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Pharmacognostic Studies and Antioxidant Properties of the Leaves of *Solanum macrocarpon*.

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Abstract

The leaves of *Solanum macrocarpon* (Solanaceae) was evaluated for its pharmacognostic characteristics and antioxidant properties. Microscopic studies showed the presence of both anomocytic and anisocytic types of stomata on both the upper and the lower epidermis, unicellular and branched clothing trichomes as well as glandular trichomes with unicellular stalk and multicellular heads. The leaf is dorsiventral with the mesophyll cells containing abundant prismatic calcium oxalate crystals. The midrib showed a crescent-shape and bicollateral vascular bundle. Microscopic evaluation established Stomatal index as 11.59±0.63 and 18.69±0.84 for the upper and lower surfaces respectively. The palisade ratio, the vein islet and vein-let termination numbers were determined as 6.22±0.36, 9.09±0.64 and 9.02±0.53 respectively. Physicochemical evaluation yielded extractive values of 1.33±0.33, 4.65±0.66, 8.66±0.32 and 7.34±0.25 for petroleum ether, ethyl acetate, ethanol (95%) and water respectively. Total Ash, Water soluble, Acid insoluble Ash were 13.96±0.06, 6.86±0.16 and 2.20±0.09 respectively. Basic phytochemical screening revealed the presence of tannins, flavonoids and alkaloids. The alcoholic extract showed radical scavenging activity with IC_{50} of 2.42mg/ml. The pharmacognostic standards and the antioxidant activity of the leaf of S. macrocarpon have been established. The pharmacognostic standards are essential for proper identification of the plant.

Key words:

Solanum macrocarpon, pharmacognostic, antioxidant, activity

INTRODUCTION:

Solanum macrocarpon is an African herbaceous plant commonly used for its nutrition and medicinal properties. It is widely cultivated in West Africa where it serves as an important fruit and leaf vegetable. Locally, the leaf is considered very nutritious and is used in preparing soups and stews. The leaves are rich in protein, fat, crude fibre, calcium and zinc [1] and are found to contain appreciable amounts of the amino acid, methionine [2]. The leaves have a variety of medicinal uses. For example, in Sierra Leone boiled leaves are chewed to treat throat problems; in Kenya the crushed leaves are taken to treat stomach problems [3]. It is also used to treat toothaches.

Proper identification of medicinal plant materials is important in ensuring their quality, safety and efficacy. Some of the parameters used to establish the identity of medicinal plants materials and also detect adulteration are the pharmacognostic parameters [4]. Despites the various uses, both as source of medicine and nutrition, there is a scanty data on the pharmcognostic standards for identification and authentication of the leaf of *S. macrocarpon.*

Besides, plants and in particular vegetables, whole grains and fruits are known to be a good source of naturally occurring antioxidants. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [5]. They thus protect body cells from damages caused by free radicals. The free radicals are considered to be contributing factor to several chronic diseases in humans such as cancers, diabetes mellitus, cardiovascular diseases [6] and several neurodegenerative disorders. In spite of its use as a vegetable, there is no data on the antioxidant capacity of this leafy vegetable, *S. macrocarpon*. In these regards, the current study aims at evaluating the pharmacognostic parameters of the leaf towards its standardization, and also assessing its antioxidant capacity to determine its usefulness as a vegetable that could contribute to the reducing of oxidative stress.

MATERIALS AND METHODS

Collection and Preparation of the Leaves

Fresh leaves of *Solanum macrocarpon* were collected from the physique garden of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST in April, 2010. Authentication was done by Prof. JP Tetteh of the School of Agriculture, University of Cape Coast. Herbarium and voucher sample were prepared and deposited in the Herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST (Voucher No KNUST/HM1/2010/L056). The fresh leaves were washed and air-dried for five days. The dried plant materials were coarsely powdered using an electric mill and the powdered material packed in air-tight container and stored in a desiccator until needed for use. Fresh leaves were however used for microscopic studies.

Microscopic Examination of the Leaf

Free-hand sections of the lamina of the leaf were cleared in warm chloral-hydrate solution in a flask over a boiling water-

bath. The cleared specimens were mounted and photomicrographs of the epidermal features were captured from the slides using an Olympus light microscope fitted with camera using AMcap software. The transverse sections (TS) of the lamina and the midrib of the fresh leaf were also cleared, mounted, observed and their features photographed.

