PHARMACOGNOSTIC STUDIES ON THE LEAVES OF AMARANTHUS VIRIDIS LINN. GROWING IN NIGERIA

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ABSTRACT

Amaranthus viridis Linn. is of the family Amaranthaceae. It is an annual herb, nearly one (1) meter high, occurring throughout the West African region and the tropics. It is widely used in Traditional Medicine as diuretic and emollient, in poultices on inflammations, boils or abscesses, as well as in the treatment of gonorrhea, piles, fever, filaria, dysentery etc. This work focused on the evaluation of various Pharmacognostic parameters including microscopic, chemomicroscopic and physicochemical properties of the leaves, as well as the phytochemical constituents based on the 70% aqueous ethanol leaf extract, of the Nigerian plant. The microscopy of the leaf revealed the presence of smaller slightly wavy-walled epidermal cells on the upper surface and larger wavy-walled epidermal cells on the lower surface, with both containing anisocytic stomata; non-glandular and glandular trichomes with unicellular head which are multicellular and uniseriate containing four cells, on both surfaces; rosettes of calcium oxalate crystals within the spongy mesophyll and around the vessels; spiral and annular vessels and a transverse section that shows it to be a dorsiventral (with distinct upper and lower surfaces) leaf type, as well as the presence of lignin, tannins, starch, proteins and calcium oxalate crystals in different specific locations within the leaf. The physicochemical properties as observed include: moisture content of 12.19 ± 0.06 % w/w; total ash value of 19.92 ± 0.10 % w/w; acid-insoluble ash value of 1.07 ± 0.08 % w/w; water-soluble ash value of 5.97 ± 0.06 % w/w; ethanol – soluble extractive value of 1.71 ± 0.26 % w/w and water – soluble extractive value of 2.7 ± 0.36 % w/w. Preliminary phytochemical screening showed the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides in the crude 70 % aqueous ethanol leaf extract The findings in this work established some Pharmacognostic standards for correct identification of the plant from among so many closely related species and/or varieties.

Keywords: Amaranthus viridis, Pharmacognostic, Microscopic, Chemomicroscopic, Physicochemical, Phytochemical.

INTRODUCTION

Amaranthus viridis Linn., syn., gracilis Desf., belongs to the family, Amaranthaceae. It is an annual herb, nearly one meter high, with a pan-tropical occurrence (Burkill,1985). It is a palatable vegetable in parts of Africa and Southern
India (Chopra et al., 1986) and also widely used in Traditional Medicine as in Asia and Africa (Burkill, 1985). The existence of so many species of the Amaranthaceae looking the same in physical appearance causing identification problems and varieties of the same plant species, due to agro-climatic conditions and locality factors has made this work necessary.

The Pharmacognostic evaluation, which includes macroscopic/organoleptic, microscopic, physicochemical and phytochemical investigations are meant to evaluate the quality and purity of crude drugs in order to establish the standard pieces of information that will form the monographs to be used for their correct identification (Evans, 2002). Khan et al (2011) have reported that the leaves of A. viridis growing in Pakistan have epidermal cells of both sides having polyhedral to hexagonal shapes with smooth walls, stomata of anisocytic type, vein termination number ranging from 44.65 to 57.25 per unit area, vein islet number ranging from 14.56 to 23.57 per unit area, palisade ratio ranging from 15.62 to 24.42 per unit area and stomatal indices of the upper and lower surfaces as 21.25 to 24.62 and 42.54 to 43.47, respectively. They have also reported that phytochemical and chemomicroscopical screenings of the leaves indicated the presence of alkaloids, saponins, starch, fat, protein and cellulose and the absence of tannins, anthraquinones, calcium oxalate and lignin. With respect to the Nigerian variety, only aspects of the microscopy of the leaves were reported by Alege and Daudu (2014), who showed that the mean stomatal number and stomatal indices for the upper and lower epidermises are 8.8000 and 10.0839, and 10.8330 and 16.9266, respectively. This study, therefore, focused on the Pharmacognostic evaluation of the leaves of the Nigerian plant, including the aspects of the microscopy of the upper and lower epidermises, transverse section and powder characteristics not reported, as well as physicochemical constants and phytochemical screening of the 70% aqueous ethanol leaf extract.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

