituents in

order to get new phytomolecules which would be helpful in target based drug therapy.

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