Vol 5 | Issue 4| 2014 | 184-188.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

PRELIMINARY PHYTOCHEMICAL SCREENING, ANIONIC AND ELEMENTAL CONTENTS EVALUATION OF *TERMINALIA AVICENNIODES* GUILL AND PERS

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ABSTRACT

This study was carried out to determine the chemical contents of leaf of *Terminalia avicennioides* Guill and Perr, of the family; Combretataceae. Air dried grounded leave was subjected to analysis using atomic absorption spectrophotometer and UV direct reading spectrophotometer for the presence of macro-elements (Mg, Na) and micro-elements (Fe, Cu, Zn, Ni, Cr, Cd, Pb) and anions (nitrate, phosphate, sulphate). The result revealed higher concentration of Na (292.00 µg/g) while Mg (3.62 µg/g) lie within the recommendation and Fe (0.20 µg/g), Cu (0.19 µg/g), Zn (0.15 µg/g), Ni (8.50 µg/g), Cr (0.20 µg/g), Cd (0.39 µg/g) were detected in low concentrations as recommendation by WHO and Pb was not detected. Sulphate was the highest (25.00 µg/g) followed by Phosphate (0.85 µg/g) among the anions studied. The air dried powdered leaves of *Terminalia avicennioides* (1 Kg) was soxhlet extracted using solvents; n-hexane, ethyl acetate, ethanol and water, the extract were filtered and concentrated yielding 3.95 % w/w, 8.31 % w/w, 13.81 % w/w and 4.05 % w/w respectively. The result of the qualitative phytochemical analysis of these extracts revealed the presence of carbohydrates, tannins, glycoside, terpenoids, cardenolide, saponin, flavonoid and aloes.

Keywords: Ethanol, Terpenoids, Cardenolide, Saponin.

INTRODUCTION

Medicinal plants produce and contain a variety of chemical substances that act upon the body found richly in leaves, flowers, stems, seeds, fruits, bark and roots of plants to prevent, relieve and treat illness. Medicinal plant can be define as a living thing that grows on the earth and usually has a stem, leaves and root with healing properties and usually used for healing [1]. Plants that have one or more of its organs containing substances that can be used for therapeutic purpose are called medicinal plant [2]. Since man took his first step on this planet, he has been sustained by plant kingdom and continues to be so. No one knows for sure when humans begin using herbs for medicinal purposed as such the use of plant as medicine predated written human history, thus many of the herbs and spices used by humans to season food also yield useful medicinal compound [3]. Plants have the ability to synthesis a wide variety of chemical compounds that are used to perform important biological functions, and as defence against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemical have beneficial effects when consumed by humans and used effectively to treat diseases.

Traditional plant- based medicine that have been historically used in different parts of the world or different cultural systems are considered in western medicine as "alternative medicine" [4]. They are referred to as herbal medicine or botanical medicine being that herbs are plants or plant parts valued for medicinal, aromatic or savory qualities used for therapeutic or medicinal purposes [5]. The use of herbs is the oldest form of health care known to mankind, it is the integral part of development and modern civilization. In many regions of the world such as Africa and Asia, plant are being traditionally used because of either been the only affordable health care option or that they are believed to be effective [6]. Medicinal plants are generally locally available and relatively cheap and there is every virtue in exploiting such local and traditional remedies when they have been tested and proven to be nontoxic, safe, inexpensive and culturally acceptable to the community. The aim of the study is to carry out phytochemical analysis

METHODOLOGY

Sample Collection, Identification and preparation

Fresh samples of the leaves of *Terminalia avicennioides* (*Guill* and *Perr*.) was collected from Jumu'a

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village in Potiskum Local Government Area of Yobe State in November 2012. Jumua village is about 7km East of Potiskum, Potiskum is a local government of Yobe state in Nigeria lying at $11^{o}42^{'}36$" North of the equator and $11^{o}4'12$" East of Greenwich prime meridian, approximately lies 1297km north of equator (NIPOST, 2009). The plant material was identified by a plant taxonomist in the Department Biological Sciences, University of Maiduguri, given a voucher specimen number of 734C and deposited at the herbarium of the Postgraduate Research Laboratory, Department of Chemistry. The sample was air dried under shade (Rates, 2001). The air dried plant material was then ground to powder and stored in cellophane bags at room temperature before use.

