

Determination of Vitamin K1 in *Aspilia africana*, *Vernonia amygdalina*, *Chromolaena odorata*, and *Alchornea cordifolia* Extract

Ere Diepreye^{1*}, Eboh Abraham Sisein²

¹Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta, University, Bayelsa State, Nigeria

²Biochemistry Department, Niger Delta University, Bayelsa State, Nigeria

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*Corresponding author

Ere Diepreye

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Abstract: A colorimetric method was used for the determination of vitamin K1 phyloquinone in different plant extracts (*Alchornea cordifolia*, *Aspilia Africana*, *Chromolaena odorata*, *Vernonia amygdalina*). Benzene was used to extract the plants after drying and milling. The method of vitamin k determination utilizes the coupling of 2,4-dinitrophenylhydrazine, in which a hydrazone of vitamin K, forms acid-salts in basic media giving blue coloured solutions that absorbs maximally at 635 nm. The results showed that *Aspilia Africana* 2.43 ± 1.56, *Vernonia amygdalina* 2.12 ± 1.11, *Chromolaena odorata* 6.36 ± 2.09, *Alchornea cordifolia* 8.31 ± 1.23 ug/g dry extract

Keywords: *Aspilia africana*, *Vernonia amygdalina*, *Chromolaena odorata*, And *Alchornea cordifolia* Extract, Vitamin K1.

INTRODUCTION

Vitamin K is the family name for a series of fat-soluble compounds which have a common 2-methyl-1,4-naphthoquinone nucleus but differ in the structures of a side chain at the 3-position. They are synthesized by plants and bacteria. In plants the only important molecular form is phyloquinone (vitamin K1), which has a phytyl side chain. Bacteria synthesise a family of compounds called menaquinones (vitamin K2), which have side chains based on repeating unsaturated 5-carbon (prenyl) units. These are designated menaquinone-n (MK-n) according to the number (n) of prenyl units. Some bacteria also synthesise menaquinones in which one or more of the double bonds is saturated. The compound 2-methyl-1,4-naphthoquinone (common name menadione) is widely used in animal husbandry as a cheap provitamin source of dietary vitamin K [1].

Vitamin K₁, the precursor of most vitamin K in nature, is a stereoisomer of phyloquinone, an important chemical in green plants, where it functions as an electron acceptor in photosystem I during photosynthesis. For this reason, vitamin K₁ is found in large quantities in the photosynthetic tissues of plants (green leaves, and dark green leafy vegetables such as romaine lettuce, kale and spinach), but it occurs in far smaller quantities in other plant tissues (roots, fruits, etc.). Iceberg lettuce contains relatively little. The function of phyloquinone in plants appears to have no resemblance to its later metabolic and biochemical functions (as "vitamin K") in animals, where it performs a completely different biochemical reaction [2].

Due to its fat-soluble properties, vitamin K is largely stored in the liver of adults and children. Only a small amount (approximately 1 µg/kg/day) is required in the diet. Sources of vitamin K include green leafy vegetables, vegetable oils, and cereal grains [3]. However, within neonates, a relatively small amount of vitamin K is found in the liver compared to other fat-soluble vitamins [3].

The highest concentrations of phyloquinone (in the range of 400–700 µg/100 g) are found in green vegetables corresponding to its known association with photosynthetic tissues. The next best sources are certain vegetable oils (e.g., soybean, rapeseed, and olive oils) with phyloquinone contents in the range of 50–200 µg/100 g. Other vegetable oils, such as peanut, corn, coconut, groundnut, sun flower and safflower oils, have much lower contents of phyloquinone in the range of 1–10 µg/100 g. Many food items in different food categories such as fruits, grains and dairy products also contain phyloquinone in the range of 1–10 µg/100 g [2].

Plants and their metabolites are the primary sources of novel bioactive compounds and are attractive alternative therapeutic options to synthetic drugs. The use of medicinal plants in health care is now a common practice [4].

Chromolaena odorata (L.) is an ornamental plant usually considered to be one of the top 100 most invasive environmental weeds of wastelands, roadsides

and other exposed areas in the world [5]. This flowering shrub is native to North and Central America, and was later introduced to parts of Asia, Africa and Australia. *C. Odorata* is also known by various other names such as Armstrong's weed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed and trifid weed [6]. It possesses insecticidal properties and is used as a green manure. It is also used for the preservation of dead bodies [7]. The fresh leaves of *C. odorata* or the decoction has been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, post-natal wounds and also for the treatment of leech bites, indigestion and skin infection [8].

Aspilia africana C.D.Adams (Compositae), a herb about 1 m tall covered with bristles and commonly known as "haemorrhage plant" due to its ability to stop bleeding from fresh wounds. In Nigeria, it is variously known as "Orangila" in Igbo, "Tozalin" in Hausa, "Yunyun" in Yoruba and "Edemedong" in Efik [9] and widespread in Africa. In wound care in herbal medicine, the bruised leaves and flowers of *A. africana* are used to clean the surfaces of sores with subsequent healing [10]. In addition, it is also used for the treatment of rheumatic pains as well as bee and scorpion stings [11].