Quantitative Microscopy

Quantitative leaf parameters such as palisade ratio, stomatal number, stomatal index, vein – islet and veinlet termination numbers were determined from cleared sections of the lamina [4, 7].

Basic Phytochemical Screening

Preliminary phytochemical screening for major chemical groups was performed [8].

Physicochemical Evaluation

Various physicochemical parameters such as ash values (total ash, water soluble ash and acid-insoluble ash) and extractive values (water, alcohol, ethyl-acetate and petroleum ether soluble extractives) were determined [9].

Free Radical Scavenging Activity

Free radical scavenging activity was determined as described by Blois (1958) [10], to assess the potential of the leaf extract to prevent oxidative damage to body cells by free radicals. The percentage of DPPH inhibition by the samples was calculated according to the formula:

$$\%\ inhibition = \frac{(A - A_S)}{A} \times 100$$

Where A is absorbance of control, A_s the absorbance of sample.

The IC_{50} value (the concentration at 50% inhibition) was determined from the curve of percentage inhibition and log concentration.

Determination of Total Phenolic Content of the Extract

The total phenol content of the ethanolic extracts was determined by Folin-Ciocalteu's reagent [11] with tannic acid as standard. The total phenol content was expressed as Tannic acid equivalent (TAE) mg/gram of dry weight of extract.

RESULTS

Microscopic Examination of the Leaf

The microscopic examination of the leaf revealed epidermal cells which are irregular in shape and have wavy anticlinal walls. The epidermis bears both glandular and clothing trichomes (Fig. 2). The glandular trichomes have unicellular stalk with multicellular head while the clothing trichomes are simple unicellular and branched. The leaf bears anisocytic and anomocytic types of stomata on each of its surface.

The TS of the leaf (Fig. 3) showed cuticular upper and lower epidermis. The epidemis showed single-layered, compact, horizontally-elongated cells. The leaf is dorsiventral with a single-layered, compact, perpendicularly elongated palisade cells below the upper epidermis overlying a spongy mesophyll composed of 3-5 layers of loosely packed isodiametric parenchymatous cells. At the center of the lamina is the midrib which showed upper and lower epidermis with no trichome. Underlying the upper and above the lower epidermis are thick regions of collenchymatous tissues. The stele of the midrib showed crescent shape. The vascular bundle is conjoint with the phloem occurring on both the adaxial and abaxial faces of the bundle. This makes the vascular bundle a bicollateral one. Lying between the vascular bundle and the collenchymatous tissues are large loosely arranged parenchymatous cells.

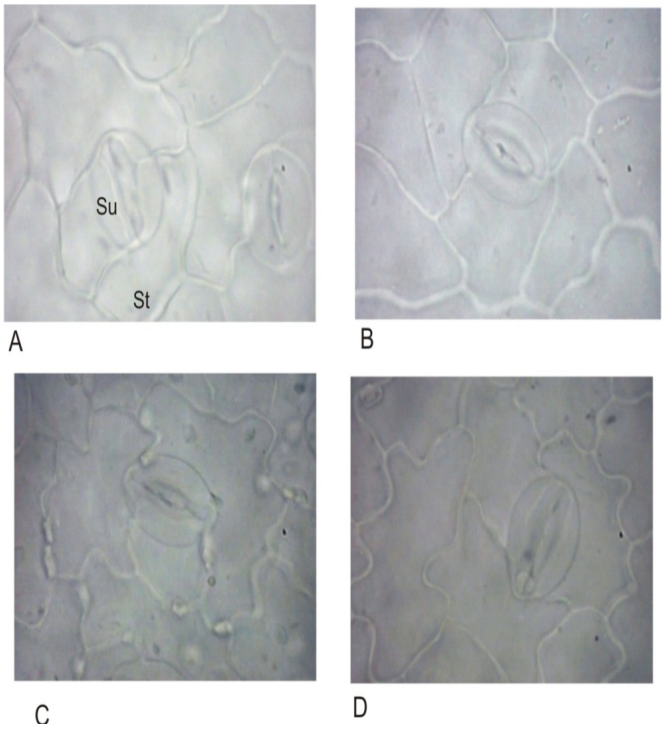

Fig. 1 Photomicrographs showing the types of stomata associated with *s. macrocarpon* leaf (x400)

