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Pharmacognostic and phytochemical characterization of *Maerua angolensis* DC.

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***Maerua angolensis* DC is a medicinal plant widely used in ethnomedicine in northern Nigeria. It is used to treat disease conditions like skin infections, sexually transmitted diseases, peptic ulcers and wounds amongst others. The plant is well known in Fulani *Fulfude* as *leggal baali* (or *leggal mbaali*). The plant was subjected to pharmacognostic and physicochemical characterization to establish standard profiles for authentication of the plant which could be useful for further study on the plant. The chromatographic (TLC and HPLC) and phytochemical profiles were conducted along with the leaf microscopy and chemomicroscopy, using standard methods. The result established the chromatographic profile of the leaf extract. The qualitative phytochemical screening showed the presence of carbohydrates, saponins, anthraquinones and cardiac glycosides. The chemomicroscopy revealed the presence of lignin, cellulose, tannin, starch and oil, while mucilage and protein were not seen. The total ash content and moisture content were 12.1 and 7.0%, respectively and were within WHO limits. Extracts of the plant showed high hygroscopic character. The result provides good information for the authentication and use of the plant in further research and development.**

Key words: *Maerua angolensis*, pharmacognostic character; phytochemicals, chromatographic profile.

INTRODUCTION

Many natural products are used in alternative medicine (Sevindik et al., 2017; Mohammed et al., 2020a). Especially in folk medicine, different plant species have been used in the prevention and treatment of diseases (Mohammed et al., 2018; Mohammed et al., 2020b). The medicinal plant *Maerua angolensis* DC. belongs to the genus *Maerua* of the Capparaceae (Capparidaceae) family. Its synonyms include *M. bukobensis Gilg & Gilg-Ben., M. currorii Hook. f., M. emarginata Schinz, M. lucida Hochst. ex A. Rich., M. retusa Hochst. ex A. Rich., M. senegalensis R. Br. ex A. Rich., M. tomentosa Pax, and M. floribunda Fenzl*. It is known as *leggal baali* (or *leggal mbaali*) in Fulfude-Fulani.

M. angolensis is a tall tree that grows in tropical Africa

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and arid regions. It is widely distributed in the savannah region of tropical Africa to South Africa and Swaziland. It is absent from the high-rainfall areas. The tree size varies from medium to big, growing up to 10 to 20 m high. It is commonly found growing in bush and rocky areas, and planted on graves in Nupe area of Nigeria (Ayo et al., 2013). *M. angolensis* has a long history of use in traditional medicine especially in Nigeria and other West African countries where it is used as antidote for pains and wounds (Iliya et al., 2014). A hydroethanol extract yields of 17.6, 7.1 and 10.4% w/w d.m have been reported for the leaf, root and stem bark, respectively (Burkil, 2004). The parts commonly used in ethnomedicine, solely or in combination, are leaf, root, leaf and branches, or leaf and root (von Maydell, 1990; Tropical Plants, 2020). It is propagated by seed (Yusuf et al., 2017). The leaves are analgesic, and used either alone or with other plants, to treat a range of stomach troubles. The powdered leaves, taken with food, are prescribed for asthenia (weakness or loss of strength) and anorexia. The leaf is used to treat skin rashes and sexually transmitted disease. An infusion of the leaves is used to treat rheumatism (Yusuf et al., 2017). A snuff made from the leaves of *M. angolensis* and *Ximenia americana* L. is used to treat headaches. The leaf-sap is dropped into fresh wounds as an antiseptic dressing (Burkil, 2004; Yusuf et al., 2017; Tropical Plants, 2020). The whole plant is compounded as medicine for treating epilepsy (Benneh et al., 2018). The roots are used to treat hydrocoele, for influenza and for toothache. The root and bark decoctions are drunk as aphrodisiacs (Yusuf et al., 2017). The leaf, fruit and seed are used as sauces, condiments, spices, and flavourings. The leaf and root are used as pain-killers, and in arthritis, rheumatism, etc. The leaf is used in paralysis, convulsions and to manage psychosis, diabetes, peptic ulcer, diarrhea and spasm. The plant is also used to treat inflammation, cancer and cellular ageing (Meda et al., 2013). The leaves contain alkaloids, saponins, tannins, anthraquinones and flavonoids (Yusuf et al., 2017). It also contains carbohydrate, reducing sugars and cardiac glycosides (Meda et al., 2013; Ayo et al., 2013). The variety in Tanganyika in Tanzania was found to have alkaloids and saponin glycosides. Reported studies on the bark revealed glycosides, terpenes, tannins, flavonoids, saponins, carbohydrates, proteins, alkaloids and other constituents (Iliya et al., 2014).

