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PHARMACOGNOSTIC STUDIES ON THE LEAVES OF *VERNONIA AMYGDALINA* DEL. (ASTERACEAE)

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ABSTRACT

Macroscopical, microscopical and chemo-microscopical studies have been carried out on the leaves of *Vernonia amygdalina* Del. of the family Asteraceae. Anomocytic stomata (in the upper and lower epidermis), calcium oxalate crystals (rosette and prism type), starch grains and covering trichomes were identified. Quantitative- leaf microscopy and extractive value evaluation of the powdered leaves were also carried out. These could serve useful in the identification of this plant species.

INTRODUCTION

Vernonia amygdalina Del. of the family Asteraceae, is a small tree between 1 and 3 m in height, commonly found throughout African countries including Nigeria and a number of others [1]. The tops of this plant are used in folk medicine as antihelmints, laxatives and fertility inducers in barren women, also in Tanzania, some wild Chimpanzees were observed to use it for the treatment of parasite related diseases [2]. Also leaves of this plant were found to be of nutritional importance [3].

In Nigeria, the plant is used as vegetable and as spices in the popularly known bitter-leaf soup and in Northern Nigeria Hausa people call it "Shiwaka" and its water extract is taken to prevent certain illnesses such as stomach ache [4]. Phytochemical screening of the plant revealed the presence of steroid, in the entire plant, sesquiterpenes in the leaves; fruits and flowers and also tannins, as well as flavonoids in the leaves [5, 6].

Chemical studies were carried out on some Nigerian vegetables, among which was this plant species. These studies revealed that, this plant species was of nutritional value worthy of consideration. This was because its mineral contents were believed to aid in the maintenance of tissue integrity [7]. This study is intended to establish, macroscopical, microscopical, chemo-microscopical, quantitative-leaf microscopical characters as well as quantitative evaluation of the powdered and fresh leaves of the plant to be used as diagnostic features in the identification, evaluation and monograph preparation of the plant.

RESULTS

The macro- morphological features of the leaves of *V. amygdalina* Del. identified were acute apex, serrated margin, symmetrical base, lanceolate shape, reticulate venation, pubescent surface, petiole (1.0 - 2.0 - 2.5 cm), with dimension of 8.0 – 12.0 – 12.5 cm x 3.0 – 5.0 – 5.9 cm. Organoleptically, the leaves were greenish, strongly bitter and have characteristic odour. Micro-morphologically, features of the fresh and powdered leaves were anomocytic stomata (2.8 – 3.5 – 4.0 μm) in the upper and lower epidermis but were more in the lower epidermis. Other features observed were phloem fibers (6.8 – 7.5 – 8.5 μm), multicellular covering trichomes (7.5 – 8.8 – 12.9 μm), fragments of lamina with palisade cells, fragment of midrib portion with conducting elements and a number of calcium oxalate crystals both the rosette (2.0 – 2.5 – 3.5 μm) and prism (1.5 – 1.8 – 2.5 μm) forms, with the rosette form being more numerous and starch grains (3.5 – 3.7 – 3.8 μm). The various longitudinal and transverse sections of the leaves (fresh) through the midrib and petiole showed exact position and arrangement of the various tissues present in the leaves of this plant species.

The results of the quantitative-leaf microscopy were palisade ratio (4.0 – 4.1 – 4.2), stomatal numbers (256 – 265.2 – 270 and 305 – 312.1 – 315) and stomatal indices (18.9 – 19.1 – 19.2) and 38.1 – 38.2 – 38.5) for the upper and lower epidermis respectively. Vein-islet number was 5.0 – 6.2 – 7.0 and vein-let termination number was 10.0 – 10.7 – 12.0.

Chemo-microscopically, features identified include calcium oxalate crystals, cellulose, lignin, lipids, starch, tannins, cutin and mucilage. Quantitatively, moisture content was 4.0 – 4.5 – 4.8, total ash was 10.5 – 11.0 – 11.5, acid-insoluble and water-soluble ash values were 1.0 – 1.5 – 1.8 and 4.2 – 4.5 – 5.0 respectively. Alcohol extractives were 13.5 – 14.0 – 14.6 where as water extractives were 22.0 – 22.5 – 23.5.

