Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 151-158 *https://dx.doi.org/10.4314/sa.v20i1.13*
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COMPARATIVE MORPHO-ANATOMICAL CHARACTERISTICS AND PHYTOCHEMICAL CONSTITUENTS OF *Aloe vera barbadensis* MILLER AND *Aloe vera* var. *chinensis* (HAW) BERGER

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Received: 15-02-2021
Accepted: 12-03-2021

ABSTRACT

This study was set to investigate the comparative morphological and anatomical characteristics of *Aloe vera barbadensis* Miller and *Aloe vera* var. *chinensis* (Haw) Berger. These are evergreen perennials belonging to Asphodelaceae Juss. in the Order Asparagales Link. The former measures up to 80±20cm in height with lanceolate leaves and rosette habit. The leaves have spiny margins decorated with whitish spots on both foliar surfaces which disappear at maturity. The tubular flowers are orange and densely clustered at the stem apex; corolla is yellowish, tubular and up to 2.5±0.5cm in length whereas the latter is 40±10cm in height with rather lightly green rosette leaves and foliar white spots on foliar surfaces maintained at maturity. The two plants are fleshy and succulent with mild bitter taste. Leaves are amphistomatic with tetracytic sunken stomata. The cells of the epidermal layer are nucleated mostly hexagonal and include pentagonal, heptagonal to square or rounded. Stomatal indices for *Aloe vera barbadensis* adaxial foliar layer is 7.92 % and abaxial 4.76% while *Aloe vera* var. *chinensis* adaxial surface is 7.92% and abaxial 3.85%, not significant. Anatomical studies revealed the cell types from the epidermis, hypodermis, general cortex to the pith are similar in mid-ribs, petioles, stems and nodes. The roots have piths and vasculation is closed type. Phytochemical studies showed the presence of Alkaloids, flavonoids, saponins and tannins in both species, whereas combined anthraquinone was observed absent in both plants. Cardenolide, phlobatannins and free anthraquinones were present in *Aloe vera* var. *chinesis* but absent in *Aloe vera barbadensis* while cyanogenic glycoside was absent in *Aloe vera* var *chinensis* but present in *Aloe vera barbadensis*. The species are used in natural medicine. The information contained in this research would further aid in the taxonomic delimitations of these plants.

Keywords: Comparative, *Aloe vera*, phytochemistry, morphology, anatomy, Asphodelaceae.

INTRODUCTION

Aloe vera is believed to have originated from the Arabian Peninsula but grows wild in tropical, semi-tropical and arid climates. The genus consists of about 500 species around the world (Viljoen, 2008). It is almost stemless or has a condensed stem [Viljoen, 2008]. The margin of leaves is serrated and has small white teeth and flower spikes up to 90cm tall with yellow tubular corolla. There is conflicting literature about the identification of white-spotted form of *Aloe vera* as *Aloe vera* var. *chinensis* (*Gao and Xiao, 1997*; *Wang et al., 2004). Aloe vera barbadensis* grows larger

than a number of other *Aloe* species including *Aloe vera* var *chinensis*. *Aloe vera* var *chinensis* and both plants have lanceolate leaves and rosette habit (Metcalfe and Chalk, 1950). *Aloe vera* leaves contain polymannans, anthraquinone, c-glycosides, anthrones and other anthraquinones (King *et al*., 1995; Eshun *et al.*, 2004). It is proven to be the source of anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes and flavonoid. Epidermal cells have sunken stomata equally distributed on both adaxial and abaxial foliar surfaces, (Olubunmi and Anthony, 2014). The relevance of the study is to enhance information on the existing literature and taxonomic properties of these plants. Thus the objective is aimed at considering the comparative morphological, anatomical and phytochemical characteristics of *Aloe vera barbadensis* Miller and *Aloe vera* var. *chinensis* [Haw] Berger.

MATERIALS AND METHODS

Geographic Location

The location of the parent plant studied was Port Harcourt, Rivers State, Nigeria.

