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Phytochemical Screening and *In vitro* Antioxidant Studies of Stem Bark Extracts of *Vitellaria paradoxa* (Gaertn.) Sapotaceae

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Abstract

Vitellaria paradoxa, which belongs to the family Sapotaceae, is a medicinal plant with vast use in traditional medicine for the treatment of various diseases such as cancer, fungal and bacterial infection. This research is aimed to carry out phytochemical screening and investigate the antioxidant potential of the stem bark extracts of *V. paradoxa*. The powdered stem bark was extracted successively by maceration using n-hexane, ethyl acetate and ethanol. The preliminary phytochemical screening was carried out using standard procedures while the antioxidant activity of the extracts was tested qualitatively by TLC Bioautography method and quantitatively using Ferric Reducing Antioxidant Power (FRAP) assay, where ascorbic acid and water were used as positive and negative control respectively. The results showed that n-hexane extract contains steroid/triterpenes, diterpenes and cardiac glycosides, while in ethyl acetate extract steroids/triterpenes, alkaloids, saponins, tannins, phenolic compounds, flavonoids, cardiac glycosides,

anthraquinones, carbohydrates were found. The qualitative antioxidant test showed that n-hexane extract does not have ferric reducing power while in the quantitative FRAP assay, ethyl acetate and ethanol extracts were found to have similar activity and compared well with the standard drug. The highest absorbance (reductive power) for ethyl acetate extract was 0.62, ethanol (0.57) and standard drug (0.70). Based on the results obtained, the plant is an effective antioxidant, hence, a potential source of drugs for diseases associated with oxidative stress such as cancer.

Keywords: *Vitellaria paradoxa,* TLC Bioautography, Ferric Reducing Antioxidant Power (FRAP) assay, phytochemical screening

Introduction

Medicinal plants are plants with scientifically proven ability to cure diseases as well as improve the well-being of humans [1]. In large parts of Africa, medicinal plants are the most readily available resources for health care for rural inhabitants. This is due to their abundance and also due to lack of access to conventional drugs [2,3]. These attributes of the so called "natural" medicines are attracting the attention of an increasingly large number of people of the world. According to Mallappa [4] about 60 % of the world's population depends on herb based and traditional medicine for health care due to their safety, affordability, and effectiveness. 80 % of the population in developing countries are still dependent on the traditional and folk systems of medicine [5], 85 % traditional medicines are prepared by the use of plant extracts and nearly more than 25 % of modern drugs currently available are derived from the plants [4]. Additionally, plants are the major source of pharmaceuticals and they provide bioactive compounds that can be used directly as drugs or as precursors for drug synthesis based on combinatorial chemistry and genomics [6].

The side effects of some synthetic drugs [7] and increase in resistant strains have led to the screening for more effective, less toxic and cost effective drugs [8] from natural sources [9]. The recent studies suggested that plant products are rich source of many biologically active phenolic compounds which have been found to possess potent antioxidant as well as antimicrobial activity [10]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide and hydroperoxide of lipid hydroxyl, thus, inhibiting the oxidative mechanisms that lead to degenerative diseases [11].

V. paradoxa is one of the major sources of medicine in traditional medicine practice in Africa, and has been accepted among the rural inhabitants for fighting diseases and infections [2]. In Nigeria, the seed oil decoction is used for curing cough and tuberculosis [12,13], the stem bark and seed nuts

for diarrhea and hemorrhoids [14,15]. Furthermore, the seeds are used as antibacterial, antifungal agent and coolant [16], while the stem is used in the treatment of skin diseases and gastric ulcers [17,18]. Additionally, the use of the leaf, fruit and bark in treating fungal diseases like rash, skin and wound infections and chicken pox [19] and the fruits and bark for toothache and rheumatism [20] have been reported. For cancer and tumor, the bark and oil of *V. paradoxa* are utilized [21,22]. These herbal remedies usually involved plant preparations, like maceration, decoction, cream, and infusion, and they may comprise the whole or part of the plant [23]. This study is aimed at conducting preliminary phytochemical screening of *V. paradoxa*, to qualitatively ascertain antioxidant potential using the method of TLC Bioautography and quantitatively ascertain same using Ferric Reducing Antioxidant Power (FRAP) assay to test three different extracts of stem bark of *V. paradoxa* obtained by successive extraction.

