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PHARMACOGNOSTIC AND ELEMENTAL ANALYSIS ON THE LEAVES OF SCOPARIA DULCIS (PLANTAGINACEAE)

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ABSTRACT *Scoparia dulcis* is a perennial herb widely distributed in tropical and subtropical regions. It is a common herb in traditional medical practices in Nigeria. The leaf is known to be used for the treatment of hypertension, diabetes, stomach discomforts and as an antisickling agent. Pharmacognostic study of the leaf was carried out to determine the macroscopical and microscopical features, chemo-microscopical analysis , physico-chemical parameters, as well as elemental content .The Leaf of *Scoparia dulcis* was observed macroscopically to be green in colour , odourless, 1.85 cm long and 0.32 cm wide while Microscopically, the powdered leaf and sections of the leaf revealed the presence of epidermal cells which were polygonal in shape and the anticlinal walls were wavy. Anomocytic stomata were observed to be present on both the upper and lower epidermis of the leaf. Calcium oxalate crystals were seen to be present and was of the prism type, Glandular trichome with multiseriate heads were also observed on the upper epidermis of the leaf only. Elemental analysis showed the presence of Mn, Co, Ni, Cu, Zn, Fe, Pb,Mg and Ca. Chemo-microscopic characters present include lignin, starch, tannins, suberin, cellulose and calcium oxalate crystal. The Physico-chemical parameters (%w/w) determined include moisture content (5.01), total ash content (7.5), acid insoluble ash content (0.83), water soluble ash content (2.83), ethanol extractives (33.0) and water soluble extractives (27.0). The results obtained from this research will provide information that may be useful for proper identification and preparation of an official monograph of the plant.

Keywords: *Scoparia dulcis* , Macroscopic, Microscopic, Physico-chemical, Elemental analysis

INTRODUCTION

Scoparia dulcis Linn (Plantaginaceae), commonly known as sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions, The plant is an erect herb with serrated leaves, producing white flowers and measuring upto a half meter in height when fully grown(Krishna et al.,2012) . In these regions, the fresh or dried *S. dulcis* plant have been traditionally used as remedies for stomach discomforts, hypertension, diabetes, bronchitis, as an analgesic, antipyretic and antisickling agent (Ratnasooriya et al.,2005 ; Ahmed at al.,2019). It is known as rumafada in Hausa, Mesenmesen gogoro in Yoruba and aiya in Ibo indigenous languages of Nigeria (Orhue and Nwanze .,2009). The plant has been reported to possess antisickling activity (Abere et al., 2015) , antibacterial and immunostimulatory activity (Abdulsalaam et al .,2013), Also in India ,Mishra et al .,2013 reported the plant to have anti-oxidant and antidiabetic activity. Evaluation of pharmacognostic and physicochemical properties of medicinal plants are essential for standardization and preparation of monographs (Fatokun at al.,2017) , standardization of medicinal products is therefore paramount because many of such natural products derived from plants have been used as starters for the synthesis , manufacture of synthetic drugs and are important factors in traditional medicine . Due to the established therapeutic activity of the plant and its broad base usage in traditional medicine, The present study, therefore aim to establish the pharmacognostical parameters of the plant to aid in its proper identification and standardization .

MATERIALS AND METHODS

Collection , Identification and Preparation of Scoparia dulcis

The leaves of the plant *Scoparia dulcis* were collected in the field around Yankarfe village, Sabon gari Local Government Area, Kaduna State, Nigeria, in the month of August 2016. The plant was identified and authenticated by Mallam Namadi Sunusi, in the Herbarium Unit of the Department of Botany, ABU, Zaria, Nigeria and voucher specimen number (32034) was deposited. The plant was carefully cleaned with water to remove any foreign matter, The leaves were carefully plucked from the whole plant, They were then air dried under shade for about two weeks , comminuted to powdered form using a pestle and mortar. The powdered leaf sample gotten was then stored in an airtight container for further use.