Plant Extraction

One kilogram (1 Kg) of the powdered air dried leaves was soxhlet extracted using the following organic solvents; hexane, ethyl acetate, ethanol and water. The crude extracts obtained were concentrated to dryness using a rotary evaporator between 40^{o}C to 45^{o}C. The extracts colour, yield and texture of extract fraction were evaluated, labeled and subjected to phytochemical screening.

Preliminary Phytochemical Screening

A small quantity each of the hexane, ethyl acetate, ethanolic and water extract fraction were subjected to phytochemical screening so as to test for the presence of the following secondary metabolites: sugars, tannins, phlobatanins, glycosides, cardiac glycosides, terpenoids, cardenolides, saponins, flavonoids, alkaloids and aloes according to the methods of Trease and Evans (2002), Harborne (1998) [8-9].

Determination of elemental and Anionic Content of the leaf of *T. avicennioides*

Ashing and Digestion of the leaf Sample

The air dried plant sample was pulverized manually into a coarse powder. Then 5g of the sample was packed into an acid washed porcelain crucible and ashed in a muffle furnace for 3 hours at 550^{O}C. The crucible was removed from the furnace and cooled. To the ashed sample (0.5g), 10 ml of 6M HCl was added and covered, and this content was then heated on a steam bath for 15minutes. Then 1 ml HNO_3 was then added and the mixture was heated for an hour in order to dehydrate silica and completely digest organic substances. Lastly 5 ml of 6M HCl and 10 ml distilled water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was then cooled and filtered through Whatman No. 1 filter paper into a 100ml volumetric flask and then made up to the mark with distilled water [10],[11].

Analysis of some elements in the leaves of *Terminalia avicennioides*

The micro and macro elements were determined using AA-6800 Shimadzu Japan atomic absorption spectroscopy (AAS) and DR/2000 direct reading spectrophotometer. The laboratory procedure for the preparation and determination of micro and macro elements was used as outlined by Radojevic and Bashkin (1999) for plant sample [12].

Determination of Anions in the leaf of *Terminalia avicennioides*

Determination of nitrate

Plant sample solution was prepared by chopping the sample into smaller sizes. About 0.5g of the sample was transferred into 100 ml flask and soaked with distilled water. The flask was corked and shaken for 30 minutes, then filtered into another 100ml volumetric flask and the volume made to the mark with distilled water[10-11]. The nitrate was determined using batch direct reading 2000 spectrophotometer [11].

Determination of phosphates

The air dried and finely powdered sample of *T. avicennioides* was used. About 0.5g was weighed into a crucible; which was then underplayed with 5ml of (20% $^{w}/_{v}$) Magnesium acetate, the crucible content was ashed at 500^{O}C for four (4) hours in muffler furnace and then removed and cooled in a desiccator. Hydrochloric acid (6M) was added to the crucible and covered, then heated on a water bath for 15minutes. The contents of the crucible were then completely transferred into evaporating basin and 1 ml of concentrated HNO_3 was added. The heating was continued for an hour to dehydrate silica. Then 1 ml of 6M HCl was further added, swirled, then 10 ml distilled water was added and again heated on the water bath for complete dissolution. The contents of the evaporating basin was then cooled and filtered through Whatman No. 1 filter paper into 100 ml volumetric flask and the volume made up to the mark with distilled water [10-11]. The phosphate was determined using batch direct reading 2000 spectrophotometer.