Different solvent extracts of the leaf had been reported to exhibit antimicrobial activities (Benneh et al., 2018; Yusuf et al., 2017; Ayo et al., 2013). Yusuf et al. (2017) reported an activity of 200 µg/mL against clinical isolates of *Staphylococcus aureus* and *Escherichia coli* for the leaf ethanol-extract. A methanol extract of the leaf was found to be active against *S. aureus* (ATCC 13704), *Streptococcus pyogenes* (Local strain), *Corynebacterium ulcerans* (Local strain), *Bacillus subtilis* (NCTC 8230), *E. coli* (NCTC 10418), *Salmonella* Typhi (ATCC 9184), *Klebsiella pneumonia* (ATCC 10031), *Pseudomonas aeruginosa* (NCTC 6750), *Neisseria gonorrhoeae* (NCTC 10341) and *Candida albicans* (ATCC 10231) at 50 mg/m (Ayo et al., 2013). The broad antimicrobial activity is believed to be responsible for the wound healing properties of the plant and its use in infectious diseases in ethnomedicine (Ayo et al., 2013).

The plant has been demonstrated to exhibit strong antioxidant activity by Meda et al. (2013). Further studies suggested that the bark is non-toxic in anti-inflammatory doses, supporting ethnomedical use of the plant in managing inflammation (Meda et al., 2013; Ayo et al., 2013).

Although there have been reported scientific studies on some biological activities on the plant, not much has been documented on the pharmacognostic and phytochemical characteristics of the plant towards aiding its authentication. This study aims at establishing pharmacognostic parameters and chromatographic profiles which could serve as reference data for authenticating the plant.

METHODOLOGY

Collection of material

The raw plant sample was submitted to NIPRD on the 7th of February 2020. The sample was authenticated by both ethnobotanist and taxonomist at the herbarium unit of the Department of Medicinal Plant Research and Traditional Medicine of NIPRD, and a voucher specimen was prepared.

Powdered leaf sample of the plant was subjected to the various studies including microscopy and chemomicroscopic evaluation, physicochemical characterization and chromatographic profiling.

Extraction

The pulverized leaf was macerated in solvent over 24 h. The solvents used were absolute ethanol and water. The ethanol extract was filtered and concentrated with the aid of rotary evaporator and dried over a water bath. The water extract was also filtered and freeze-dried.

Chromatographic profiling

Chromatographic profiling was conducted using the ethanol extract. The TLC profiling of the sample was done using TLC glass plate pre-coated with silica gel G60 F254, 0.2 mm layer. The plate was developed using the mobile phase composition of ethylacetate/petroleum ether of 6/4. Detection was in daylight, and under UV light at 366 and 254 nm. The retardation factors (R_f) of each spot were calculated.

For HPLC profiling, the HPLC system used was Shimadzu HPLC system consisting of Ultra- Fast LC-20AB prominence equipped with SIL-20AC autosampler; DGU-20A3 degasser; SPD-M20A UV-Diode array detector (UV-DAD); column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto, Japan); column, VP-ODS 5 µm and dimensions (150 × 4.6 mm). The chromatographic conditions included mobile phase solvent A: HPLC grade water and solvent B: HPLC grade methanol; mode: isocratic; flow rate 0.8 ml/min;

Figure 1. Voucher specimen.

injection volume 2 µl of extracts solution (50 µg/ml) in the mobile phase; detection was at UV 254 nm wavelength. The HPLC operating conditions were programmed to give the following: solvent B: 20% and column oven (Adamu et al., 2020).

Phytochemical characterization

Qualitative phytochemical screening was carried out on the pulverized samples to test for carbohydrate, phenols, tannins, saponins, flavonoids and anthraquinones using standard methods as described by Evans (2005), Sofowora (1993), and Trease and Evans (1989).

Physicochemical characterization

Physicochemical parameters such as moisture content, total ash and water and alcohol extractive values were determined following African Pharmacopoeia (1986) and WHO Guidelines (1992).