Morphological Studies

The meter rule was used to ascertain the plant height, leaf length and width etc.

Micro-morphological (Epidermal) Studies

Fresh leaves and young stem collected for this study were peeled and subjected to alcohol solutions in the ratio of 50%, 75% and absolute alcohol respectively. The cleared epidermal layers obtained were stained with safranin for 5 minutes washed and counter stained with Alcian blue for same time interval, l washed and temporarily mounted in aqueous glycerol solution. Photomicrographs were taken from good preparations. The Stomatal Index [S.I.] was obtained using the formula:

$$\text{S. I.} = \frac{S}{S+} \quad X \quad \frac{100}{1}$$

Where *S* and *E* are mean numbers of stomata and epidermal cells respectively within the particular area under investigation. Likewise the Trichome Index (T.I) was obtained using:

$$\text{T. I.} = \frac{T}{T+} \quad X \quad \frac{100}{1}$$

Where T and E are trichomes and epidermal cells respectively within the study area.

Anatomical Study

Two weeks to one month old plants were used for this study. Harvested stems, leaves, petioles, flowers, fruits and roots were fixed in FAA in the ratio of 1:1:18 of 40% formaldehyde, acetic acid and 70% alcohol for at least 48 hours following the method of Johansen, (1940). The free hand sectioning using a systematic arrangement of 5 razor blades as described by Wahua (2020) was also adopted. Microphotographs were taken from good preparations using Sony camera of 7.2 Mega pixels having 2.411 LCD monitor and High sensitivity ISO 1250.

Phytochemical Studies

Leaves of the *Aloe vera* species studied were sun dried for 72 hours (3 days) and weighed. Fifty grammes (50g) of the dried leaves were macerated in 96% ethanol using a pestle and a mortar. The extract was thereafter filtered and evaporated to dryness (constant weight) using a rotary evaporator set at 45^0C. Residue yields were noted and

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a portion was used for the phytochemical screening.

Phytochemical Screening for Saponins

Frothing tests were done following the method described by Wall *et al*. (1952). The ability of saponins to produce frothing in aqueous solution and to haemolyse red blood cells was used as screening test for these compounds. 0.5g of the plant extract was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins. The disc was then washed in ether, dried and placed on a 7% blood nutrient agar. Complete haemolysis of red blood cells around the disc after 6 hours was taken as further evidence of presence of saponins.

Test for alkaloids

This was carried out using 0.5g of the plant extract which was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated (Harborne, 1973 and Trease *et al.*, 1989). A modified form of the thin-layer chromatography (TLC) method as described by Farnsworth *et al.* (1962) was used. One gram (1g) of the extract was treated with 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10ml portions of chloroform. The extracts were combined and concentrated to 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvent systems were used to develop each plant extract, these were: methanol, ethanol, ethyl acetate and water respectively. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. A positive reaction on the chromatograms (indicated by an orange or darker colored spot against a pale yellow background) was confirmatory evidence that the plant extract contained alkaloid.

Test for tannins

Five grammes (5g) of each portion of plant extract was stirred with 10ml of distilled water, filtered, and 5% ferric chloride reagent was added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Shoppee, 1964).

Test for anthraquinones

Bontrager's test was used. Five grammes (5g) of each plant extract were shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution (10mls of NH_3 and 90mls of distilled water) added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated the presence of free hydroxyanthraquinones

Test for combined anthraquinones

Five (5g) of each plant extract was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammonia phase (lower layer) indicated the presence of

anthraquinone derivatives in the extract (Trease and Evans, 1989).

Test for phlobatannins

The deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Trease and Evans, 1989).

Test for cardiac glycosides

Lieberman's test was used in which 0.5g of the extract was dissolved in 2ml of acetic anhydride and cooled in ice. One milliliter (1ml) of Sulphuric acid was carefully added in drops until a color change from violet to blue to green indicated the presence of a steroidal aglycone portion of the cardiac glycoside (Shoppee, 1964).