The whole plant materials were collected, following descriptions from literature and with the assistance of a traditional medicine practitioner, from the Babale area of Jos North Local Government Area, Plateau State, Nigeria. The correct identity of the plant was later authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University (A.B.U.), Zaria, by Mallam U. S. Galla, with the specimen voucher number given as 335. A herbarium specimen of the plant was also prepared and deposited at the herbarium of the Department of Pharmacognosy, University of Jos. The leaves were separated from the other parts of the plant and shade-dried for about 2 weeks to ensure sufficient drying, by spreading them well on a flat drying surface in the laboratory, with adequate aeration, to prevent fungal growth. The well-dried leaves were pulverized using a blender and 150g of the powder was extracted by cold maceration in sufficient volume of 70% Ethanol, with the aid of a rotary shaker, for about 48 hours. Afterwards, the extract was concentrated or dried, with the aid of a rotary evaporator and kept in the refrigerator for further use, while the remaining powder was then stored in an airtight container, for further use.
Plate I: *Amaranthus viridis* Linn. growing in its natural habitat, Jos, Plateau State.

**Microscopy**

Carefully derived tiny sections of the fresh leaf, including: transverse sections of the lamina with the midrib, other portions of the whole leaf as well as the leaf margin and surface preparations of both epidermises, as well as small quantities of the leaf powder were cleared using few drops of chloral hydrate solution with gentle heating. These were then mounted in dilute glycerol and observed under a compound microscope using suitable magnifications (x100 and x400) (Evans, 2002; Kokate, 1994; Wallis, 1985) and photomicrographs taken using a microscope digital camera (FotoWinJoe®).

**Chemomicroscopy**

Anatomical sections of leaf and powder were variously treated (mounted) with appropriate chemical reagents on microscope slides and observed under the microscope for the presence and tissue distribution of chemical substances as follows: phloroglucinol plus conc. hydrochloric acid for lignin, ferric chloride solution for tannins, sudan iv solution for oil, N/50 iodine solution for starch, millon’s reagent for proteins and chloral hydrate plus hydrochloric acid for calcium oxalate (Evans, 2002; Kokate, 1994).

**Physicochemical Studies**

The moisture content (Loss on drying), ash values and extractive values were determined using the leaf powder using standard procedures (Evans, 2002; B. P., 1993; Agrawal and Paridhavi, 2007).

**Phytochemical Screening**

The 70% aqueous ethanol leaf extract was subjected to preliminary chemical tests for the presence of various metabolites, including carbohydrates, tannins, flavonoids, alkaloids, cardiac glycosides, saponins, anthraquinones and steroids using standard
procedures (Sadasivam and Balasubramanian, 1985; Ibrahim, 1990; Evans, 2002; Sarker et al., 2006; Agrawal and Paridhavi, 2007; Sofowora, 2008).

RESULTS AND DISCUSSION

Microscopic Features of the Leaves

The findings from the microscopic examinations provide vital data to aid the correct identification and description of the plant, *A. viridis* Linn., growing in Nigeria. In this work, microscopy of the upper epidermis of the leaf, as revealed by the photomicrographs, showed the presence of slightly wavy–walled epidermal cells, glandular and non-glandular trichomes having uniseriate (multicellular) stalk with four cells and unicellular head, with waxy surface, as well as anisocytic type of stomata, being surrounded by about three epidermal cells with one being markedly smaller (Plates II, III and IV).

Examination of the lower epidermis also revealed the presence of anisocytic type of stomata, but it is composed of epidermal cells with wavy anticlinal walls, that seem to have larger surface areas than those of the upper epidermis (Plates V and VI) (Evans, 2002; Kokate, 1994). The lower epidermis also seem to contain more stomata than the upper epidermis, as reflected in the work by Alege and Daudu (2014). It should be noted that Khan et al. (2011), reported that the leaf has epidermal cells on both sides, having polyhedral to hexagonal shapes with smooth walls, as well as stomata of anisocytic type.