Microscopy and chemomicroscopic evaluation

Chemomicroscopic studies of the pulverized leaf was done using reagents and stains like iodine, concentrated sulphuric acid (98%), concentrated hydrochloric acid (36%), ferric chloride, Sudan III, ruthenium red and phloroglucinol with conc. HCl (1:1) to test for the presence of lignin, cellulose, tannin, starch, oil, mucilage and protein. A quantity of the powdered sample was cleared in chloral hydrate, mounted in diluted glycerol on a microscope slide and viewed under the microscope at different magnifications (Ibrahim et al., 2015).

Elemental analysis

The powder leaf sample was subjected to elemental analysis to determine the level of some heavy metals using atomic absorption spectrometer (AAS) following the method described by Association of Official Analytical Chemists (AOAC) (Egharevba et al., 2015; AOAC, 1995, 1980).

RESULTS AND DISCUSSION

Authentication

A voucher specimen number NIPRD/H/7100 was prepared and deposited at NIPRD herbarium. The photograph of the voucher specimen is as shown in Figure 1. The plant was phenotypically identified as *M. anglolensis* after comparison with literature information from Tropical Plants (2020) and Burkil (2004).

Microscopic profiling of the leaf powder

The photomicrograph of the microscopic evaluation is as depicted in Figure 2. The characteristics of epidermal cells, trichomes, paracytic and anomocytic stomata, and the presence of prismatic calcium oxalate crystals, and presence of fibres amongst others, can also be used as diagnostic features for the authentication and standardization of the plant samples in relation to members of the same family (Adeshina et al., 2008; Chukwunonye et al., 2017; Olotu et al., 2018).

Chemomicroscopy

The results of chemomicroscopic evaluation of *M. angolensis* are shown in Table 1. The plant showed the presence of lignin, cellulose, tannin, starch and oil, but mucilage and protein were not detected.

Phytochemical characterization

Phytochemical screening result revealed the presence of carbohydrates, saponins, anthraquinones and cardiac glycosides, while phenols, flavonoids, tannins, and alkaloids were absent (Table 2). This did not correspond completely with earlier report of Ayo et al. (2013) and Yusuf et al. (2017) as flavonoids and alkaloids were not detected in this sample. Yusuf et al. (2017) reported the absence of anthraquinones. These phytochemicals act separately or additively and synergistically to elicit the observed pharmacological effects in living organisms (Chandra et al., 2012; Richards et al., 2016).

Physicochemical parameters

The total ash content was 12.1±0.1%, while the moisture content was 7.0±0.0%. These values are within the WHO limits of 8.0 and 15.0% for total ash content and moisture content, respectively (African Pharmacopoeia, 1986). The total ash is indicative of the amount of inorganic mineral salts that may be present. The moisture content is indicative of the residual or retained moisture after drying for storage. A high moisture content will promote microbial growth and early spoilage of the stored materials. A less moisture content keeps the material microbiologically safe (Murali, 2014).