RESULT

Morphological Study

The morphological features of *Aloe vera barbadensis* Miller and *Aloe vera* var *chinensis* (Haw) Berger are thus: The former attains up to 80±20cm in height and the leaves possess sharp apex that are lance-shaped and rosette in structure, having spiny margins decorated with whitish spots on both foliar surfaces which disappear at maturity whereas the latter is 40±10cm in height with rather lightly green rosette leaves and foliar white spots on foliar surfaces maintained at maturity. The plants have tubular flowers which are orange and densely clustered at stem apex. The yellowish corolla is tubular up to 2.5±0.5cm in length for both plants. They are succulent. Plate 1 and 2.

Plate 1: *Aloe vera barbadensis* Miller

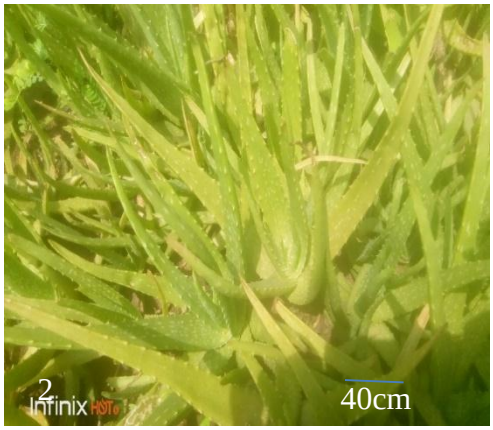

Plate 2: *Aloe vera* var. *chinensis* (Haw) Berger

Epidermal Study

Plants are amphistomatic, stomata are sunken and tetracytic. Epidermal cells are nucleated, mostly hexagonal but include pentagonal, heptagonal to square or rounded. Stomatal indices for *Aloe vera barbadensis* adaxial foliar layer is 7.92 % and abaxial 4.76% while *Aloe vera var. chinensi*s adaxial surface is 7.92% and abaxial 3.85%. See plates 3, 4, 5 and 6

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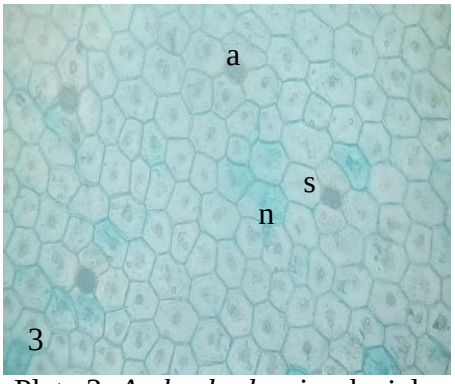