Plate II: Upper epidermis (EP1) with stomata (x 400)

Plate III: Upper epidermis (EP1) with trichome (x 400)
Plate IV: Upper epidermis showing non-glandular multicellular uniseriate trichome with warty surface (x 400)

Plate V: Lower epidermis (EP2) showing epidermal cells with wavy anticlinal walls and slightly larger surface area than the upper epidermal cells (x 400)

Plate VI: Lower epidermis with more numerous anisocytic stomata, surrounded by wavy-walled epidermal cells (x 400)

Plate VII: Transverse section of the leaf through the lamina and midrib, showing a dorsiventral leaf type with cuticle, upper epidermis, upper palisade layer, spongy mesophyll and lower epidermis (Ep1P1SmEp2), also including xylem and phloem vessels as well rosettes of calcium oxalate crystals, cleared with chloral hydrate, mounted in dilute glycerol and viewed under the compound microscope (Leica®) (x 100)
The transverse section of the leaf through the midrib and sections of the lamina revealed the presence of cuticle, trichomes, upper epidermal layer (Ep1), palisade layer (P1), parenchyma, vascular bundle, xylem vessels of spiral and annular types, collenchyma, spongy tissue (Sm) and lower epidermal layer ((Ep2)), showing the leaf type to be dorsiventral.

**Plate VIII:** Transverse section of leaf showing upper part of lamina with glandular trichomes, a stoma, layer of palisade cells, spongy parenchyma and an annular vessel (x 400)

**Plate IX:** Transverse section of leaf showing lower part of the lamina close to the midrib, with glandular trichomes, vascular bundle and calcium oxalate crystal sheath (x 400)

**Plate X:** Transverse section of leaf around the midrib, showing spongy mesophyll and vascular bundle with spiral and annular vessels (x 400)

**Plate XI:** Transverse section of leaf around the midrib showing lower epidermal layer, spongy parenchyma, spiral vessel surrounded by bundle sheath cells, annular vessels and rosettes of calcium oxalate crystals (x 400)
Plate XII: Transverse section of leaf showing vascular bundles with both spiral and annular vessels (x 400)

Plate XIII: Leaf powder showing single fibres with pointed ends (x 400)

Plate XIV: Leaf powder showing bundle and single fibres (x 400)

Plate XV: Leaf powder showing stomatal fragments (x 400)
Microscopy of the powder characteristics of the leaves showed the presence of single fibres, having narrow lumen and pointed ends (Plates XIII), bundle of fibres (Plate XIV) and stomatal fragments (Plate XV) (Evans, 2002; Kokate, 1994; Wallis, 1985). No previous published report on the powder characteristics of the leaf was seen. Hence, these findings will aid in the identification and standardization of the plant.

Chemomorphic Properties of the Leaves

The chemomicroscopy of the leaf, treated with various test reagents provide information with respect to the distribution of important chemical components within the leaf as a morphological part of the plant; which can be diagnostic.

**Table 1 : Chemomorphic features of A. viridis L. leaf powder**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Result</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>Present</td>
<td>Vessels and fibres</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
<td>Spongy tissues and fibres</td>
</tr>
<tr>
<td>Starch</td>
<td>Present</td>
<td>All through</td>
</tr>
<tr>
<td>Proteins</td>
<td>Present</td>
<td>Around vessels</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>Present</td>
<td>Scattered, but mainly in spongy mesophyll</td>
</tr>
</tbody>
</table>

The results, as summarized in Table 1 above, showed the presence of lignin, due to red colouration mostly within and around the vessels and fibres, for fortification. Tannins were also shown to be present, due to greenish colouration mostly around the calcium oxalate crystals and fibres. Tannins are known to be some of the most abundant secondary metabolites occurring in plants, across the families, for mostly defensive and protective purposes. Starch was observed to be present, due to blue colouration almost throughout the leaf structure, while proteins were indicated, due to blue-black colouration mostly within and around the vessels; but oil was not shown to be present. These attest to the nutritional value of *A. viridis* as a food vegetable. The presence of calcium oxalate crystals was also confirmed, and were mostly within the spongy mesophyll (Plates VII, XI and XII). The crystallization of excess calcium in this form, occurs to a large extent in plants generally, and the nature and type are often diagnostic. Khan *et al* (2011) reported that the leaves of *A. viridis* also contain starch, protein and fat, but lacks lignin and tannins. The difference may be attributed to differing climatic and soil conditions, which can lead to phytochemical variations in plants, within families, genera or even species, especially, quantitatively. The findings here will be useful in building a standard monograph for the Nigerian plant.

