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PHYTOCHEMICAL ANALYSIS, PROXIMATE, MINERAL AND AMINO ACID COMPOSITION OF THE LEAVES OF *WEDELIA TRILOBATA* (L.) HITCHC.

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ABSTRACT

Phytochemicals present in plants are responsible for the biological activities and therapeutic properties of the various medicinal plants. They function as antioxidants in health promotion by preventing oxidant-damage which is the main cause of many diseases. This study reveals the phytochemicals in different solvents of the leaves of wedelia trilobata growing in Yola north local government area of Adamawa State Nigeria. The dried leaves of W. trilobata was used to determine the proximate composition by standard methods, minerals composition was determined using AAS and the amino acid composition and the quantitative composition of the phytochemicals using HPLC. The proximate composition shows the plant is rich in crude protein and fibre. The adequate ash content accounts for the abundance of minerals. In this study, heavy metals were not detected. Manganese was the most abundant with a value of 207.15 mg/100 g, copper was the least 2.77 mg/100 g, the elements detected were Ca, Fe, Mg, Zn, Cu, Mn. Nineteen (19) amino acids were detected in the leaves of W.trilobata. all the essential amino acids were present in reasonable quantities. Tyrosine 9.2 mg/100 g was the most abundant followed by phenylalanine, glycine, and aspartate with values 8.78, 7.83 and 5.14 mg/100 g respectively. The percent of ideal essential amino acids in W.trilobata are higher than World Health Organization (WHO) standards except for leucine, lysine and threonine with values 6.6/2.29, 5.8/4.53, 3.4/3.36 percent of total W.trilobata/WHO respectively. The quantitative phytochemical composition determined using HPLC showed that phenolics had the highest concentrations in all the solvents used, followed by tannins and phlobatanins was the least. The phytochemical, mineral, proximate and amino acid contents of this plant must have contributed to the medicinal properties of the plant.

Key words: Wedelia trilobata, phytochemicals, amino acids, proximate analysis.

INTRODUCTION

Plants contain a range of phytochemicals that are useful to prevent and treat human diseases. They are considered a good source of complex individual mixtures. To explain the medicinal value of plants, the bioactive components found in plants require identification and characterization of the metabolites (Azizan *et al.*, 2015). *Wedelia trilobata* (now with a new name *Sphagneticola trilobata*) is of the Asteraceae family

and is native of South America and widely distributed in many wet tropical areas (Invasive species compendium, 2015; Weeds of Australia, 2011). *Wedelia trilobata* is a fast- growing perennial mat-forming herb. The plant is known for its beautiful flowers that is year-round and has been used widely as decorative ground cover in gardens, paths and in public areas. It is an invasive plant and can be a threat to neighboring flora (Invasive species compendium, 2015; Qi *et al*, 2014). Like other genera of *Wedelia, W. trilobata* has been widely used in folk medicines in many countries to treat a variety of diseases such as headaches, muscle cramps, rheumatism, stubborn wounds, common cold cough, hepatitis, tumour, central nervous system problem, indigestion, infections, inflammation and fever (Invasive plant compendium 2015).
W. trilobata has been reported to contain potential bioactive compounds such as sesquiterpene lactone, diterpenoids and Phyto steroids which exert important biological activities towards cancer, microbial infection and inflammation (Balekar *et al.,* 2012a; Balekar *et al.,* 2012b; Keerthiga *et al.,* 2012; Ren *et al.,* 2015). In order to determine the potential use of herbal medicine, it is important to emphasize the study of medicinal plants that was found in folklore.
In Nigeria *W.trilobata* is mainly found in premises where they are used mainly as ornamental plants to beautify the environment whereas in other countries it is also known for its medicinal properties. With this background of the uses of *Wedelia trilobata,* these preliminary experiments were conducted to evaluate the hypoglycemic/antidiabetic activities of the plant herein the phytochemicals, proximate, mineral and amino acid profile of *Wedelia trilobata* obtained from Yola, Adamawa State Nigeria are reported.

MATERIALS AND METHODS

Collection, preparation and extraction of *W. triolobata leaves*

The leaves of *Wedelia trilobata* were collected from the premises of Meridian Hotel in Jimeta, 9° 15′40.66″ N and 12° 27′16.97″ E Yola North Local Government Area of Adamawa State. The botanical identification and authentication were done by Dr. Akinnibosun Henry Adewale of the Department of Plant Biology and Biotechnology, University of Benin. The voucher specimen was deposited at the Department of Plant Biology and Biotechnology, University of Benin Herbarium and was assigned specimen number UBH-W529.

