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Evaluation of Phenolic and Antioxidant Vitamin Contents in Different Leaf Extracts of *Acalypha wilkesiana*

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ABSTRACT

This study investigated the total phenolic and the different antioxidant vitamins contents in the ethanol and acetone extracts of the leaf of *Acalypha wilkesiana* using standard analytical methods, in order to authenticate the medicinal potentials of this plant. The results obtained indicated that ethanol extracted the highest phenolic content. The analysis of vitamin concentration showed that acetone extract had the highest vitamins A and E contents (0.812±0.45 and 108.97 ± 0.79 mg/100 g respectively), while the ethanol extract contained the highest concentration of vitamin C (79.595±0.926 mg/100 g). Results from this study have shown that *Acalypha wilkesiana* leaves are a good source of antioxidant - vitamins and polyphenols, and are potential source of natural antioxidants.

1. Introduction

In recent times, there is growing interest in exploiting plants for nutritional, therapeutic as well as for antioxidant activities [1, 2]. This is because the presence of various types of antioxidant compounds makes these plants a valuable source of nutraceuticals [3, 4]. Fortunately, chemical composition diversity in plants also includes many compounds that are beneficial to humans such as vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value [5]. Natural antioxidants endogenous to food of plant origin can scavenge reactive oxygen and nitrogen species (RONS); evidence suggests that these may be of great importance in preventing the onset of oxidative diseases in the human body such as cancer, anaemia, diabetics and cardiovascular diseases [6, 7]. Lately, great interest has been focused on the following dietary antioxidant vitamins (A, C and E) particularly because of their likely role in prevention of coronary heart disease and cancers and delaying ageing processes [8-10]. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid peroxidation is initiated. Vitamin C has been cited as being capable of regenerating vitamin E [11]. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. It also plays a role in resistance to viral infection. Beta carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. *Acalypha wilkesiana* is one of several medicinal plants used in Nigeria, therefore the aim of this work is to determine the concentrations of antioxidant vitamins and polyphenols present in the various leaf extracts under study.

2. Experimental Methods

2.1 Collection and Extraction of Medicinal Plants

Fresh leaves of *A. wilkesiana* were collected from Ufeh Street, Federal housing estate, Uyo, Akwa Ibom State. The plant was identified and authenticated by Mr. Etefia, department of Pharmacognosy and Natural Medicine, University of Uyo. The leaves were thoroughly washed under running tap water to remove debris and later air dried under shade at room temperature for 14 days. The dried samples were pulverized to powder using electric blender and stored in polythene bag. Approximately 8 kg of the powdered plant material was extracted by cold maceration method with ethanol and acetone and left for 72 hours with intermittent shaking. The plant extracts was filtered and then concentrated using rotary evaporator at 40 °C, and each extract was transferred into well labelled sterile glass vials and stored at 4 °C before use.

2.2 Determination of Polyphenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method [12]. Sample extract was dissolved in methanol (1 mg/mL). An aliquot of 0.5 mL of each plant extract (1 mg/mL) was mixed with 5 ml of Folin- Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v). The mixture was shaken slightly and allowed to stand at 22 °C for 5mins. After, 4 mL (75 g/L) of sodium carbonate (Na_2CO_3) was added, and the tubes containing the mixtures were allowed to stand for 30 min at 40 °C to develop colour. Absorbance was then read at 765 nm using the spectrophotometer. Results were expressed as Gallic acid equivalent in (mg/g) of extracts. All samples were analyzed in triplicate.

2.3 Determination of Vitamin A

Vitamin A was determined using Antimony trichloride method by AOAC, [13]. About 0.5 g of each extract was taken into a beaker. 10 mL of chloroform was added to the extract and tested with 1 mL antimony trichloride reagent to develop blue colour. Reading was done using UV spectrophotometer at 620 nm.

2.4 Determination of Vitamin C

The extraction of the ascorbic acid was carried out with 0.5 g of each sample in 50 mL of oxalic acid. The mixture was filtered to remove suspended particles. 0.1 g of diatomaceous earth was added to the filtrate and agitated for 15 minutes. The mixture was filtered and colour development was done with 2, 4 – dinitrophenyl hydrazine and absorbance read at 520 nm.

2.5 Determination of Vitamin E (Tocopherol)

This was determined spectrophotometrically using a modified standard method of AOAC [13]. 0.5 g of each of the plant extracts was extracted with 0.5 mL of ethanol and shaken for 1 minute. 3 mL of xylene was then added and centrifuged to separate extract. 1 mL of the extract was added to 1 mL

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of 2, 2'- dipyridyl reagent with 5 mL $FeCl_3$ and 5 mL of H_3PO_4 and an orange colouration was observed. The blank and standard (1 tablet of vitamin E) was prepared in the same way excluding the extract. Absorbance of test sample and standard sample was read at 539 nm.

3. Results and Discussion

The results obtained in this study are presented in Tables 1-3.