Extraction characteristics

The extraction yields of the ethanol and water extraction

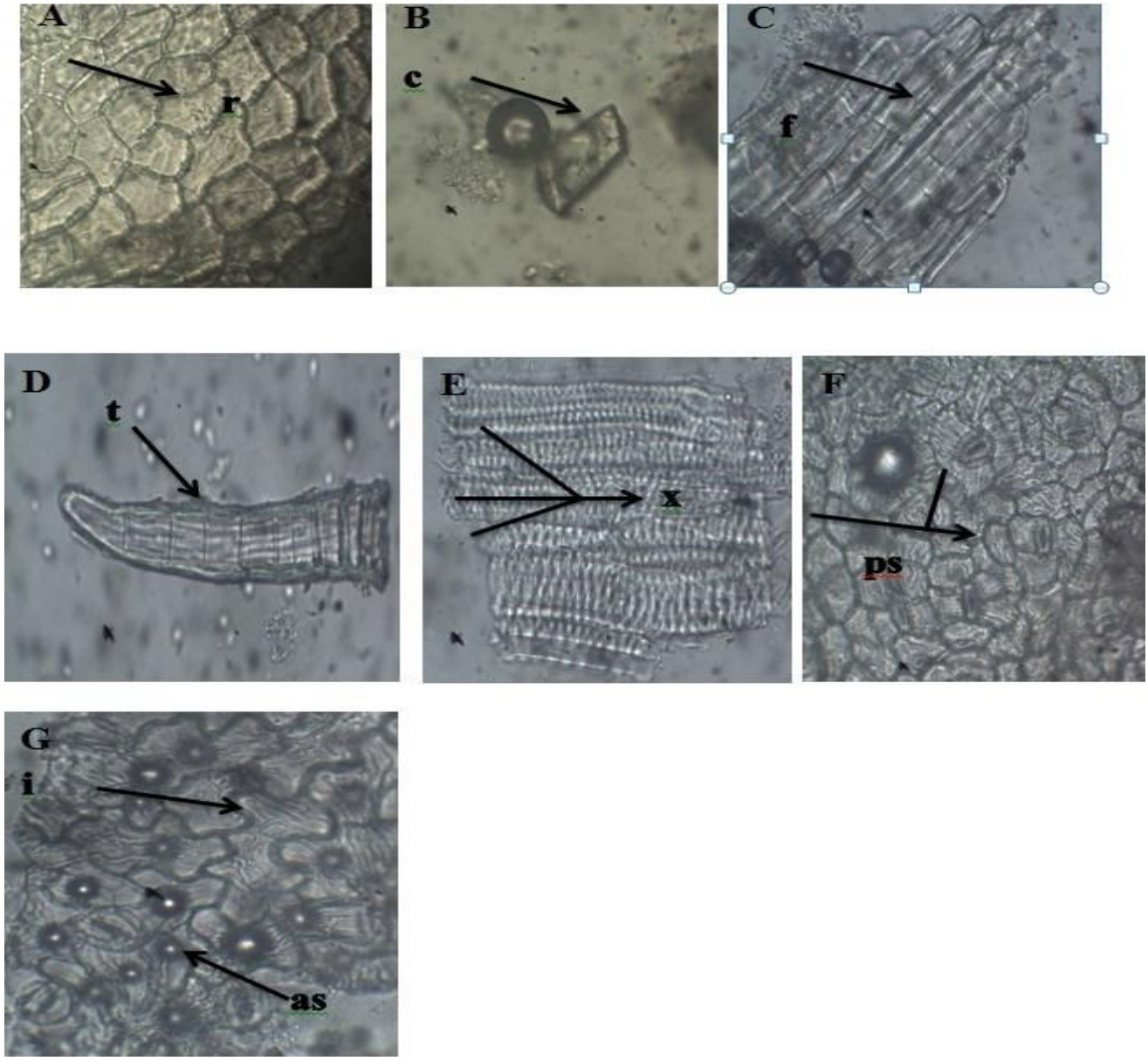

Figure 2. Microscopy of leaf powder (x400): 'A' showing regular/polygonal epidermal cell (r); 'B' showing prism calcium oxalate crystal (c); 'C' showing fiber (f); 'D' showing trichome (t); 'E' showing xylem vessels (x); 'F' showing paracytic stomata and 'G' showing anomocytic stomata (as) and irregular/wavy epidermal cell (i).

Table 1. Results of chemomicroscopic evaluation of *M. angolensis*.

Parameter	Results
Lignin	+
Cellulose	+
Tannin	+
Mucilage	-
Starch	+
Oil	+
Protein	-
Calcium oxalate crystal	+

Table 2. Results of phytochemical characterization.

Parameter	Results
Alkaloids	-
Anthraquinones	+
Carbohydrates	+
Cardiac glycosides	+
Flavonoids	+
Phenols	-
Tannin	+
Saponin	+

Table 3. Retardation factors (Rf) and colour of TLC in daylight, 366 and 254 nm.

Rf value	Daylight	UV 366	UV254
0.92	Light yellow	No colour	Yellow
0.75	Pale green	Pink	Grey
0.69	Light yellow	No colour	Yellow
0.65	Pale blue	Dark pink	Grey
0.59	Greenish yellow	Pink	Yellow
0.46	Green	Light pink	Yellow