Preparation of sample:

The fresh leaves of the plant were washed thoroughly with clean water and shed dried for a few days. The dried leaves were pulverized in an electric blender into coarse powder and stored for further analysis. Some of the powder was extracted in different solvents (methanol, ethanol, ethyl acetate, hexane, and water), concentrated using a rotary evaporator and the solid mass obtained and preserved for further use.

Phytochemical Screening

Standard methods described by Trease and Evans (1989), Harborne (1973 and 1987) and Sofowora (1993), AOAC (2000).

Test for Cardiac Glycosides
5mls of extract was treated with glacial acetic acid (2ml). One drop of 1% ferric chloride ($FeCl_3$) was added to it. The solution was carefully under layered with 1ml of concentrated sulfuric acid. A brown ring was formed at the interface that indicated the presence of deoxy-sugar (Keller Kelliani's Test).

Test for Steroids
0.5ml crude extract was mixed with 2ml of chloroform followed by the careful addition of 3ml concentrated sulfuric acid (H_2SO_4). A layer of reddish-brown coloration formed indicates the presence of steroid (Salkowskis Test).

Test for Flavonoids
5ml of dilute Ammonia (NH_3) solution was added to a portion of aqueous filtrate of the extract. To this concentrated H_2SO_4 was added. The appearance of a yellow color indicated the presence of flavonoids. Yellow color usually disappears on standing.

Test for Saponins
Frothing test, the best test for the detection of saponin was applied 0.5ml of extract was added to 5ml of distilled water in a test tube. The solution was shaken vigorously and the stable persistence of 'froth' indicated the presence of saponins.

Test for Tannins
0.5ml of extract was boiled in 10ml of distilled water for 1 hour and then filtered. After adding a few drops of 1% Ferric Chloride ($FeCl_3$) and allow to stand for some time, a blue or greenish black precipitate indicates the presence of tannins.

Test for Alkaloids
a) Meyer's test: to 1ml of the extract solution was added a few drops of Mayer's reagent (Potassium mercuric iodide). Appearance of white (creamy yellow) precipitate or turbidity indicated the presence of Alkaloids.
b) Wagner's test: to 2ml of extract was added few drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodine in 100mls of water). Formation of a reddish-brown (brown/reddish) precipitate indicates the presence of alkaloids.

Test for Terpenoids
5mg of dry crude plant extract was dissolved in 2ml of chloroform ($CHCl_3$), and then 1ml of acetic anhydride was added to it. Concentrated sulphuric acid (H_2SO_4) 3ml was added. A reddish violet color shows the presence of terpenoids.

Test for Phlobatanins
0.25g methanol extract was dissolved in 10ml distilled water and filtered. The solution was boiled with 1% aqueous HCl. The formation of red precipitate indicated the presence of phlobatanins.

Test for Anthraquinone

Borntrager's test for free anthracene derivatives. The powdered leaves (0.5g) was taken in a test tube and 5ml of chloroform was added and shaken for 5 minutes, filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink/red or violet color in the aqueous layer after shaking indicated the presence of free anthraquinone.

Test for Carbohydrates

a) Benedict's test: to 0.5ml of extract in a boiling tube was added 0.5ml of Benedict's reagent and heated gently (on a boiling water bath) for 2 minutes. A characteristic orange-red precipitate indicated the presence of sugars.
b) Iodine test: to 2ml of extract solution, 2ml of iodine solution was added and mixed. A dark blue or purple coloration will indicate the presence of iodine.

Elemental Analysis

0.5g was accurately weighed and placed in a digestive tube. The sample was digested with concentrated HNO_3 (5ml) at 175°C for 40 minutes then at 150°C for 90 minutes. To the cooled mixture, 1ml HNO_3 and 0.5ml H_2SO_4 were added and the mixture was heated at 170°C for 60 minutes. To the cooled mixture H_2O_2 (2ml) was added dropwise and heated at 140°C for 10 minutes to remove any remaining NO_2 that might interfere in the measurement. The resulting mixture was transferred to a calibrated flask and HCl (8ml) was added and the filtrate diluted with deionized water to 25ml. sample was immediately analyzed using Atomic Absorption Spectrophotometer (AAS) Buck 210 for (Pb, Cd, Mg, Fe, Ca, Zn, Cu, Mn, V, Cr) and Jenway ME 882 Flame Photometer (Na, K).

Proximate Analysis

Proximate analysis was determined according to standard methods as described by AOAC (2015).

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was carried out using HPLC.

Determination of Amino acids

Amino acids profile was determined using HPLC.

RESULTS AND DISCUSSION

Table 1a: Preliminary Phytochemical screening of *Wedelia trilobata* leaves using different solvents.