Plate 3: *A. barbadensis* adaxial foliar

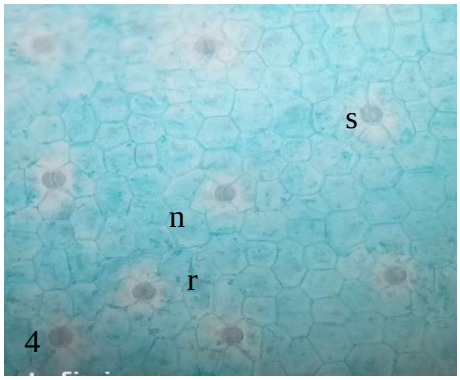

Plate 4: *A. barbadensis* abaxial foliar

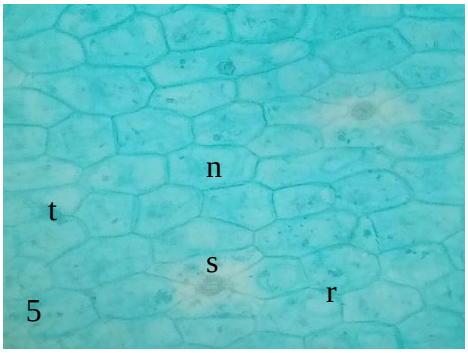

Plate 5: *A.* var. *chinensis* adaxial foliar

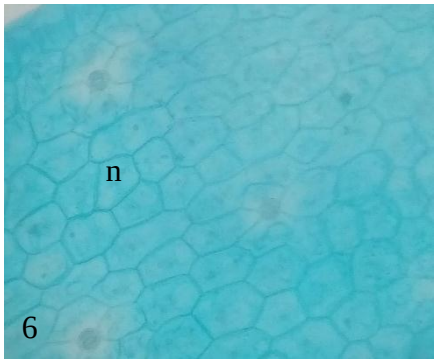

Plate 6: *A.* var. *chinen*sis foliar abaxial

Key: 'S' represents sunken stoma, 'n' stands for nucleated epidermal cells, 't' for Trichome, 'r' for raphides.

Anatomical Study

Anatomical studies revealed the cell types from epidermis, hypodermis, general cortex to the pith are comparatively similar in the mid-ribs, petioles, stems and nodes. The roots have piths and vasculation is closed. Plates 6, 7, 8 and 9.

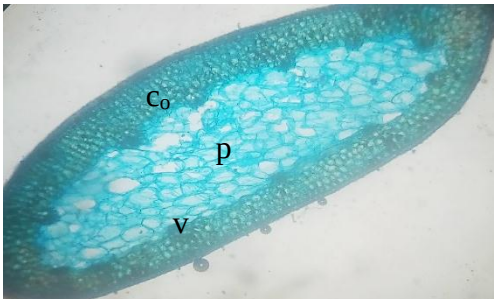

Plate 6: *A. barbadensis* Leaf anatomy. T. S.

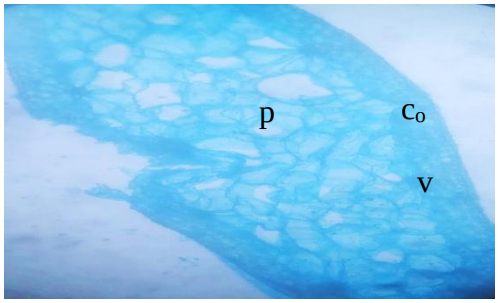

Plate 7: *A.* var. *chinensis* Leaf anatomy. T. S.

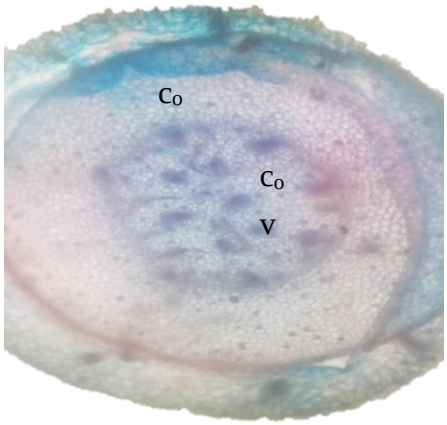

Plate 8: *A. Barbadensis* stem anatomy. T. S.

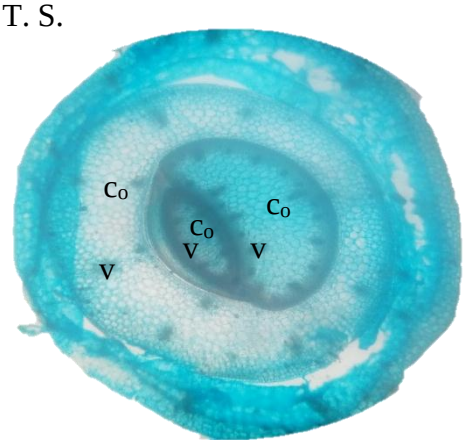

Plate 8: *A.* var. *chinensis* stem anatomy. T. S.

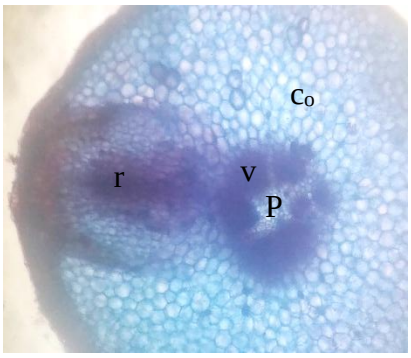

Plate 8: *A. barbadensis* root anatomy. T. S.