A: anisocytic stomata on the upper epidermis, B: anomocytic stomata on upper epidermis;
C: anisocytic stomata on the lower epidermis, D: anomocytic stomata on lower epidermis; st: stomata, su: subsidiary cell;

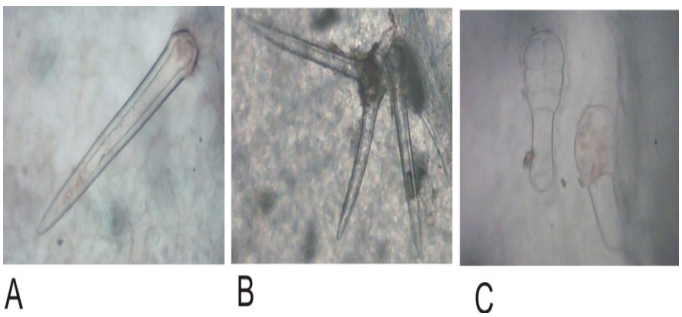

Fig. 2 Photomicrograph of the types of trichomes as seen under the microscope:

A: unicellular clothing trchome (x400); B: Branched clothing trichome (x200); C: unicellular stalk and multicellular head glandular trichomes (x400)

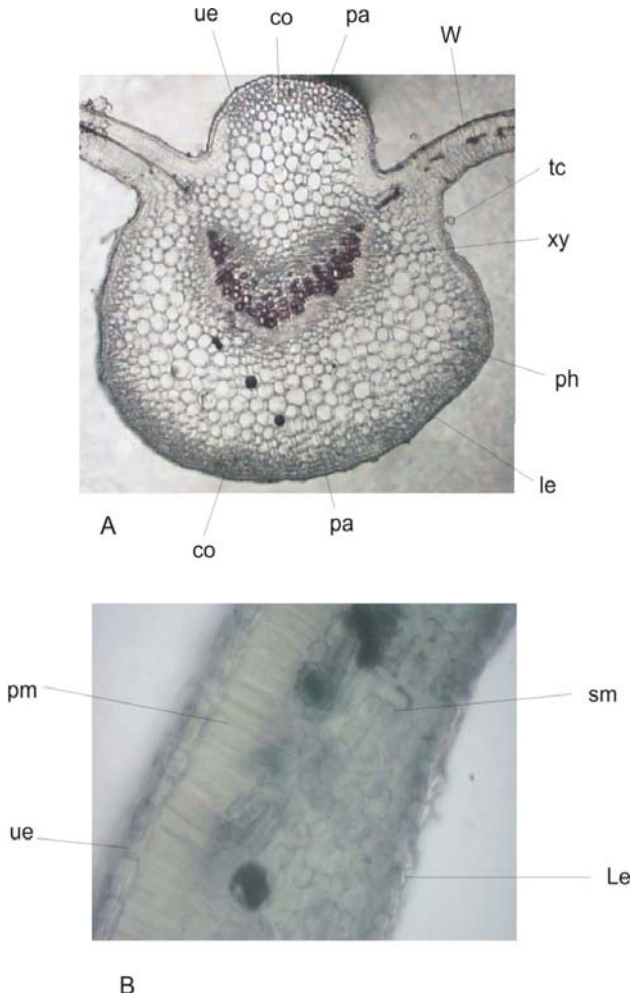

Fig. 3. Photomicrographs of the ts of the leaf

A: The midrib region (x40); B: enlarged portion of the TS of the lamina (x100)

ue: Upper epidermis; le: lower epidermis; co: collenchymatous tissue; pa: parenchymatous tissue; ph: Phloem; xy: xylem; pm: palisade mesophyl; sp: spongy mesophyl; tc: trichome; w: lamina