**Physicochemical Properties of the Leaves**

Physicochemical evaluations carried out for *A. viridis* leaves, will be expected to be relatively constant, at least for the variety grown in this region. Hence, they are diagnostic for the purposes of correct identification and prevention adulteration or substitution. Values obtained were recorded as mean ± standard...
error of mean (SEM). The moisture content (loss on drying) for the crude leaf drug powder of *A. viridis* (2g) was determined as 12.11 ± 0.06 % w/w (about 0.2g) (Table 2), while that of the whole herb (100g) of the plant was reported to be 87.90% (Sharma *et al*, 2012). Other parts like stem and roots, should be more moisture retaining; usually taking longer periods to dry. Baral *et al* (2011) have reported a moisture content (loss on drying) of 0.3g for *Amaranthus spinosus* leaves. The pharmacopoeial limit for dry herbal drugs is 14 % (African Pharmacopoeia, 1986). This implies that, the moisture content of this drug is minimal and could guarantee the stability of active chemical constituents, as well as minimize the tendency of microbial spoilage during storage. As a result, this drug can be said to have the potential of remaining in stable conditions on long term storage. The value of the moisture content will be expected to be relatively constant for the drug grown in this region.

Table 2: Physical (Numerical) Constants for *A. viridis* Leaf

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value (Average) % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content</td>
<td>12.19 ± 0.06</td>
</tr>
<tr>
<td>Total Ash</td>
<td>19.92 ± 0.10</td>
</tr>
<tr>
<td>Water-soluble Ash</td>
<td>5.97 ± 0.06</td>
</tr>
<tr>
<td>Acid-insoluble Ash</td>
<td>1.07 ± 0.08</td>
</tr>
<tr>
<td>Ethanol-soluble Extractive</td>
<td>1.71 ± 0.26</td>
</tr>
<tr>
<td>Water-soluble Extractive</td>
<td>2.17 ± 0.36</td>
</tr>
</tbody>
</table>

Ash values are useful in determining the quality and purity of crude drugs, especially in the powdered forms (Kokate, 1994). In this work, the total ash value was determined as 19.92 ± 0.10 % w/w (Table 2), which represents both ‘physiological ash’ (ash from the plant tissue) and ‘non – physiological ash’ (ash from extraneous matter) (African Pharmacopoeia, 1986). The total ash value of a crude drug reflects the degree of care taken in its preparation. The physiological ash usually consists of the carbonates, phosphates, silicates, nitrates, sulphates and chlorides of metal elements taken up by the plant during its growth. Elements taken up by plants from the soil include sodium, potassium, calcium, magnesium, cobalt, iron, manganese, carbon, phosphorus, nitrogen, sulphur, oxygen, chlorine and silicon (Shellard, 1958). The result here means that, 2 g of this plant crude drug (leaves), contains only about 20 % of residual substances, including impurities, that do not volatilize when ignited. Sharma *et al* (2012) have reported that the ash content of the whole herb (100g) of *A. viridis* that was washed free of debris before drying was found to be 1.85%. The variation could also be attributed to the presence of non-physiological debris on the plant used here. Baral *et al* (2011) also reported a total ash value for *Amaranthus spinosus* leaves as 14.9% w/w. The water–soluble ash value was found to be 5.97 ± 0.06% w/w (Table 2), which is composed of physiological aspects or parts of the plant tissue where the active constituents reside. That of *Amaranthus spinosus* was found to be 7.3% (Baral *et al*, 2011). The acid – insoluble ash value was determined as 1.07 ± 0.08 % w/w (Table 2), while that of *Amaranthus spinosus* was found to be 2.4% (Baral *et al*, 2011) and represents silica/sand (non – physiological...
A high acid – insoluble ash in leaf drugs indicates contaminations with earthy material (impurities). The result obtained is favourable, because it shows that impurities make up only about 1% of the 2 g of the drug powder; which becomes very significant, especially where the drug is to be used directly as the powder, as observed in traditional medicinal practice (Evans, 2002).

The determination of extractive values helps in measuring the amount of chemical constituents in a drug, that are extractable by a chosen solvent (water or alcohol) under specified conditions. It helps to also tell the chemical nature of the contained constituents (their degree of polarity) and the choice of the most suitable solvent for extraction and phytochemical studies. The evaluation is important as it aids to standardize the drug and guard against wrong identification and adulteration or substitution with other drugs. The result of this work shows that, the ethanol – soluble extractive and the water – soluble extractive values are 1.71 0.26 % w/w (Table 2) and 2.17 0.36 % w/w (Table 2), respectively. This shows that the leaves of *A. viridis* contain more of water – soluble (very polar) constituents. Baral *et al* (2011) reported that the ethanol – soluble extractive and the water – soluble extractive values for *Amaranthus spinosus* (its Genus relative) are 1.601 and 1.811, respectively. In both cases water appears to be a better solvent for the constituents. No previous published report of the physicochemical properties of *Amaranthus viridis* leaves was seen. These findings will be useful for a standard monograph for the plant, which will also aid in the detection of adulteration or substitution.