Materials and Methods

Sampling, Preparation of Plant Material and Extraction

The sample collection, identification, preparation and extraction procedures and the formular used to calculate the percentage yield of the extracts for the current study was reported in the previously published paper [24].

Preliminary Phytochemical Screening

The extracts were screened for the presence of secondary metabolites such as steroids/triterpenes, alkaloids, tannins, flavonoids, saponins, and proteins as described [25, 26].

Qualitative antioxidant activity studies

The extracts were analyzed by FRAP/ thin-layer chromatography (TLC) bioautography as described by Silva *et al.* [27] with slight modification (5 % ferric chloride in alcohol was sprayed on the plate before viewing). n-Hexane, ethyl acetate and ethanol extracts (0.5 g each) were dissolved in 10 cm^3 of methanol and spotted on TLC plates (7 cm length) and developed using ethyl acetate: ethanol (8:2) as solvent system. After drying, the plates were sprayed with 5 % ferric chloride in ethanol. The chromatogram was then observed for immediate appearance of bluish color on spraying. Ascorbic acid was used as positive control.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing power of the extracts was measured using the method [28] with slight modification. Five concentrations (5,10, 25, 50 and 125 mg/cm^3) of each of the extracts of ethyl acetate and ethanol were prepared and mixed with phosphate buffer (2.5 cm^3, 0.2 M, pH 6.6) and 2.5 cm^3 of 1 % potassium ferricyanide ($K_3Fe(CN)_6$). Then the mixture was incubated at 50 °C for 20 min, 2.5 cm^3 of 10 % trichloroacetic acid (TCA) was added to above mixture and centrifuged at 3000 rpm for 10 min. The

upper layer of the solution (2.5 cm^3) was mixed with 2.5 cm^3 distilled water and 0.1 % ferric chloride (0.5 cm^3). The UV absorbance of the reaction mixture was measured at 700 nm. Increase in absorbance of the reaction mixture indicated reducing power. Ascorbic acid and water were used as positive and negative control, respectively.

Graphical Representation of the FRAP Result

Line graph of the Ferric Reducing Antioxidant Power (FRAP) assay result was prepared using Microsoft Office Excel version 2016.

Results

Extraction and Percentage Yield

The yield, percentage yield and color of the three extracts obtained is given in Table 1. n-hexane extract gave a yellowish, viscous substance with percentage yield of 4.72 %. Ethyl acetate gave a reddish-brown powder (percentage yield of 6.45 %). While ethanol extract appeared crystalline, reddish brown (percentage yield of 6.57).

Table 1: Result of extraction and percentage yield

Solvent	Weight (g)	Yield (%)	Color/Appearance	Weight of residue (g)
n-hexane	18.90	4.72	Yellow/ viscous	362.00
EtOAc	25.80	6.45	Reddish brown/ powder	313.80
EtOH	27.50	6.57	Reddish brown/ crystal	

Key: EtOAc=ethyl acetate, EtOH=ethanol

Preliminary Phytochemical Screening of the Extracts

Preliminary phytochemical screening of stem bark extracts of *V. paradoxa* revealed the presence of steroids/triterpenes, alkaloids, diterpenes, tannins, flavonoids, saponins, carbohydrates, cardiac glycosides and anthraquinones as presented in Table 2. The metabolites were distributed among the various extracts with n-hexane having steroids, triterpenes, diterpenes and cardiac glycosides while ethyl acetate has all but diterpenes. Ethanol on the other has showed presence of phenolic compounds, flavonoids, anthraquinones, carbohydrates and tannins.