Table 1: Surface data of *s. macrocarpon* leaf

Parameter	Mean ±SEM	Range
Veinlet termination number	10.83 ±0.82	7.84 – 13.72
Veinislet number	11.80 ±1.06	8.23 -15.67
Palisade ratio	6.22 ± 0.36	3.45 - 9.20
Stomatal index (%)		
Upper	11.59 ± 0.63	4.00 – 18.75
Lower	18.69 ± 0.84	15.00 – 25.00
Stomatal number		
Upper	181.50±11.58	52.08 - 312.50
Lower	257.70±23.39	156.30 - 468.80

Table 2: Basic phytochemistry screening of the leaf

Constituent	Indication
Tannins	Positive
Flavanoids	Positive
Alkaloids	Positive
Anthraquinone glycosides	Negative
cardiac glycosides	Negative
Cyanogenetic glycosides	Negative
Saponin	Negetive

Table 3: Physicochemical parameters

Parameter	Value (Mean ±SEM) / %w/w
Ash	
Total ash value	13.96±0.06
Acid insoluble ash value	2.20±0.09
Water soluble ash value	6.86±0.16
Extractives	
Ethanol extractive value	8.66 ± 0.32
water extractive value	7.34 ± 0.25
Ethyl-acetate extractive value	4.65± 0.66
Petroleum ether extractive value	1.33 ± 0.33

Free Radical Scavenging Activity

IC_{50} for N-propyl gallate and *S. macrocarpon* extract were 0.0035 and 2.42 mg/ml respectively.

Determination of total phenolic content of extracts

The total phenolic content expressed as tannic acid equivalent (TAE) in mg/g weight of 1g of the extract was 79.69±2.36.

DISCUSSIONS

Traditionally, the leaves of *S macrocarpon* have been used to treat various disease conditions and also used as vegetable. However, it has no standards for proper identification. The standardization of a crude drug is essential for establishing its correct identity. This requires the establishment of pharmacognostic parameters and standards which are characteristic, to a larger extent, of the plant. Among the methods used to establish pharmacognostic standards are the quantitative and qualitative microscopy, extractive and ash value determinations [4, 7].

Microscopic Examination

In this study, we discovered the presence of both anisocytic and anomocytic stomata on each of the leaf surfaces of the plant (Fig. 1 A, B, C, and D). This is contrary to an earlier report by Mbagwu *et al*, 2007 [12] that the leaf of *S macrocarpon* has only anomocytic stomata. Other important features of the leaf surface include the presence of the unicellular and branched clothing trichomes and glandular trichome with unicellular stalk and multicellular heads. These features are valuable characteristics for the establishment of the identity of the plant.

Quantitative Microscopy

The leaf surface parameters as indicated in table 1 is the first report of the palisade ratio, vein islet and vein-let termination numbers of *S. macrocarpon*. However, Mbagwu *et al* (2007) has reported on the stomatal indices and stomatal numbers. In that report, they found the stomatal indices of the lower and upper epidermis to be in a range of 10.14-32.35% and 6.41-6.80% respectively. Nonetheless, this study has established the stomatal indices of the lower and upper epidermis to be in a range of 15.00 – 25.00% and 4.00 – 18.75% with mean values of 18.69 ± 0.84% and 11.59 ± 0.63% respectively. Mbagwu *et al* (2007) [10] established the stomatal numbers to be 137.40 for lower and 39.20 for upper surfaces. However, in this study we have established the stomatal numbers for the lower and upper epidermis to range between 156.3 - 468.8 and 52.08 - 312.50 with mean values of 257.70±23.39 and 181.50±11.58 respectively. This is not surprising since the stomatal number varies with age of plant/leaf and environmental conditions. Except stomatal number, the other quantitative leafy parameters including stomata index, Vein islet number, Vein termination number and palisade ratio are not affected by age of plant, size of leaf and environmental conditions. These are relatively constant and hence more frequently used in the evaluation of a leaf drug [4]

Basic Phytochemical Screening

The results of the preliminary phytochemical analysis are indicated in table 2. Phytochemical screening indicated the presence of tannins, flavonoids and alkaloids. These phytoconstituents may contribute to the various medicinal as well as nutritional properties of the plant.