Determination of Sulphates

5 ml of Magnesium nitrate solution was added to about 0.5 g of ground sample in the crucible and was heated to 180^{O}C on a hot plate. The heating process was continued until the colour of the sample was changed from brown to yellow (AOAC, 2000). The sample was then transferred to the furnace at a temperature of 500^{O}C for 4 hours. Magnesium nitrate was then added to prevent loss of sulphur, the content of the crucible was carefully transferred to different evaporating basin. 10ml of concentrated HCl was added and covered with a watch glass. On cooling 10 ml of distilled water was added to the basin and the content was filtered into a 100 ml volumetric flask and the volume made up to the mark with distilled water[10-11]. The sulphate was determined using batch direct reading 2000 spectrophotometer

RESULT AND DISCUSSION

The yield of the crude extracts of the soxhlet extraction of the dried powdered leaf of *Terminalia Avicennioides* using n- hexane, ethyl acetate, ethanol and water is shown on table 1. The result indicated the highest yield of 13.8% ($^{w}/_{w}$) from ethanolic extract, followed by

ethyl acetate with 8.3% ($^{w}/_{w}$) while the aqueous extract and n- hexane extract had 4.0% ($^{w}/_{w}$) and 3.9% ($^{w}/_{w}$) respectively. The resulting leaf residue after the extractions yielded 77.7% ($^{w}/_{w}$).

The extract (hexane, ethyl acetate, ethanol and aqueous) were subjected to chromogenic (qualitative) phytochemical analysis and the result is shown in table 2 The result revealed the presence of glycosides, cardiac glycosides, cardenolides and terpenoids in all the extract, tannins, saponin glycosides, flavonoids, aloes and carbohydrates are present in ethyl acetate, ethanol and aqueous extract. While phlobatanins, alkaloids and resins are not present in all the extract as can be in the table 2.

Elemental and anion content of the leaf of *T. Avicennioides*

The elemental analysis of *Terminalia avicennioides* leaf is shown in table 3. The leaf showed higher concentration of sodium (Na) – 292.00µg/g, Magnesium (Mg) - 3.62µg/g though it was within the recommended limit, Nickel (Ni) - 8.50µg/g, Chromium (Cr) - 0.02µg, Iron (Fe) - 0.20µg/g, Zinc (Zn) - 0.15µg/g, Copper (Cu) - 0.19µg/g and Cadmium (Cd) - 0.39µg/g were detected in low concentrations compared to the standard values whilst lead (Pb) was not detected. For the anions Phosphate (PO_4^{-3}) was found to be 0.85µg/g, Sulphate (SO_4^{-2}) - 25.00µg/g, Nitrate (NO_3^{-}) - 0.10µg/g and Cyanide (CN^{-}) - 0.24µg/g.

Table 1. The extraction profile of air dried powdered of the leaf of *Terminalia Avicennioide*

S/N	Fraction	Mass (g)	% yield ($^{w}/_{w}$)	Colour	Texture
1	n- hexane	39.48	3.94	Green	Gummy mass
2	Ethyl acetate	83.15	8.31	Dark green	Gummy mass
3	Ethanol	138.14	13.81	Brown	Gummy mass
4	Water	40.47	4.04	Light brown	Powder

Weight of final residue = 777g, ($^{w}/_{w}$) = weight by weight.

Table 2. Phytochemistry of crude extract of n- hexane, ethyl acetate, ethanol and aqueous of leaf of *Terminalia avicennioides*