Microscopical examination

The microscopical evaluation of the anatomical section of the leaf as well as its powdered sample was carried out using standard methods (Brain and Turner .,1975; Evans .,2009) . The prepared sections were cleared using 70% chloral hydrate solution and heated on a water-bath for about thirty minutes to remove obscuring materials. To the cleared sample, dilute glycerol was added and observed under a microscope . Appropriate images were taken and documented. The micrometric evaluation of some of the diagnostic feature was also carried out.

Quantitative leaf microscopy analysis

Quantitative leaf microscopy analysis was carried out on thin epidermal layer and examined under a microscope with the aid of Camera Lucida based on the method outlined by (Brain and Turner .,1975; Evans .,2009)

a) Stomatal Number

A section of the leaf from the middle of the lamina was cleared with chloral hydrate solution, with the aid of a camera lucida and a stage micrometer, a clean white paper was divided into squares of 1mm x 1mm using (x10) objective. The stage micrometer was then removed and replaced by the cleared preparation of the leaf. The number of stomata was counted by markings on the paper. The total number of stomata in one square of 1mm x1mm was then determined (Evans, 2009).

b) Stomatal Index

A section of leaf was cleared with chloral hydrate solution, mounted in dilute glycerol and viewed under the microscope using 4mm (x40) objective. The number of stomata divided by summation of epidermal cells and stomata multiplied by 100% gives the stomata index.

(c)Veinlet Number, Veinlet Termination Number, Vein -islet Number

A section of the leaf from the middle of the lamina was cleared with chloral hydrate solution, with the aid of a camera lucida and a stage micrometer, a clean white paper was divided into squares of 1mm x 1mm using (x10) objective. The stage micrometer was then removed and replaced by the slide of the cleared preparation of the leaf. The number of veinlet, veinlet termination and vein-islet were counted and recorded accordingly.

Chemomicroscopic Examination

The powdered leaf of *Scoparia dulcis* was cleared using chloral hydrate solution and little quantity of it was mounted on a clean slide using dilute glycerol and observed under a compound microscope for the presence of cell inclusions such as lignin, cellulose, starch, tannins, and calcium oxalate crystals according to the method outlined in Evans (2009).

Physicochemical parameters

Powdered sample of the dried plant was subjected to physicochemical analysis such as total ash, acid insoluble ash, water soluble ash ,moisture content, water and alcohol soluble extractives as outlined by the method in Evans (2009).

Determination of elemental analysis

Elements such as manganese, cobalt, nickel, copper, zinc, cadmium, iron, lead, magnesium and calcium were qualitatively and quantitatively analysed using Atomic Absorption Spectrophotometry (AAS) (Rajurkar et al., 1997)

RESULTS AND DISCUSSION

Macroscopic examination of the leaf showed the leaf to be simple ,green in colour, odourless and slightly sweet in taste with an acute apex. The average leaf size was measured to be 1.85cm long and 0.32cm wide, the shape of the leaf is ovate with serrate edge.

Qualitative Leaf Microscopy

The Microscopical examination of the powdered and anatomical sections of the leaf of *Scoparia dulcis* revealed various cellular features. An abundance of

epidermal cells were seen in the cell, this was observed on both the upper and lower surface of the leaf in high frequency, the epidermal cells were seen to be generally polygonal in shape with distinct wavy anticlinal walls (Fig 1). Anomocytic stomata was observed in the cell on both the upper and lower surface of the leaf, however the lower surface of the leaf had an abundance of stomata while the upper surface had a lower number, the presence of numerous stomata on both surfaces of the leaf is an indication that transpiration occurs on both surfaces for photosynthesis and water loss . The mean average size of stomata was measured to be 2.38 µm x 1.72 µm.

The powdered sample of the leaf showed the presence of calcium oxalate crystals which were of the prism type, the crystals were also observed in the upper epidermal surface of the leaf, they were seen to have a high frequency of occurrence and scattered generously in the cell, A recordable number of cystoliths of calcium carbonate was also observed scattered in the cell, (Fig 2 and 3). Glandular trichomes with multiseriate heads were also observed to be present in the leaf.(Fig 4).