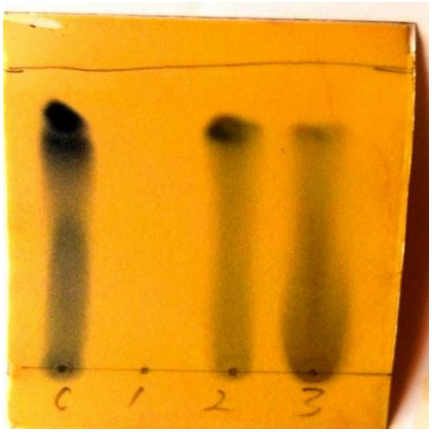
Table 2: Preliminary Phytochemical Screening of extracts of stem bark of *V. paradoxa*

Constituent	Test	n-Hexane Extract	EtOAc Extract	EtOH Extract
Steroids/Triterpenes	Salkowski's	++	+	-
	Liebermann-Burchard's	++	+	-
Alkaloids	Mayer's	-	++	-
	Wagner's	-	++	-
Diterpenes	Copper acetate	+	-	-
Phenolic compounds	Lead acetate	-	+	+
Tannins	Lead acetate	-	++	++
Flavonoids	Ferric chloride	-	++	++
	Sodium hydroxide	-	++	+
Saponins	Frothing's	-	+	+
Cardiac glycosides	Ferric chloride	+	+	-
Anthraquinones	Bontrager's	-	+	+
Carbohydrates	Molisch's	-	++	++

Key: EtOAc=ethyl acetate, EtOH=ethanol, + = moderately present, ++ = high presence, - = absent

Qualitative antioxidant activity

The result of the qualitative antioxidant activity of the three extracts and control using the method of TLC Bioautography is shown in Plate 1. It is observed that, out of the three extracts and control, only n-hexane extract did not show any sign of activity.



Key: C=control, 1=n-hexane extract, 2=ethyl acetate extract, 3=ethanol extract

Plate 1: TLC plate of qualitative antioxidant activity

Ferric Reducing Antioxidant Power (FRAP) Assay

Table 3 shows the reducing powers of ethyl acetate and ethanol extracts and that of the control. The absorbance of the various concentrations of the extracts were measured at 700 nm.

Table 3: Result of Ferric Reducing Antioxidant Power Assay

Sample/Extract	5 mg/ml	10 mg/ml	25 mg/ml	50mg/ml	125 mg/ml
Ethyl acetate	0.24	0.29	0.36	0.41	0.62
Ethanol	0.20	0.25	0.30	0.39	0.57
Ascorbic acid	0.26	0.30	0.38	0.49	0.70

Graphical Presentation of FRAP Result

Figure 1 shows the line graph of the Ferric Reducing Antioxidant Power (FRAP) assay result for EtOAc and EtOH extracts as well as the control (ascorbic acid). It is observed that EtOAc extract showed more activity than the EtOH extract while the control drug showed slightly higher activity than the two extracts

