Pharmacognostic Standardization of the Leaves and Root Bark of *Caesalpinia benthamiana*

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ABSTRACT

Caesalpinia benthamiana (Baill.) Herend. and Zarucchi (*Mezoneuron benthamianum* Baill.) (Caesalpiniaceae) has been traditionally used in management of erectile dysfunction, dysentery, urethral discharges, skin diseases and wounds. Despite a long tradition of use in the treatment of various ailments, no systematic pharmacognostic standardization work has been carried out on *C. benthamiana*. One major obstacle in the systematic exploration of the plant may be the non-availability of authentic plant material. In the present investigation, various pharmacognostic standards for the plant have been generated including the macro and micro morphological studies, one of the WHO accepted parameters for identification of medicinal plants by way of establishing the salient diagnostic characters and constants. This was carried out on the leaves and root bark of *C. benthamiana.*

The leaves were bipinnate, oblong, with entire margin. The apex was obtuse, and possessed a symmetric base and reticulates venation. The terminal leaves were however obovate in shape. Actinocytic and paracytic types of stomata were observed. Trichomes were uniseriate and unicellular and epidermal cells were found to be wavy. Surface data analysis revealed the stomatal index to be 1.69% to 11.11% for the upper surface and 16.94% to 28.52% for the lower surface. Vein-islet number was recorded as 12.5 to 16.5 whilst the veinlet termination number was 22.25 to 35 with palisade ratio ranging between 11.25 and 13.75. The water- soluble extractive value was 9.2% and 3.7% for the leaves and root bark respectively. Whilst the alcohol-soluble extractive value for the leaves was found to be 6.7% and 2.6% for the root bark. The total ash value determinations were observed to be 5.6% for the leaves and 7.9% for the root bark. The result of this study may be useful in setting diagnostic indices for the identification and preparation of a monograph for the plant.

Key words: *Caesalpinia benthamiana*, Pharmacognostic studies, Quantitative microscopy and Extractive values

INTRODUCTION

Caesalpinia benthamiana (Baill.) Herend. and Zarucchi (*Mezoneuron benthamianum* Baill.) (Caesalpiniaceae),[1] is an African tropical shrub found mostly in the secondary forest zones.

The roots of *C. benthamiana* are considered to be an effective dysentery remedy in Ghana.[2] The powdered roots are used mixed with shea butter or palm kernel oil to treat skin diseases and wounds in Ghana.[3] A decoction of the root, bark, and leaves is used in Guinea for urethral discharges.[2]

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An infusion of the dried root is drunk or used as a bath for general malaise in Senegal.[4] The aqueous decoction of the roots is used in traditional medicine as aphrodisiacs and the vasorelaxant properties have been reported.[5]

The anti-diarrhoeal effects of the aqueous extract of the plant have also been studied. The results obtained showed that the plant possessed anti-diarrhoeal activity due to its inhibitory effects on gastrointestinal propulsion and intestinal fluid accumulation.[6] The analgesic, antipyretic and anti-inflammatory effects of the aqueous extract of the plant have been also been evaluated in mice, rats and rabbits. The data obtained show that *C. benthamiana* root bark extract possesses analgesic and antipyretic activities but lacked anti-inflammatory properties.[6] In a phytochemical investigation conducted by Dickson *et al.*,[7] the bioactivity-guided fractionation of the light petroleum extract of the root bark of the plant led to the isolation of two novel cassane diterpenoids, designated as benthaminin 1 and 2. A third compound, a deoxy form of caesaldekarin C (also

referred to as methyl vouacapenate) which had previously been isolated from *Caesalpinia major*, *Caesalpinia bonducella, Vouacapoua americana and Vouacapoua macropetala*, was also isolated, together with beta-sitosterol and stigmastenone. The antibacterial and antioxidant activities of these cassane diterpenoids have also been assessed.[7] The resistance modifying activities of extracts from this plant on standard antibiotics against *Staphylococcus aureus* have also been assessed by the same group of researchers. A 4-fold potentiation of the activity of norfloxacin was observed for the ethanol extract, whilst the petroleum spirit extract resulted in a 2-fold potentiation.[8]

Despite a long tradition of use in the treatment of various ailments, *C. benthamiana* has not been explored properly by way of establishment of standards in the identification and quality control of this plant. The cassane-type diterpenoids possessing antimicrobial, antioxidant and wound healing properties isolated from the plant could serve as leads in the search for new biomolecules as drugs. The need to standardize this plant can therefore not be overemphasized. It is also worthwhile to note that some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been prepared appropriately and standardized. The use of *Artemisia annua*, *Digitalis* and *Senna* leaves are a few examples. The establishment of the pharmacognostic profile of the leaves and root bark of *C. benthamiana* will assist in standardization, which can guarantee quality, purity and identification of samples to ensure that only the authentic plant is explored properly for its traditional claims.

MATERIALS AND METHODS

The fresh leaves and roots of the plant was collected from the Ayeduase in the Ashanti Region of Ghana and authenticated by Mr. Henry Sam of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, where voucher specimens were deposited with the numbers RADCB 01 and RADCB11 respectively for the leaves and roots of the plant.

Organoleptic evaluation

In the organoleptic evaluation, various sensory parameters of the plant material such as colour, odour, taste and texture were investigated.[9]

Macroscopic evaluation

The following macroscopic characters for the fresh leaves were noted: the type of leaves, its arrangement, colour, shape, length and width of the leaves, size and surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture.[9,10]

Microscopic evaluation

Leaves were cut into smaller sizes and cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence /absence of the following were observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse section through the fresh petiole of the leaf was also examined.