S/N	Chemical compound	Result			
		TANH	TAEA	TAET	TAWE
1	Carbohydrate				
	i. Molisch's Test	-	+	+	+
	ii. Barfoed's Test	-	+	+	+
	iii. Fehling's Test	-	-	+	+
	iv. Combined reducing sugar	-	-	+	-
	v. Ketoses (Selivnoff's test)	-	-	+	+
	vi. Pentoses	-	-	+	+
	vii. Soluble starch	-	-	+	+
2	Tannins				
	i. Ferric chloride test	-	+	+	+
	ii. Lead acetate test	-	+	+	+
	iii. Hydrochloric test	-	+	+	+
3	Phlobatannins	-	-	-	-
4	Glycosides				
	i. Free anthraquinone	+	+	+	-
	ii. Combined anthraquinone	-	-	+	+
5	Cardiac glycoside				
	i. Salkowski' test	+	-	+	+
	ii. Liebermann-Burchard's test	+	+	+	-
6	Terpenoid	+	+	+	-
7	Cardenolide				
	i. Keller-Killiani's test	+	+	+	+
8	Saponin glycoside				
	i. Frothing test	-	+	+	+
	ii. Fehling's test	-	+	+	+
9	Flavonoid				
	i. Shinoida's test	-	-	+	-
	ii. Ferric chloride test	-	-	+	+
	iii. Lead acetate test	-	+	+	+
	iv. Sodium hydroxide test	-	-	-	-
10	Alkaloid	-	-	-	-
11	Aloes	-	+	+	+

Key: (-) = Absent, (+) = Present, TANH = hexane extract, TAEA =ethyl acetate extract, TAET = ethanol extract TAWE = water extract

Table 3. Elemental and anionic content of leaf of Terminalia Avicennioides.

S/N	Elements/ Anions	Concentration (µg/g)
1	Sodium (Na)	292.00
2	Magnesium (Mg)	3.62
3	Copper (Cu)	0.19
4	Nickel (Ni)	8.50
5	Chromium (Cr)	0.02
6	Cadmium (Cd)	0.39
7	Iron (Fe)	0.20
8	Lead (Pb)	Not detected
9	Zinc (Zn)	0.15
10	Phosphate (PO_4^{3-})	0.85
11	Sulphate (SO_4^{2-})	25.00
12	Nitrate (NO_3^-)	0.10
13	Cyanide (CN^-)	0.24

Table 4. WHO guidelines for elemental concentration (WHO standards 1996)

S/No	Element	WHO Standard Concentration (mg/L or ppm)
1	Sodium	4-5
2	Potassium	0.1-1.0
3	Cadmium	10-35
4	Calcium	360-800
5	Copper	1-3
6	Lead	-2
7	Iron	0.5-50
8	Zinc	15-20
9	Phosphorus	0.05-0.3
10	Sulphur	0.08-0.5

DISCUSSION AND CONCLUSION

The phytochemical studies on *Terminalia avicennioides* gave useful classes of chemical compounds such as tannins, glycosides, cardiac glycosides, terpenoids, saponin glycosides, flavonoids [13], cardenolides and aloes. These classes of chemical compounds were known to exert pharmacological and antagonistic effects and some are even capable of protecting the active ingredients in herbs from decomposition either chemically or physiologically [13].

Many trace/heavy element are known to influence various functions due to their direct or indirect action in physiological or toxic concentration [14] Iron occurs as a natural constituent in plants and animals The presence of elements in the leaf of *T. avicennioides* could be an indication of the types of mineral present in the soil. The toxic elements Cadmium (Cd) was found in very low concentration in plants [15] and this may be due to its low deposit in the soil. Human exposure occurs mainly from consumption of contaminated food, fossil fuel and municipal waste incineration thus resulting into increase in toxic elements (such as cadmium) level in soil and crops [16]. The concentration of some elements in the leaves of *T. avicennioides* from elemental analysis in this study showed them to be within safety limit as compared with the report by WHO [15]. Since these elements occur within safety limit, and are essential for growth and metabolic functions of the body, this plant (*T. avicennioides'* leaf) when ingested can play same role.

ACKNOWLEGMENT

The authors wish to acknowledge Messers Fine Akawo and Shehu Jauro of Department of Chemistry, Faculty of science and Animal Science, faculty of Agriculture University of Maiduguri. Also thanks goes to the University of Maiduguri for providing necessary laboratory facilities for the research.

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