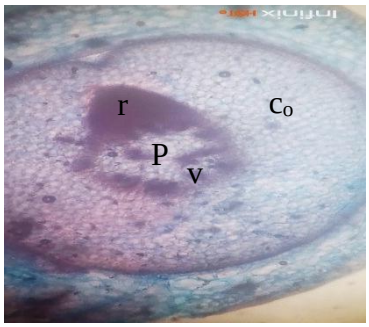

Plate 8: *A.* var. *chinensis* root anatomy. T. S.

Key: 'P' represents pith, 'v' stands for vascular bundle, 'r' represents root hair emerging from the pericycle and 'C_o' stands for cortex

Phytochemical Study

Phytochemical studies showed the presence of Alkaloids, flavonoids, saponins and tannins in both species, whereas combined anthraquinone was observed absent in both plants. Cardenolide, phlobatannins and free anthraquinones were observed present in *Aloe vera* var. *chinesis* but absent in *Aloe vera barbadensis* while cyanogenic glycoside is revealed in *Aloe vera barbadensis*. The species are used in natural medicine. See table 1.

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Table 1: Phytochemical Studies of *Aloe vera barbadensis a*nd *Aloe vera* var. *chinensis*.

PHYTOCHEMICAL TEST	*Aloe vera barbadensis*	*Aloe vera var. chinensis*
Alkaloids Mayer's test	 +ve	 +ve
Flavonoids NaOH test	 +ve	 +ve
Tannins $FeCl_3$ Phlobatannins	 +ve -ve	 +ve +ve
Anthraquinones[Bontrager's test] free anthraquinone combined anthraquinone	 -ve -ve	 +ve -ve
Cardenolide Keller-Killiani test Cyanogenic glycosides	 -ve +ve	 +ve -ve
Saponins Frothing test	 +ve	 +ve

DISCUSSION

Observation on vegetative and floral features of *Aloe vera barbadensis* Miller and *Aloe vera* var. *chinensis* (Haw) Berger revealed both species as evergreen perennials with absence of mid-ribs. Stomata are amphistomatic and sunken type, tetracytic for both species, which is in line to the findings of (Olubunmi and Anthony, 2014). They have very short stem, and sometimes condensed for very young ones while corolla appears yellowish with terminal flowers, this is also in agreement with the description of Viljoen (2008). *Aloe vera barbadensis* grows larger than *Aloe vera* var *chinensis* and both plants have lanceolate leaves and rosette habit as also supported by Metcalfe and Chalk (1950). *Aloe vera* var *chinensis* retained their whitish spotted characteristics whereas those of *Aloe vera barbadensis* lose their whitish spots at maturity; this is in concordance to the reports of *Gao and Xiao, (1997) and Wang et al. (2004).* Anatomical studies revealed the cell types from the epidermis, hypodermis, general cortex to the pith are similar in mid-ribs, petioles, stems and nodes. The roots have piths and vasculation is closed type, this is in line with the work of Metcalfe and Chalk (1950). Stomata are almost evenly distributed on both foliar surfaces. Cardiac glycosides, and other anthrax quinones haven been identified in both species in line with the finding of King *et al.* (1995). Saponins and flavonoids are present in both species.

CONCLUSIONS

Aloe vera bardensis Miller and *Aloe vera var. chinensis* (Haw) Berger are two related plants especially at early growing stages, observed with whitish spots on both foliar epidermis but at flowering some differences

are spotted. They are both useful in tradomedicine. In as much as morphological, anatomical and phytochemical studies on these plants may not be altogether new, the karyotypes, quantitative aspect of phytochemistry and DNA barcodes may be essential area of future interest.

Acknowledgement

We commend Onwuna, Omasiri B. for her effort in providing the initial field collection of the plants and some of the Laboratory work.

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