Diagnostic cellular features such as stomata (anomocytic) , cystoliths of calcium carbonate and glandular trichome with multiseriate heads were also observed in the plant and reported by Okhale et al.,2010. The occurrence of some of the above mentioned characteristics have been observed among members of the Plantaginaceae (Jain et al., 2016).

Transverse section of the leaf across the midrib showed the presence of vascular bundles and collenchyma cells(Fig 5). The presence of these anatomical features in the leaf of *Scoparia dulcis* can serve as a diagnostic tool for proper identification of the plant, pharmacognostic evaluation of crude medicinal plant is also important for determination of its identity, quality and purity.

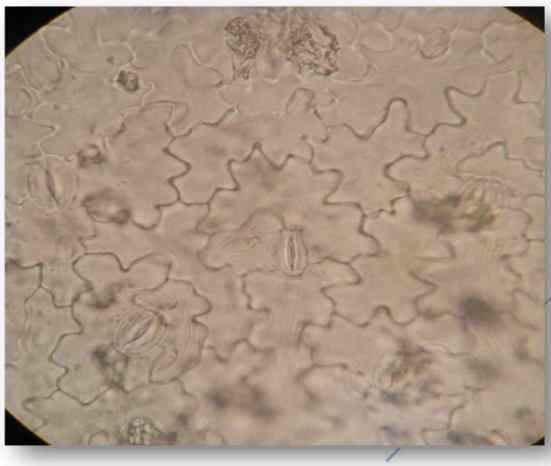

Fig I: Photomicrograph of the lower surface of the leaf of *Scoparia dulcis* showing Anomocytic stomata(ST). (Mag. ×400)

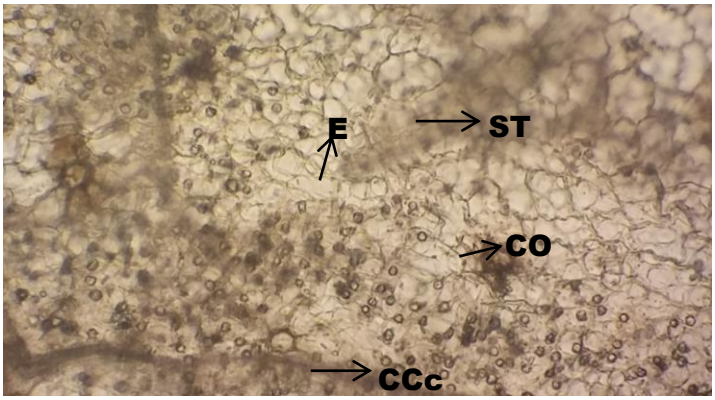

Fig 2: Photomicrograph of the upper surface of *Scoparia dulcis* leaf showing epidermal cell(E); calcium oxalate crystal (CO); cystolith of calcium carbonate (CCc) (Mag. ×400)

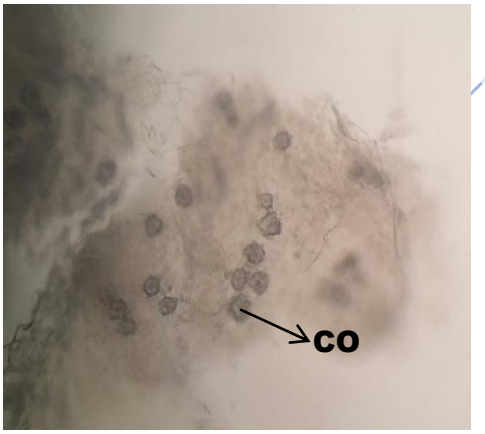

Fig 3: Photomicrograph of the powdered leaf of *Scoparia dulcis* showing calcium oxalate crystals (CO) . (Mag. ×100)