**Phytochemical Properties**

Phytochemical screening, which involved preliminary chemical tests of the crude 70% aqueous extract of the leaves of *A. viridis* growing in Nigeria, would confirm the chemotaxonomic linkage with other members of the Amaranthaceae family, and also establish the phytochemical profile for its identity and standardization, as well as provide the basis for its traditional medicinal applications.

**Table 3: Results of Preliminary Phytochemical Screening of EtOH of the Leaves of A. viridis L.**

<table>
<thead>
<tr>
<th>Constituent/Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molisch’s</td>
<td>+</td>
<td>Carbohydrates present</td>
</tr>
<tr>
<td>Barfoed’s</td>
<td>+</td>
<td>Monosaccharides Present</td>
</tr>
<tr>
<td>Fehling’s</td>
<td>+</td>
<td>Free reducing sugars Present</td>
</tr>
<tr>
<td>Combined reducing sugars</td>
<td>-</td>
<td>Combined reducing sugars absent</td>
</tr>
<tr>
<td>Pentose</td>
<td>+</td>
<td>Pentose Present</td>
</tr>
<tr>
<td>Selivanoff’s</td>
<td>-</td>
<td>Ketoses absent</td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayer’s</td>
<td>++</td>
<td>Alkaloids Present</td>
</tr>
<tr>
<td>Dragendorff’s</td>
<td>++</td>
<td>Alkaloids Present</td>
</tr>
<tr>
<td><strong>Saponins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frothing</td>
<td>++</td>
<td>Saponins Present</td>
</tr>
<tr>
<td>Haemolysis</td>
<td>++</td>
<td>Saponins Present</td>
</tr>
</tbody>
</table>
Tannins
Ferric chloride ++ Phenols present
Lead subacetae ++ Tannins present
Gelatin-salt ++ Tannins present
Phlobatannins ++ Condensed tannins present

Flavonoids
Lead acetate ++ Flavonoids present
Sodium hydroxide ++ Flavonoids present
Shinoda ++ Flavonoids present

Steroids
Salkowski’s + Steroids present

Anthraquinones
Borntrager’s - Anthraquinones absent
Combined anthraquinones - Anthraquinones absent

Cardiac glycosides
Lieberman-Burchard’s ++ Steroidal nucleus present
Keller-Kiliani’s ++ Deoxy sugars present
Kedde’s ++ Cardenolides present
Legal’s ++ Cardenolides present

Key:
++= Present in high amount
+= Present in low amount
-= Absent
EtOH = Aqueous Ethanol extract

The results, as shown in Table 3 above, indicated the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides in the crude 70% aqueous ethanol leaf extract of the plant. Ashok Kumar et al (2010), in their work, reported that preliminary phytochemical analysis showed the presence of carbohydrates, steroids, alkaloids, phenolic compounds, flavonoids, saponins and amino acids in the methanol extract of the whole A. viridis plant. Also, Krishnamurthy et al (2011) and Ahmed et al (2013) have reported the presence of tannins, saponins, alkaloids, proteins, glycosides and appreciable levels of total phenols and total flavonoids, in the methanol extract of the leaves. This shows substantial compliance, the use of different solvents of similar polarities and plant parts as well as locality differences notwithstanding, and lays credence to the fact that flavonoids and saponins commonly occur in this plant and its relatives of the Amaranthaceae (Ashok Kumar et al, 2009; Muller and Borsch, 2005; Ghani, 2003; Olufemi et al, 2003). Thus, the findings here will, help in building the phytochemical profile of this plant in the monograph, give credence to its various traditional medicinal uses and brighten the prospects for new drug development following possible bioassay guided isolation and purification.

CONCLUSION

This work has been able to establish some Pharmacognostic characters of the leaves
of *Amaranthus viridis* Linn. growing in Nigeria, including microscopic features of upper and lower epidermises, transverse section and drug powder, as well as diagnostic physicochemical and phytochemical properties, to serve as useful standards for its proper and correct identification.

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