Preliminary phytochemical investigation

The leaf and root bark powders of the plant were separately subjected to Soxhlet extraction using 70% ethanol. Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (Ferric chloride test), cardiac glycosides (Keller-Killiani and Kedde tests), alkaloids (Mayer's; Dragendorff's; Wagner's and 1% picric acid reagents), Saponin glycosides (frothing and haemolysis tests), anthracene derivatives (Borntrager's test for combined and free Anthraquinones) and Cyanogenetic glycosides (sodium picrate paper test).[10,11,12,13]

Quantitative investigations

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein- islet number and veinlet termination number were carried out on cleared sections of the leave. Other parameters determined for the powdered leaves were moisture content, total ash, acid - insoluble ash, water - soluble ash, alcohol (90% ethanol) and water soluble extractive values.[14] The moisture content of the powdered leaves and root bark of the plant was also determined using the Dean-Stark apparatus.[9]

Determination of total ash

2 g of the powdered leaves and root bark of *C. benthamiana* were weighed separately in a pre-weighed ash-less filter paper and incinerated at 400°C for about 3-4 min or until the vapours completely ceased. The temperature was gradually reduced to come to normal and then the ash was collected and weighed.

Determination of alcohol soluble extractive

Accurately weighed powder (10 g) of both leaves and root bark were taken separately and macerated with 100 ml of 95% alcohol for 24 hours. The contents were frequently shaken during the first 6 hours and allowed to remain for another 18 hours. After 24 hours, the extract was filtered and 20 ml of the filtrate was evaporated to dryness. The extract was dried at 105°C to a constant weight.

Determination of water soluble extractive

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration in this instance.

RESULTS

Macroscopically, the plant possess compound leaves which are bipinnate, alternate in arrangement, apex and base are obtuse, margin is entire, venation is reticulate, shape oblong with terminal leaves being obovate. Full length of leaves 33-38 cm, Six (6) pairs of pinnae, with length of each pinnae being 6.5-7.5 cm and four (4) pairs of leaflets on each pinnae. The average size of individual leaf is 2.0-5.0 cm ± 0.5 (length) and 1.5-3 cm ± 0.3 (width). Fresh leaves are green in colour, odourless with a gritty texture. It is petiolated with the length of petiole between 4-5 cm, shape of petiole cylindrical and brownish-green in colour.

The leave arrangement may be similar to that of *C. spinosa* but whereas the leaves of *C. spinosa* lack petioles those of *C. benthamiana* are petiolated.[15]

The length of the leaves of *C. benthamiana* falls between 33 to 38 cm which is within those of *C. pulcherrima* which are also between 20 cm and 40 cm but are longer than those of *C. gilliesii* which falls between 10 cm and 15 cm long.[16]

The leaves of *C. benthamiana* have two types of stomatal arrangements and these are the actinocytic and paracytic types. The types of trichomes observed were uniseriate clothing hairs and unicellular hairs. The epidermal cells were found to be wavy.

Micromorphological features revealed that anticlinal walls are thin and wavy. The type of stomata revealed actinocytic arrangement and these were few as compared to the paracytic ones which were more. Uniseriate covering trichomes are present on both surfaces. It has Isobilateral leaf arrangement. The midrib bundle is surrounded by a zone of pericyclic fibres possessing double layered parenchymatous cells. The roots are brown in colour and the texture is gritty. Vein islet number was determined to be 12.5 to 16.5, veinlet termination number ranging from 22.5 to 35, stomatal index of 1.69% to 11.11% for the upper surface and 16.94% to 28.52% for the lower surface and palisade ratio of 11.25 to 13.75. Water- soluble extractive values were 9.2% and 3.7% for the leaves and roots respectively. The alcohol-soluble extractive value of 6.7% was obtained for the leaves and 2.6% for the roots. Also, the total ash values for both leaves and roots were 5.6% and 7.9%. Phytochemical evaluation revealed the presence of true tannins, pseudotannins and terpenoids mainly for both leaves and roots. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the various actions of *C. benthamiana*. See Tables 1 to 6 numerical and quantitative values as well as morphological descriptions.

DISCUSSION

The standardization of a crude drug is an integral part of establishing its correct identity.[17] *Caesalpinia benthamiana* is employed in ethnomedicine in the management of various disease states without standardization. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs.[18] The vein islet, and vein termination numbers and the other parameters determined in the quantitative microscopy, are relatively constant for plants and can be used to differentiate between closely related species.[19] Also, the physical constant evaluation of the crude drugs is an important parameter which is a valuable tool in detecting adulteration or improper handling of crude drugs. The moisture content of the powered drugs may be said to be high since the BP stipulates an allowable value of not more than 10%. Any value beyond this encourages microbial growth and subsequent deterioration of the stored powdered drugs.

Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly significant in the evaluation of purity of the crude drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.[20] The macro - and micro – morphological features of the leaf described, distinguishes it from other members of the genera. For example whereas the leaves of *Caesalpinia spinosa* lack petioles those of *Caesalpinia benthamiana* are petiolated.[15] The length of the leaves of *Caesalpinia benthamiana* falls between 33 to 38 cm which is within the range for those of *Caesalpinia pulcherrima* which are also between 20 cm and 40 cm but the leaves of *Caesalpinia gilliesii* are shorter, falling within the range of 10 cm and 15 cm.[16]

By and large, the pharmacognostic constants including extractive values, ash values and the phytochemical profile of *Caesalpinea benthamiana* obtained for the leaves and root bark of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for the proper identification and use of this plant.

CONCLUSION

These parameters which are being reported for the first time, could serve as useful information in preparing a monograph of the plant which can be locally incorporated into the Ghana Herbal Pharmacopoeia which may ultimately serve as a significant addition to international herbal pharmacopoeias. Any crude drug which is claimed to be

Caesalpinea benthamiana but whose characters significantly deviate from the accepted standards above may be considered to be contaminated, adulterated, substandard or fake.

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