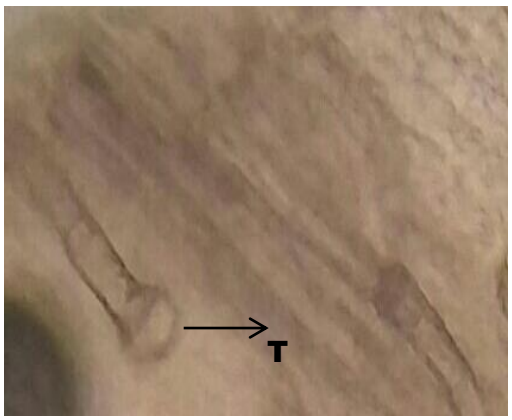

Fig 4: Photomicrograph of the powdered leaf of *Scoparia dulcis* showing Glandular trichome (T). (Mag. ×400)

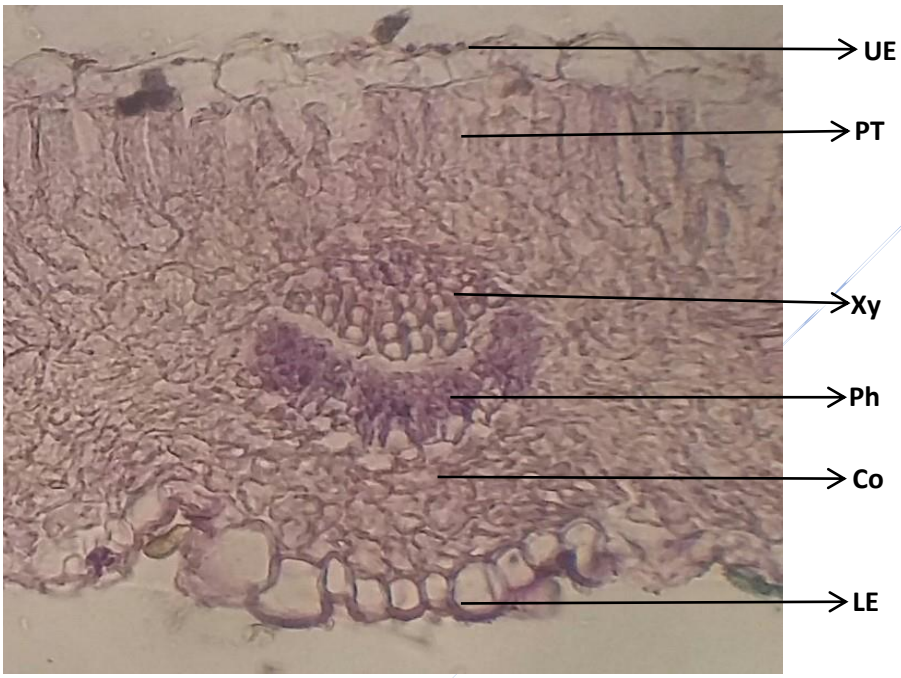

Fig 5: Photomicrograph of the transverse section through the midrib of the leaf of *Scoparia dulcis* showing upper epidermis(UE); palisade tissue(PT); xylem(Xy); phloem(Ph); collenchyma(Co); lower epidermis (LE) . (Mag. ×100)

Quantitative Leaf Microscopy

The results of the average stomatal number, stomatal index, palisade ratio, veinlet termination number and veinlet number for the leaf were determined under a microscope. The results are as shown in table 1. Leaf constants such as stomatal number, stomatal index, palisade ratio, vein-islet number and veinlet termination number are of diagnostic importance and they vary from one plant species to another. The stomatal index value in addition to other factors (e.g leaf constants values) can make a positive identification possible and is less subjected to variations by by external conditions. (Brian and Turner., 1975)

Table 1: Quantitative Microscopical Values for the Leaf of *Scoparia dulcis*

Parameters	Values* ± SEM
Stomatal number	9.2 ± 1.39
Stomatal index	24.7± 2.32
Palisade ratio	3.75± 0.92
Veinlet termination number	1.0 ± 0.61
Vein islet number	1.75 ± 0.24

*Mean value of five counts

Chemomicroscopical Features of the Powdered Leaf of Scoparia dulcis

Various cell wall materials and cell inclusions such as starch, lignin, tannins, calcium oxalate, calcium carbonate, cellulose, suberin and aleurone grains were observed to be present in the powdered sample of the plant. (Table 2)