Physicochemical Evaluation

Total ash, which is the inorganic ash left after a vegetable drug is burnt usually consist of carbonates, phosphates, silicates and silica. It measures the purity and quality of a crude drug. The acid-insoluble ash consists of mainly silica and it indicates contamination with earthy material [5]. From the result the acid-insoluble ash of 2.20± 0.09 % is low. This low value suggests the absence of earthy material contamination of the plant material. The Results for extractive value of the leaves (Table 3) showed ethanol (96%) to yield the highest extractive of 8.66±0.32, an indication that *S. macrocarpon* leaves contains mostly polar to mid-polar phytoconstituents.

Free Radical Scavenging Activity

The free radical scavenging activity of the extract expressed as percentage inhibition of DPPH show dose-dependent activity. The IC_{50} values of the N-propyl gallate and *S. macrocarpon* extract were 0.0035 and 2.42 mg/ml respectively. Though the IC_{50} value for the free radical scavenging activity of the extract is significantly low compared to that of the reference (N-propyl gallate), the leaf of *S. macrocarpon* could still offer some protection against oxidative damage to body cells. It nutritional and medicinal uses are therefore very relevant.

Total Phenol Content

The total phenol was assayed based on the reduction of phosphomolybdate salts to form a blue complex that was detected quantitatively at 700 nm. The total phenolic content expressed as tannic acid equivalent (TAE) in mg/g weight of 1g of the extract was 79.69±2.36. This implies that the total phenolic content of the leaf could contribute significantly to the antioxidant capacity of the leaf.

CONCLUSION:

This study established the pharmacognostic characteristics of the leaf and antioxidant activity of the alcoholic extract of the leaf of *S. macrocarpon*. Key microscopic and physiochemical parameters have been established. The findings could be useful diagnostic features for the standardization of *S. macrocarpon* leaf. The antioxidant properties indicate the usefulness of the leaf as a vegetable that can contribute to reducing oxidative stress.

REFERENCES

1. Oboh, G. Ekperigin, M. M. and Kazeem, M. I. Nutritional and haemolytic properties of eggplants (*Solanum macrocarpon)* leave. Journal of Food Composition and Analysis. Volume 18, Issues 2-3, 2005, 153-16
2. Messiaen, C.M, The Tropical Vegetable Garden, 4th Ed. Macmillan Press Limited, London and Basingstoke, 1992, 232-233
3. Bukenya-Ziraba, R. and Bonsu, K.O., *Solanum macrocarpon* L. In: Grubben, G.J.H. & Denton, O.A. (Editors). PROTA 2: Vegetables/Légumes, 2004, [CD-Rom]. PROTA, Wageningen, Netherlands.
4. Evans W.C, Trease and Evans, Pharmacognosy Textbook, Elsevier Limited, United Kingdom, 15th Ed, 2006
5. Velioglu Y. S., Mazza G., Gao L., and Oomah B. D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. Journal of Agriculture and Food Chemisty. **1998,** *46,* 4113□4117
6. Khandelwal K. R. Practical Pharmacognosy-Techniques and experiments.Nirali Prakashan, india, 19th Ed, 2009.
7. Moskovitz J, Yim MB, Chock PB. Free radicals and diseases. Ach Biochem Biophy, 2002. 397;354-9
8. Harborne, J.B. Phytochemical analysis: A Guide to Modern Techniques of Plant Analysis. Chapman and hall. London, 2nd ed. 1991.
9. Khandelwal, K. R., Practical Pharmacognosy, techniques and experiments. Nirali Prakashan, Abhyudy Pragati, 19th Ed, 2008, 157-159.
10. Blois MS., Antioxidant determination by the use of a stable free radical. Nature **26**, 1958, 1199-1200.
11. Slinkard, J. and Singleton V.L. Total phenol analysis: automation and comparison with manual methods, American Journal of Enology and Viticulture 28, 1977, 49-55.
12. Mbagwu, F. N; Nwachukwu, C. U; Okoro, O O., Comparative Leaf Epidermal Studies on *Solanum Macrocarpon* and *Solanum Nigrum*. Nature and Science, 5(3), 2007. Available from http://www.sciencepub.net/nature/0503/01-0266-Mbagwu-LEAF-ns-0503.pdf; accessed on 19/03/2011