DISCUSSION

Transverse section of the fresh leaf through the midrib showed that, the plant has a nodal vascular structure and this conforms with the literature of the family Asteraceae which stated that, the most common type of nodal vascular structure among dicots; occurring in many primitive as well as advanced families such as the Rosaceae, Compositae (Asteraceae), Fagaceae and a number of others, is the three trace-trilacunar type as reported by [12]. The occurrence of anomocytic stomata both abaxially and adaxially, is of diagnostic importance; likewise results obtained on the average, from the quantitative leaf microscopy as palisade ratio (4.1), stomatal numbers (265.2 and 312.1) for the upper and lower epidermis respectively.

The chemo-microscopical examinations confirmed the presence of tannins and possibly other phenolic compounds, and this was supported by the literature that indicated, the presence of flavonoids with characteristic antioxidant and antimicrobial activities and its saponins (bitter active constituents) and these supported the use of the plant by the wild Chimpanzees to treat parasite-related diseases by chewing the plant to obtain the bitter tasting constituents [5]. The presence of calcium in form of oxalate testified the literature that, the plant has small/ low amount of soluble calcium in which it was noted that, plant growing on low calcium soil contained little soluble calcium. Hence, the presence of oxalic acid rather than malate, in these species ensures that any calcium that does not find its way into cell sap is precipitated as oxalate salt. On this basis, it was suggested that plant families as Umbelliferae, Compositae (Asteraceae) and others are regarded as potassium type because of their high content of potassium when compared with the presence of calcium [13]. The presence of both major and minor elements in the plant as well as oxalate and cyanide (minute amount) confirmed the plant to be of nutritional importance, as it is used traditionally as both vegetable and spices in the popularly known bitter-leaf soup [3]. These evaluative values could be used in differentiating and identifying the plant. The moisture content (4.5% w/w) seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. The total ash that was coming from the plant tissues was reasonably high whereas the corresponding acid-insoluble ash happened to be small, to indicate the physiologic state of the plant. Both water and alcohol soluble extractive values were also, reasonably high to indicate the solubility of the active constituents in these extractive solvents. These could be seen when compared with certain results obtained in [8,11].

Based on these pharmacognostic studies carried out, the results obtained could reasonably aid in the identification and evaluation of *Vernonia amygdalina* Del. of the family Asteraceae, in crude drug processing in due curse.

MATERIALS AND METHODS

Plant Collection and Identification.

V. amygdalina Del. (Asteraceae) was collected in May, 1998 from Samaru, Zaria, Nigeria. The collected plant material was authenticated by the taxonomists (M. Musa and U.S. Gallah) at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Its voucher specimen's number is 675.

Macroscopical Examinations

The macro- morphological features of the plant parts (leaves) were observed under magnifying lens. They were described using the terminologies in [8].

Microscopical Examinations

Fresh leaves of the plant were studied transversely and longitudinally, using surface preparations and sections. They were cleared with chloral hydrate and mounted on the microscope slide with dilute glycerol. Quantitative evaluations and quantitative-leaf microscopy were also carried out as outlined by [8, 9].

Chemo-microscopical examinations were also carried out, following thorough clearing of the powdered leaves with chloral hydrate solution and a subsequent mounting with dilute glycerol on a microscope slide, and tested with various detecting reagents. Various cell inclusions/ chemical constituents were identified in accordance with [10,11].

Quantitative Evaluations of the Crude Vegetable Drugs (powdered leaves)

Moisture content of the powdered leaves was determined based on the loss on drying method. The ash values (acid-insoluble ash and water-soluble ash) were determined, to find out about the physiological state and level of extraneous matter. Solvent extractives (alcohol and water) were determined as described in [8].

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