Phytochemical constituents	Aqueous	Methanol	Hexane	Ethanol	Ethyl Acetate
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Protein	+	+	-	+	-
Terpenoids	+	+	-	-	+
Tannins	+	+	+	+	+
Saponins	+	+	+	+	-

Anthra-Quinones	+	+	+	+	+
Phlobatanins	+	+	+	-	+
Cardiac glycosides	+	+	-	+	+
Triterpenes	+	+	+	+	+
Phyto-Steroids	+	+	+	+	+
Fixed oil/fats	+	+	+	+	+
Amino acids	+	+	-	+	+

Detected = +, Below detectable level = -

Table 1b: Quantitative Phytochemical composition mg/100 g.

Parameters	Ethanol	Aqueous	Methanol	Hexane	Ethyl acetate
Tannins	415 ± 0.00	183 ± 0.01	487 ± 0.01	493 ± 0.02	496 ± 0.01
Alkaloids	298 ± 0.01	164 ± 0.01	244 ± 0.01	380 ± 0.00	519 ± 0.01
Saponins	323 ± 0.01	940 ± 0.00	529 ± 0.01	463 ± 0.02	414 ± 0.02
Flavonoids	162 ± 0.00	111 ± 0.01	211 ± 0.01	385 ± 0.01	131 ± 0.02
Phenolics	1534 ± 0.01	151 ± 0.01	2396 ± 0.01	2679 ± 0.01	2940 ± 0.00
Glycosides	135 ± 0.01	12 ± 0.01	200 ± 0.01	292 ± 0.01	311 ± 0.01
Terpenes	263 ± 0.01		395 ± 0.00	300 ± 0.00	351 ± 0.01
Phlobatanins	15 ± 0.01		115 ± 0.00	113 ± 0.00	181 ± 0.01

Values are means ± standard deviation of triplicate determinations

Phytochemicals are secondary metabolites that contribute to biological activities of medicinal plants (Yadav *et al* 2014). Phytochemical screening showed that all the phytochemicals were detected in most of the solvents used for extraction. The hexane extracts showed the absence of protein, amino acid and terpenoid were not detected, while in ethyl acetate extract, carbohydrate was not detected. Terpenoids, were also not detected in the hexane and ethanol fractions. Water, methanol, hexane and ethyl acetate were good for extracting most of the phytochemicals as shown in Table 1.

Phytochemicals are known to react with nutrients and dietary products, scavenge free radicals and thus diminish the risk of dreadful diseases such as arthritis, cancer, osteoporosis, cardiac ailments and early aging. Among the natural phenolic compounds found in plants, the flavonoids are the most important ones.

Flavonoids are usually referred to as antioxidants and function to provide protection against diseases (Ding *et al* 2010*).* Flavonoids get easily absorbed into cell membrane, thereby protect the cells from the damage of free radicals. Also, they show inhibitory activity against peroxidation of lecithin (Ding *et al.,* 2010). They possess diverse biological activities such as antiviral, antiulcer, cytotoxic and anti-inflammatory ones (Lee *et al.,* 2011; Ghasemzadeh *et al.,* 2011; Husain and Kumar, 2015). Terpenoids are significant in plant growth and metabolism (Husain and Kumar, 2015).

Alkaloids are the basic natural products which are regarded as the most efficient therapeutic agents among plant metabolites. They have physiological activities; hence they are widely used in medicines for their analgesic and anti-bacterial activities (Pradeep *et al*, 2014). Many alkaloids are terpenoids in nature and they function as growth regulators or as insect repellents and attractants. Steroids, saponins and

cardiac glycosides are three of the important group of terpenoids. Anthraquinones on the other hand are considered as one of the most potent agents in metastatic breast cancer (Thamaraselvir and Jayanthi,2012). Flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic activities (Thamaraiselvi and Jayathi2012, Ding *et al*, 2010, Husain and Kumar 2015 and Lee *et al*,2011). Tannins exhibit potent anti-microbial (Thamaraiselvi and Jayathi 2012 and wound healing properties (Pradeep *et al,* 2014). Terpenoids possess a wide range of bioactivities including anti-hyperglycemic, anti-allergic, anti-inflammatory, anti-microbial, anti-viral and immunomodulatory activities (Pradeep et al 2014). *Wedelia trilobata* is a good source of phytochemicals.

Table 2: Proximate composition of samples (%).

Parameters	*Wedelia trilobata*
Moisture	6.15 ± 0.00
Ash	7.75 ± 0.00
Fibre	17.55 ± 0.01
Fats	6.00 ± 0.00
Protein	16.36 ± 0.00
Carbohydrate	46.19 ±0.01

Values are means ± standard deviation of triplicate determinations.