Table 1 The percentage yield of crude extracts of *Acalypha wilkesianna "godseffiana"*

Solvents	Weight of dried plant material (kg)	Weight of extract (kg)	% yield of extraction
Ethanol	8.0	0.532	6.65
Acetone	8.0	0.569	7.11

Table 2 Results of polyphenolic content of different extracts of *Acalypha wilkesianna*

Extracts	Total phenolic content (mg gae/g)
Acetone extracts	13.24±0.02
Ethanol extracts	15.862±0.023

Values are means of triplicate determination ±standard deviation

Table 3 Antioxidant vitamin contents of different extracts of *Acalypha wilkesianna*

Vitamins (mg/100 g)	Acetone extract	Ethanol extract
Vitamin A	0.812±0.45	0.040±0.024
Vitamin C	32.897 ± 0.264	79.595±0.926
Vitamin E	108.97 ± 0.79	19.07 ±0.53

Values are means of triplicate determination ±standard deviation

3.1 Percentage Yield

Table 1 shows the percentage yield of the different crude extracts (acetone and ethanol) of the leaf part of *Acalypha wilkesianna.* The acetone extract exhibited the highest yield (7.11%). The extract yields, polyphenolic contents, and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent and method, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Percentage yield may also depend on solvent concentration, extraction temperature and particle size of samples [14-16]. From the results obtained it is suggested that acetone may be a better solvent for the extraction of *Acalypha wilkesiana*.

3.2 Total Phenolic Content

Plant-derived antioxidants, especially polyphenolic compounds, have gained considerable importance due to their potential health benefits. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators as well as stabilizing of the resulting antioxidant-derived radicals [17-21]. The interest in utilizing natural antioxidants has increased in recent times because commonly used synthetic antioxidant agents such as butylated hydroxytoluene, rutin, Gallic acid and butylated hydroxyl anisole usually have adverse effects on living cells [22-23].

The results of the total phenolic content in the different extracts are presented in Table 2. From the results obtained, the total phenolic content was highest in ethanol extract. This variation in the phenolic contents of extracts may be attributed to the polarities of different solvents. The result of total phenolic content obtained in this study varied from 238.89±19.48% GAE and 9.44±4.76% GAE reported by Novia et al., [24] for methanol and chloroform leaf extracts of *Acalypha wilkesianna* respectively. Kingsley and Marshall, [25] carried out a quantitative estimation of bioactive compounds in the leaves of *Acalypha wilkesianna.* The result obtained revealed that the aqueous extracts contained 0.05 ± 0.01% phenolics while ethanol and the powdered extracts contained 0.26 ± 0.01 and 0.25 ± 0.01% phenolics respectively, which was far less than what was obtained in this study. The Phenolic content, antioxidant and anti-denaturing activities of defatted and non-defatted methanol extract of *Acalypha wilkesiana*, *Cnidoscolus aconitifolius* and *Vernonia amygdalina* leaf were studied by Anokwuru et al., [26]. The result obtained indicated that the phenolic content of *Acalypha wilkesiana* was 285.9 ± 1.92 and 157.3 ± 0.35 mg GAE/g for defatted and non-defatted extracts respectively. Phenolic compounds are natural antioxidants that have the character of quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical. In addition, these compounds have anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities [27, 28], which substantiates the use of *Acalypha wilkesiana* leaves in the treatment of various illnesses.

3.3 Antioxidant Vitamins

Vitamins are biologically active organic compounds that are essential micronutrients involved in metabolic and physiological functions in the human body [29]. It has been reported that dietary antioxidant vitamins (A, C and E) work both or singly and synergistically to prevent or delay oxidative reactions that lead to degenerative disease, including cancers, cardiovascular disease, cataracts and other disease [30]. The results of antioxidant vitamin contents of the ethanol and acetone extracts of *Acalypha wilkesiana* are presented in Table 3. From the results obtained, acetone extract contained the highest concentration of Vitamins A and E, while vitamin C content was highest in ethanol extract. Vitamin C, a well-known antioxidant inhibits, minimizes and terminates the propagation of the free radicals by donating hydrogen and electron thus changing its structure from ascorbic acid to dehydroascorbic acid [31]. They also act synergistically with tocopherol to regenerate the tocopheryl radicals. Vitamin C is required for maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine [32]. It also helps in the development of bones, teeth and prevention of scurvy [33]. The recommended daily allowance of ascorbic acid is 60 mg (for adults) and 20 mg (for children) [34]. From the result obtained, the vitamin C concentration in ethanol extract was higher than the recommended daily allowance for both adult and children, while the concentration in acetone extract was lower than the daily allowance for adult but above the allowance for children. This indicated that *Acalypha wilkesiana* leaves are rich sources of vitamin C. Vitamin A is a natural antioxidant that inhibits free radicals and is essential for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions [35]. In this study, the highest value of vitamin A was seen in acetone extract. However, the concentration of this vitamin in both extracts was lower than the recommended dietary allowance (RDA) for vitamin A which is 900 µg. Vitamin E as an antioxidant protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [36, 37]. It exerts it antioxidant activity by donating the hydrogen or the hydroxyl group of its chroman ring to neutralize the free radical [38]. Vitamin E is vital to the formation and normal function of red blood cell and muscles [35]. The concentrations of vitamin E in this study exceeded the recommended dietary allowance of vitamin E which is15 mg/day of α – tocopherol. The considerable levels of these antioxidant vitamins in *Acalypha wilkesiana* leaves is indicative of the plant nutritional and therapeutic importance, thus the consumption of these leaves may contribute in meeting the daily vitamin requirement in human beings.

4. Conclusion

Polyphenolic and antioxidant vitamin contents in ethanol and acetone leaf extracts of *Acalypha wilkesiana* were determined. The results obtained in the present study revealed the presence total phenolic compounds and an appreciable content of vitamin C, vitamin E and low content of vitamin A in all the extracts, indicating that *Acalypha wilkesiana* leaves are promising source of natural antioxidants. This study further justifies both its nutritional and ethnomedicinal benefits to human health.

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