Table 2: Chemomicroscopical Evaluation of the Powdered Leaf of *Scoparia dulcis*

Parameters	Inference
Starch	+
Lignin	+
Tannins	+
Calcium oxalate	+
Calcium carbonate	+
Cellulose	+
Suberins	+
Aleurone grains	+

Physicochemical Constants of the Powdered Leaf of Scoparia dulcis

The various physical constants determined for the leaf of *Scoparia dulcis* were as follows: Moisture content was calculated to be 5.01% .The percentage total ash, water soluble ash and acid insoluble ash values were 7.5, 2.83 and 0.83% respectively. The mean of the ethanol extractive value was calculated to be 0.33 g while that of the water extractive value 0.27 g. The percentage yield when calculated for the ethanol extract was 33.0 % while the percentage yield of the water extract was 27.0 % (Table 3).

The above values are useful as a criteria to judge the identity and purity of crude drugs (W.H.O.,1996) The ethanol extractive value (33.0%) gotten was higher than the water extractive value (27.0%) suggesting that ethanol is a better extracting solvent for the plant sample.

Moisture content obtained was 5.01 % indicating the drug has low chances of undergoing microbial degradation during storage and also the general requirement of moisture content in crude drug is that, it should not be more than 14 % (B.H.P 1990), the value obtained in this study was within the accepted range.

The values gotten for total ash, acid-insoluble ash and water-soluble ash (7.5 %, 0.83 % and 2.83 % respectively) indicates low amounts of impurities such as carbonate ,silica and sand in the plant sample, The total ash value is used as criteria to judge the identity and purity of drugs (WHO., 1996; Prasad *et al*., 2012).

Table 3 : Physicochemical Constants of *Scoparia dulcis* Leaf Powder

Parameters	**Values (%w/w) ± SEM***
Moisture content	5.01 ± 0.33
Total ash value	7.5 ± 0.50
Acid Insoluble ash	0.83 ± 0.33
Water Soluble ash	2.83 ± 0.76
Ethanol Extractives	33.0 ± 1.00
Water Extractives	27.0 ± 0.20

***Average values of three determinations

Elemental Analysis

Ten elements were screened as shown in Table 4; Mn, Co, Ni, Cu, Zn,Fe, Mg and Ca have been classified as essential elements, their concentration obtained in the plant was quite high with Ca recording the highest amount (3963.0) while Cd has been classified as a non- essential element , this is of no surprise as the concentration obtained in the plant was quite low (1.45), Pb element was gotten to be (100.7), this is still within the acceptable limit in a given sample and the concentration recorded could also be attributed to mining activities around area of plant collection.

The presence of Mn, Co, Ni, Cu, Zn,Fe, Mg and Ca reflects their function as essential nutrient elements ,often as co-factor activators in metal-ligand enzyme complexes (Valkovic .,1975) , these elements are known to play important roles in the body such as normal growth and development, aiding in white blood cell formation and neutralizing free radicals .

The active constituents of medicinal plant or the metabolic products of plant cells wherein a number of trace elements play an important metabolic role can be used medicinally for their therapeutic effect (Rasheed .,1995 ;Zafar et al.,2010). The presence of the above elements in the plant under study gives an insight into its potential as a dietary supplement to solve issues of elemental deficiencies in man. The toxicity levels for the elements analysed were found to be within WHO permissible safety baseline limit in man.

Table 4: Elemental Analysis of *Scoparia dulcis*

Elements	Concentration(ppm)
Mn	71.80
Co	100.7
Ni	631.2
Cu	28.50
Zn	15.76
Cd	1.45
Fe	914.6
Pb	100.7
Mg	1181.5
Ca	3963.0

CONCLUSION

The results of this study has provided information that will be useful in the positive identification and quality control of the leaf of *Scoparia dulcis* as well as aid in the preparation of a standard monograph of the plant.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

REFERENCES

Abdulsalaam I.A, Ehinmidu J.O, Igbadi E.O 2013. Evaluation of antibacterial properties, acute toxicity and immunostimulatory potential of

Scoparia dulcis. *Nigerian Journal Biotech*, 26 :21-25.