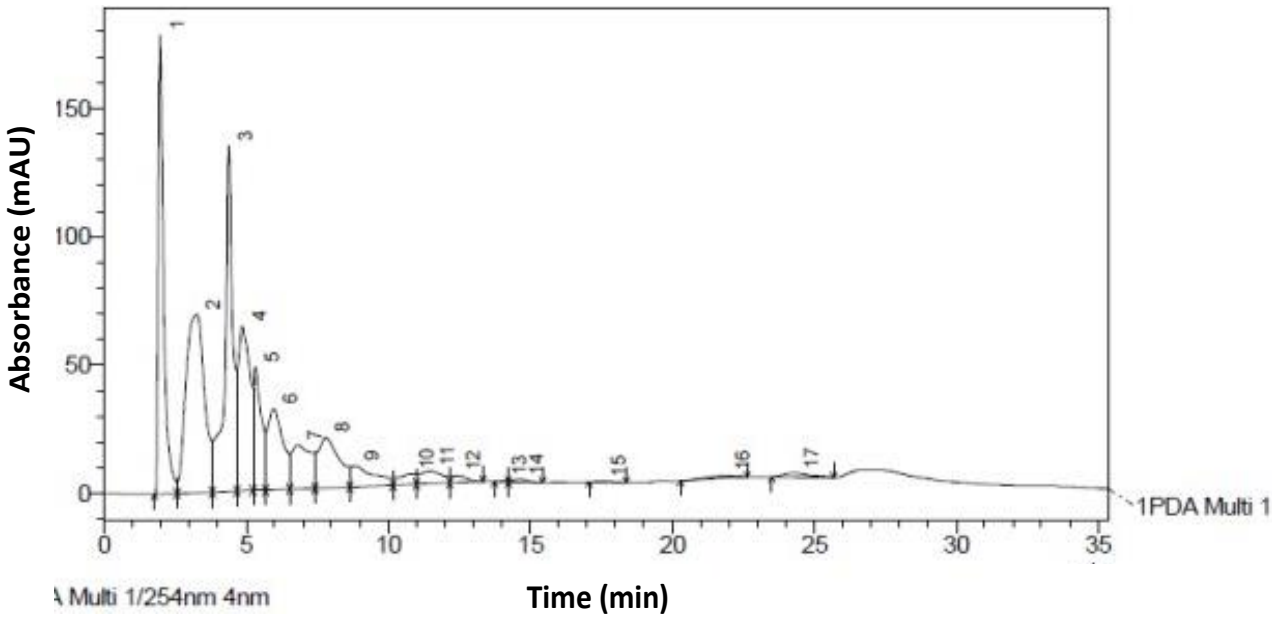

Figure 3. HPLC chromatogram and profile of the leaf of *M. angolensis*.

were found to be 12.75 and 10.60%, respectively. This indicates that alcohol will be a better solvent of extraction than water if bulk yield is of major consideration during extraction. Most plant materials give this trend due to the nature of both solvent. Water which is mostly polar, allows polar organic and mineral solutes to be readily extracted. Ethanol on the other hand is more lipophilic and allows mostly polar and some not so polar organic compounds to be readily dissolved in the extraction process. The ethanol extract was observed to be highly hygroscopic, sticky and viscous. The dry water extract obtained by freeze-drying was glassy-solid crystals, and also very hygroscopic. Moisture accelerates natural products' degradation by hydrolysis (Roy et al., 2018). This information is necessary for formulation of a stable product. The hygroscopic nature indicates that strong moisture absorbents or film coating may be required in addition to pH adjustments, in the formulated products (Roy et al., 2018).

Chromatographic profiles

The TLC profiling of the sample gave 6 spots in day light. No new spot was observed at 366 and 254 nm. The visible spots under daylight were at Rf values of 0.92, 0.75, 0.69, 0.65, 0.59 and 0.46, with the corresponding colours of light yellow, pale green, light yellow, pale blue, greenish yellow and green, respectively. However, four spots were observed at 366 nm as pink, dark pink, pink and light pink corresponding to Rf values of 0.75, 0.65, 0.59 and 0.46, respectively. At UV 254 nm, the six spots appeared as yellow, grey, yellow, grey and yellow, respectively. The Rf values and observed colours are as depicted in the Table 3.

The HPLC profile of the water extract showed 17 peaks. The retention time for the major peaks were 1.973, 3.253, 4.385, 5.969 and 7.804 min, respectively (Figure 3).

The TLC chromatogram showed 6 spots under daylight

and UV 254 nm, and 4 spots under UV light at 366 nm. The HPLC profile showed about 17 peaks, out of which the first 8 were prominent. The established TLC and HPLC profiles can serve as authentication guide in identity where adulteration is suspected.

Conclusion

The study established the pharmacognostic and phytochemical characteristics of the leaf of *M. angolensis* DC. It also established the chromatographic profiles of the leaf extract. These data will serve as useful reference for authentication of the plant especially during economic exploitation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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