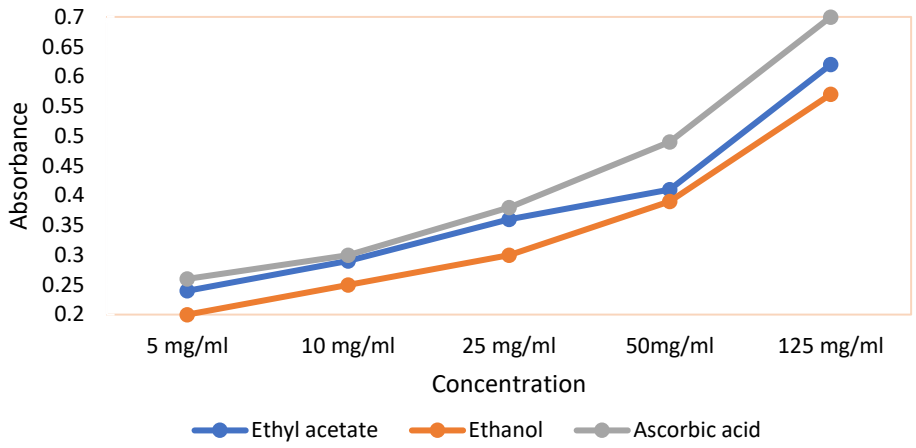


Figure 1: Graphical Presentation of FRAP Result

Discussion

The results obtained indicate that ethanol is a better solvent of extraction as earlier reported [29]. This suggests the possibility of an increase in solubility of active ingredients of plant materials in polar solvents such as ethanol [30,31]. The yellow color of n-hexane extract and percentage yield (4.72) in the current study are similar to results reported [32] for similar sample (stem bark of *V. paradoxa*). It was observed that ethyl acetate and n-hexane extracts contain steroids and triterpenes. The presence of these groups of compounds in extracts obtained from solvents of various polarities was reported by [33, 34]. Polyphenolic compounds such as tannins and flavonoids were present in the extracts of polar solvents (ethyl acetate and methanol extracts). Among the different phytochemicals, phenolic compounds have been applied in different areas such as pharmaceutical, health, food, and cosmetic industries. These compounds are widespread in the plant kingdom as part of our daily diet and are attractive as natural antioxidants [35,36]. Flavonoids belong to the group of polyphenolic compounds and are typically known for health-promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [37,27]. They exist widely in the plant kingdom and displayed a positive correlation between increased consumption of flavonoids and reduced risk of cardiovascular and cancer diseases [38,27]. Correspondingly, ethyl acetate and ethanol extracts also tested positive for phenolic compounds. The phenolic compounds are aromatic secondary metabolites that impart colour, flavour and are associated with health benefits such as the reduced risk of heart and cardiovascular diseases [39,40] and equally account for most of the antioxidant activities in plants [40]. Alkaloids were detected in ethyl acetate extract only. Presence of alkaloids in extracts of moderately polar solvents and their absence in both polar and nonpolar solvents was reported [32].

The result of the qualitative antioxidant activity of the three extracts and control (Plate 1) showed that ethyl acetate and ethanol extracts have ferric reducing power due to the appearance of bluish color while, the n-hexane extract showed converse result. However, the most intense bluish color was observed in the ascorbic acid.

Free radicals can cause damage to essential proteins, DNA, lipids and also be attributed to various human diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, etc. due to oxidative stress. The most important role of antioxidants is to suppress free radical mediated oxidation by inhibiting the production of free radicals [41]. Antioxidants which are compounds known to possess the ability to inhibit oxidation and neutralize the effects of free radicals can be produced in the body, readily supplied through diet, supplement drugs or through the use of extracts from plants that have medicinal effect [42]. However, several enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase etc. act as antioxidants to influence oxidative stress [42]. Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts [43]. The great number of antioxidants in the extracts from *V. paradoxa* stem resulted in the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by donating an electron. The amount of ferrous ion (Fe^{2+}) or the extent of ferric reduction can be indicated by the Perl's blue colour appearance and determined by the absorbance at 700 nm [44]. The extracts of *V. paradoxa* produced blue color with potassium ferricyanide, trichloro acetic acid, and ferric chloride, with the color intensity and absorbance under 700 nm increasing with increase in concentration, indicating the possibility of using these extracts as potential antioxidants. Similar result has been reported [45]. The values of absorbance for ethyl acetate extract, ethanol extract and ascorbic acid at 5 mg/cm^3 were 0.24, 0.20 and 0.26 respectively. These values increased with an increase in concentration (Table 3 and Figure 1). The highest absorbance at 125 mg/cm^3 for ethyl acetate extract was 0.62, ethanol (0.57) and ascorbic acid (0.70). The control showed slightly higher activity than the two extracts as shown by the line graph (Figure 1). This is because it is a pure drug [46,31,47]. There is a significant correlation between the phenolic compounds and antioxidant activity [48]. Reducing power is mainly caused by the presence of the high phenolic compounds of the plant [49].

Conclusion

In this research, phytochemical screening, qualitative (using TLC Bioautography) and quantitative (using FRAP assay) antioxidant activities were conducted on the three stem bark extracts (n-hexane, ethyl acetate and ethanol extracts) of *V. paradoxa* obtained by successive (sequential) extraction. Based on the results

obtained, it can be concluded that the plant is rich in polyphenolic compounds and is an effective antioxidant, hence, a potential source of drugs for diseases associated with oxidative stress such as cancer.

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