Abere TA, Okoye CJ, Agoreyo FO, Eze GI, Jesuorobo R.I, Egharevba C.O, Aimator PO 2015. Antisickling and toxicological evaluation of the leaves of *Scoparia dulcis linn*. *BMC Complement Altern med*, 15: 1-7.

Ahmed R, Nuhu H.D, Ibrahim H, Nuhu A, Idris M.M 2019. Toxicological assessment of Aqueous and methanol leaves extract of *Scoparia dulcis* Linn (Plantaginaceae) in wistar rats. Trop J Nat Res ., 3(3):64-70.

British Herbal Pharmacopoeia 1990. British Herbal Medicine Association Bournemonth: Dorset 1st edition 1:1-2.

Brain KR, Turner TD 1975. The practical evaluation of phytopharmaceuticals. Bristol: Wright Scientechnica, p 4,36

Evans WC. Trease and Evans pharmocognosy 2009. 16th ed. St. Louis: Elsevier's Ltd.,

Fatokun Omolola T, EsievoKevwe B, Ugbabe Grace E and Kunle Oluyemisi F 2017. Microscopic, physicochemical and chromatographic fingerprints of leaves of Nigerian Cassia Tora Linn. Jopat .,16(1) :86-94. ISSN 1118-1028.

Jain P.K, Das D, Jain P, Jain P 2016. Pharmacognostic and pharmacological aspect of Bacopa monnieri. Innovare journal of Ayurvedic sciences., 4(3): 7-11.

Krishna M, Mayank P, Poonam T, Singh R 2012. Pharmacological properties of *Scoparia dulcis*: A Review. Pharmacologia., 3:344-347.

Mishra MR, Mishra A, Pradhan DK, Panda AK, Behara RK, Jha S 2013. Antidiabetic and antioxidant activity of *Scoparia dulcis* linn. *Indian J. pharm sci.*, 75(5) :610-614

Orhue NEJ and Nwanze EAC 2009. Antianaemic properties of *Scoparia dulcis* in trypanoma brucci infected rabbits. African J Biochem Rev ., 3:245-249.

Okhale S.E, Amanabo M.O, Jegede I.A, Egharevba H.O, Muazzam I.W, Kunle O.F 2010 .Phytochemical and Pharmacognostic investigation of antidiabetic Scoparia dulcis Linn Scrophulariaceae whole plant grown in Nigeria. Researcher ., 2(6):7-16.

Prasad, S. K, Laloo, D, Sahu, A. N, Hemalatha S. 2012 . Cytomorphological and physiochemical evaluations of *Cryptocoryne spiralis* (Retzius) Wydler. *Journal of Herbs Spices and Medicinal Plant.*,8: 304-307.

Rajurkar NS, Damame MM 1997. Elemental analysis of some herbal plants used in the treatment of cardiovascular diseases by AAS and NAA. J Radioanalytical Nucl Chem .,219(1): 77-80.

Rasheed MN 1995. Trace elements in some wild plants from the shores of the high dam lake and the adjacent desert, as determined by atomic absorption spectroscopy. J. Arid. Environ., 29: 185-197.

Ratnasooriya W.D., Jayakody J.R., Premakumara G.A., Ediriweera E.R. 2005. Antioxidant activity of water extract of *Scoparia dulcis* . Fitoterapia .,76:220-222.

Valkovic VV 1975.Trace Element Analysis. London: Taylor and Francis. pp. 5-83

World Health Organization . Quality of Pharmaceuticals. A compendium of guidelines and related materials, good manufacturing practices and inspection. World Health Organization Geneva 2 ;1996.

Zafar M, Khan M.A, Ahmad M, Jan G, Sultana S , Ullah K, Marwat S.K, Ahmad F, Jabeen A, Nazir A, Abbasi A.M, Ur Rehman Z, Ullah Z 2010. Elemental analysis of some medicinal plants used in traditional medicine by atomic absorption spectrophometer(AAS). J. med. Plant Res.,4(19): 1987-1990.