The proximate composition of *Wedelia trilobata* in Table 2 show high fibre content. Fibre is good for the digestive system. The ash value determines the inorganic composition. Ash content of 7.75% is slightly higher than 6.32% obtained by Langhi *et al*. (2020) from *Wedelia trilobata* in India.

Table 3: Mineral composition of samples (mg/100 g).

Minerals	RDA	UL	*W. trilobata*
Ca	1000 mg	250 mg	61.05 ± 0.01
Fe	9 – 15 mg	25 mg	4.05 ± 0.00
Mg	280 – 350	350	207.15 ± 0.01
Pb			BDL
Cd			BDL
Zn	7 – 9 mg	25	2.77 ± 0.01
Cu	900 µg	5 mg	0.35 ± 0.01
Mn	3 mg	1.8 mg	42.15 ± 0.00
Phosphorous			
Na	1.5 g	2300 mg	22.92 ± 0.02
Potassium	3.5 g	3000 mg	98.56 ± 0.03

Values are means ± standard deviation of triplicate determinations.
RDA – Recommended daily dietary allowance per day for adults.
UL – Tolerable upper intake level per day for adults.
BDL – Below detectable level.

The mineral composition in Table 3 shows the plant has high amount of magnesium (207.15 mg/100 g) meets RDA for adults. The amount of copper was the least (0.35 mg/100 g) which is below the tolerable upper intake level. Zinc, iron and calcium are all below the daily recommended allowance. The heavy metal Cd and Pb are below

detectable limits. Several minerals are co-factors for signaling intermediaries of insulin action and key enzymes of glucose metabolism. Plants rich in minerals have been shown to enhance glycemic control in diabetic patients. Micronutrients function as co-enzymes and co-factors for metabolic reactions and thus help support basic cellular reactions such as glycolysis, citric acid cycle and amino acid metabolism required to maintain energy production and life. Magnesium is a co-factor in the glucose transport mechanism of the cell membrane and various enzymes in carbohydrate oxidation and is thought to play a role in the release of insulin. Copper, Cr, Fe and Zn are essential micronutrients for human health. In addition, these elements play an important role in human metabolism, and interest in these elements is increasing together with reports of relationship between trace element status and oxidative diseases (Tresina and Mohan, 2012). The table also displays the Recommended Dietary Allowances (RDA) and Upper Tolerance Levels (UL) of most of the elements and thus it can be deduced that the existing profile of the trace and other elements, based on WHO, 2008 and WHO, 2011 reports, were within permissible limits (Dhonukshe-Rutten 2012).

Table 4a: Amino acids composition of samples mg/100 g protein

Amino acids	*W. trilobata*
Tryptophan	4.84 ± 0.01
Histidine	1.34 ± 0.01
Leucine	1.84 ± 0.01
Isoleucine	4.01 ± 0.01
Phenylalanine	8.78 ± 0.01
Valine	3.87 ± 0.01
Lysine	3.64 ± 0.01
Methionine	3.71 ± 0.01
Threonine	2.70 ± 0.00
Asparagine	3.62 ± 0.01
Arginine	3.71 ± 0.01
Alanine	2.52 ± 0.00
Aspartate	5.14 ± 0.00
Glutamate	2.42 ± 0.01
Glycine	7.83 ± 0.01
Tyrosine	9.62 ± 0.01
Cysteine	3.13 ± 0.01
Proline	2.84 ± 0.01
Serine	4.85 ± 0.01
Total	80.41

Values are means ± standard deviation of triplicate determinations.

Table 4b: Comparison of the essential amino acid content of *W. trilobata* with that of the World Health Organization idea pattern (% of total amino acids).

Amino acid	WHO ideal pattern	*Wedelia Trilobata*	EAAS
	(% of total)	(% of total)	(% of ideal)
Tryptophan	1.1	6.02	547
Leucine	6.6	2.29	34
Isoleucine	2.8	4.97	178

Phenylalanine + Tyrosine	6.3	10.92	173
Valine	3.5	4.81	78
Lycine	5.8	4.53	184
Methionine+ Cysterin	2.5	4.61	98
Threonine	3.4	3.36	99

EAAS – Essential amino acid score

Table 4a and 4b shows the amino acid profile of *W. trilobata.* The essential amino acids in *W. trilobata* are higher than WHO ideal pattern except for leucine and lysine, showing that the plant is very rich in essential amino acids. The plant *W. trilobata* has all the essential amino acids and can be considered a 'complete protein' for human nutrition (Penuel *et al*, 2014).

CONCLUSION

This study concludes that the leaves contain a number of pharmaceutically important chemicals like alkaloids, saponins, flavonoids, terpenoids, tannins, carbohydrates and protein. The plant is a good source of nutrients and these may be responsible for observed pharmacological activities.

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