Vernonia amygdalina is a multipurpose and rapid regenerating soft wooded shrub of 2 to 10 m tall with petiolate leaves of around 6 mm in diameter. *V. amygdalina* can be commonly found along drainage lines and in natural forests or at home and commercial plantations. *V. amygdalina* is a common homestead farming vegetable and fodder tree in Nigeria [12] and has been used as an ingredient to prepare Nigerian (Ogbono soup) or Cameroon (Ndole) dish after removal of its bitter taste through soaking in several changes of water or by boiling [13].

Alchornea cordifolia is a plant which is found growing along water courses in many parts of Africa, especially West Africa, is used as an ingredient in several well-known remedies. The powdered leaves of the plant are used in the treatment of wounds, ulcers and sores, and it is also taken with palm wine or lime in effecting a cure for gonorrhoea [14]. These uses suggest that *Alchornea cordifolia* possesses antibacterial activity. Evidence to support this has been provided by Ogunlana and Ramstad [15] who reported the activity of the methanolic extract of this plant against *Staphylococcus aureus*, *Staphylococcus albus* and *Escherichia coli*. The decoction of the leaves has also been reported to provide remedies for bronchial problems [14] suggesting smooth muscle relaxing properties.

Preparation of plant materials

The leaves of various plants *Alchornea cordifolia*, *Aspilia Africana*, *Chromolaena odorata*, *Vernonia amygdalina* were harvested in a farm near the Niger Delta University, Bayelsa State. The leaves were botanically identified at the Department of Botany, Niger Delta University, Nigeria. Thereafter, they were shade-dried for one week. The leaves were later milled into a fine particle size. The different plant extracts (20 g) were extracted with 200 ml of benzene for 3 h. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield benzene extract which was stored in the freezer for further analysis.

MATERIALS AND METHODS

Chemicals

Phylloquinone, 2, 4-dinitrophenylhydrazine, ethanol, hydrochloric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Reagents

The method of Novelli 1941 was adopted for the estimation of vitamin K1

1. Standard solution of phylloquinone (vitamin K1) .25 mg of phylloquinone in 100 ml of benzene.

Dinitrophenylhydrazine

50 mg of 2, 4-dinitrophenylhydrazine were dissolved in 20 ml of ethanol-hydrochloric acid. The latter was made from 2 ml of concentrated hydrochloric acid ($d = 1.9$) which are mixed with 31 ml of absolute alcohol.

3. Ethanolic ammonia solution. (A solution of equal parts of ethanol (96%) and concentrated ammonia, $d = 0.90$).

Determination of the Calibration Curve

Dilute solutions are made from the standard solution of phylloquinone in benzene so that they contain 5, 7.5, 10, 12.5, 15, 20, μg vitamin K, per ml. Each solution is then tested as follows: 2 ml of the vitamin solution are pipetted into a little flask. 1 ml of 2,4-dinitrophenylhydrazine solution is then added, followed by 2 ml of absolute alcohol. The mixture is then heated under reflux for 80 - 90 minutes for 80°C , and then blown dry in a current of air at $40-50^{\circ}\text{C}$. The residue is taken up in warm alcohol and washed into a 25 ml measuring flask, in which 2.5 ml of alcoholic ammonia have already been introduced. The mixture is then allowed to cool slowly to room temperature; meanwhile, a blank was made for calibrating the colorimeter. For this purpose 1 ml of hydrazine solution was placed in a 25 ml measuring flask, 2.5 ml of alcoholic ammonia was added and the flask was filled up with alcohol. It was not necessary to heat the contents in this case. The blue colour was measured at 635 nm.

Analysis of the plant sample

2 ml of the benzene extract was coupled with 1 ml of 2,4-dinitrophenylhydrazine solution and followed by 2 ml of absolute alcohol. The mixture was then heated under reflux for 80 - 90 minutes for 80^o C, and then blown dry in a current of air at 40-50^o C. The residue was taken up in warm alcohol and washed into a 25 ml measuring flask, in which 2.5 ml of alcoholic

ammonia have already been introduced. The mixture is then allowed to cool slowly to room temperature. The blue colour is measured at 635 nm and the content of vitamin K3 calculated and expressed in ug/g dry extract [16].

RESULTS**Table-1: Vitamin K1 contents of different extracts.**

Plant Extract	Concentration (ug/g)
Alchornea cordifolia	8.31 ± 1.23
Aspilia Africana	2.43 ± 1.56
Chromolaena odorata	6.36 ± 2.09
Vernonia amygdalina	2.12 ± 1.11

Each experiment was repeated three times. Results have been expressed as mean ± standard deviation.

DISCUSSION

Vitamin K is a substance that our body needs to form clots and to stop bleeding. Vitamin K₁, also known as phyloquinone, is made by plants, and is found in highest amounts in green leafy vegetables because it is directly involved in photosynthesis. It may be thought of as the plant form of vitamin K. It is active as a vitamin in animals and performs the classic functions of vitamin K, including its activity in the production of blood-clotting proteins. Animals may also convert it to vitamin K₂ [1].

The results of this study showed that the amount of vitamin K in Alchornea cordifolia > Chromolaena odorata > Aspilia Africana > Vernonia amygdalina as depicted in table 1 above. Therefore these plants can serve as nutraceuticals for the management of bleeding

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