Elemental composition and morphological studies of *Quassia undulata*

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Abstract. *Quassia undulata* is a perennial medicinal plant species belonging to the family *Simaroubaceae*. It is mostly used in traditional medicine for treatment and management of a wide range of diseases. In this study, the elemental composition and micromorphological characteristics of the leaves of *Q. undulata* were are evaluated by standard methods. The data obtained for the elemental and chemo-microscopic composition of the powdered leaves, and the foliar anatomical features of the fresh leaves with their distinct characteristics will be useful in the systematics, proper identification, authentication, and standardization of this plant for medicinal purposes.

Key words: chemoscopy, macromineral, micromineral, morphology

Introduction

Quassia undulata (Guill. & Perr.) D. Dietr. commonly known as Akan-asantehotoro in Ghana and Oriji in Southwest Nigeria belongs to the family *Simaroubaceae* (Odubanjo & al. 2018). It is a fast-growing perennial shrub or a small to fairly large tree that can attain a height of up to 24 meters (Gyakari & Cobbinah 2008). It is commonly found in the grasslands in tropical and subtropical Africa, Asia, Australia, and America (Iko & Eze 2012). In general, minerals, an essential component of a plant like *Q. undulata,* are inorganic chemical constituents required by the living organisms for a variety of life-dependent functions and processes. Minerals are mostly taken up by plants from the soil, while other organisms (man and animals) acquire them from the plants (Aliero & Usman 2016). The knowledge of nutritional composition of the studied plant species is of utmost importance and may be helpful in addressing mineral deficiencies in rural locality. Also, the importance of minerals in human and animal nutrition cannot be undermined as they play crucial roles in a vast array of metabolic processes, help in maintaining the acid-base balance and are also involved in muscle contractions (Soetan & al. 2010).

Apart from the earlier mentioned importance of mineral composition of plants and the pharmacological uses of *Q. undulata*as highlighted by Odubanjo & al. (2018) and Ajaiyeoba & al. (2000), knowledge of micromorphological features of leaves is also essential in plant taxonomy, as it serves as a useful anatomical tool in resolving taxonomic conflicts in different groups of plants. Plant characteristics such as orientation, size and length of the ultra-structures of epidermal cells, trichomes and stomata are of high importance in taxonomy and phylogeny (Albert & Sharma 2013). Chukwuma & al. (2014) have reported that the leaf epidermis studies have immensely improved the taxonomic researches of some taxa. Foliar micromorphological studies of a wide array of plants have been reported that aided their identification (Sonibare & al. 2014). Despite the immense medicinal importance of *Q. undulata*, very few reports are available on its micromorphology, which is one of the well-established techniques for pharmacognostic evaluation. Hence, this study is aimed to determine its mineral

composition and micromorphological characteristics, which may be of taxonomic importance in the species identification and its nutritional potential.

Material and methods

Sample collection and preparation of plant materials

Fresh leaves of *Q. undulata* were collected from the Herbal Garden of Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. They were identified and authenticated at the Forest Herbarium of Ibadan, with a voucher number FHI-102099. The leaves were air-dried to constant weight at room temperature for two weeks. They were pulverized with the aid of Ricoh grinder (MG601 model) and stored in an air-tight bottle prior to mineral analysis and microchemical testing. Another set of collected fresh leaves was used for the macroscopic study, transverse and epidermal sectioning.

Mineral analysis

Elemental composition of *Q. undulata* leaves was determined according to the standard analytical method (AOAC 2010), where a pulverized sample was digested using nitric and perchloric acids. Calcium, magnesium, iron, copper, manganese, zinc, and phosphorus were determined from the filtered aliquots using Atomic Absorption Spectrophotometer (Buck Scientific; 210VGP Model), while sodium and potassium were determined with the aid of a Flame photometer (SearchTech British; FP640 Model). All analyses were done in triplicate.

Microchemical analysis

A pinch of powder samples was placed on a grease-free microscopic slide, along with a drop of glycerine and water (1:1) and then covered with a clean cover slip and observed under the compound microscope (×10 magnification). The powder samples were also mounted and stained with phloroglucinol, concentrated hydrochloric acid (HCl), diluted iodine solution, Ruthenium Red, Sudan Red and diluted HCl.

Macromorphological study

The macromorphological examinations of fresh leaves of *Q. undulata* such as dimensions, surface, point of attachment, lamina, venation, shape, base, presence or absence of petiole, the apex, margin, base, lamina, texture, including the organoleptic profile (colour, odour and taste) were carried out according to described methods (WHO 2007; Evans 2009).

Transverse sectioning

Transverse section of the leaf sample was done by the sectioning method according to Kolawole & al. (2017). The leaves were sliced with a sledge microtome (Reichert Austria model S105). Samples were stained in Safranin-O for two minutes and then dehydrated in increasing concentrations of ethanol (30 %, 50 %, 70 % and 90 %). Clove oil was used for clearing, and Canada balsam was used as mounting agent before observations under a light microscope (Olympus MR1606762 Model and Amscope digital camera MD35).

Epidermal sectioning

Fresh leaf samples of *Q. undulata* (4 cm long) were soaked in concentrated HNO_3 in covered glass Petri dishes for about 4 hours. After tissue disintegration of the leaves was confirmed by the presence of bubbles, the epidermises were placed in Petri dishes containing distilled water and rinsed. The adaxial and abaxial layers were separated by forceps and tissue debris was removed with a fine camel brush. The separated adaxial and abaxial layers were transferred for 2 minutes into Petri dishes containing 50 % ethanol to harden the cells. The epidermises were thereafter stained with Safranin-O for 5 minutes and rinsed in distilled water to remove excess stain. The stained tissues were then mounted in 25 % glycerol onto clear-glass microscope slides and covered with cover slips. The edges of the cover slip were sealed with nail varnish to prevent dehydration. A slide was prepared for each epidermis and were labelled appropriately, prior to viewing under the microscope (Olympus MR1606762 and Amscope digital camera MD35). Images of each samples were taken for qualitative microscopic analysis (Chukwuma & al. 2014; Kolawole & al. 2017).

The quantitative microscopic determination of epidermal surfaces of *Q. undulata* was done according to the standard method (Kolawole & al. 2017). Clean microscopic slides with cover slips containing the already prepared abaxial and adaxial epidermal sections were mounted on a set-up camera lucida with ×10 objective (a small stage micrometer division equals 10 μm; calibration factor equals 2.7) on a light microscope. The stomata number, stomata index, cell density, and

palisade ratio were all determined from the different views observed on the microscope and by appropriated formulae. The length and width of stomata and cells were measured by calibration of the microscope eyepiece by a stage micrometer. The mean value and standard error for all microscopic parameters were also calculated on the basis of occurrence of each examined character in a total of 20 fields of view, as quantitative microscopic determinations.

Statistical analysis

Data obtained from examinations of all epidermal sections were computed into Microsoft excel 2013 and means ± standard deviation was calculated. Values were presented as means ± standard deviation at 95 % confidence level.

Results and discussion

Elemental analysis

The mineral composition of the analysed *Q. undulata* leaves has revealed that the studied species contains both macro and micro elements in varying amounts (Table 1). The results have shown that the macro elements calcium, magnesium, potassium, phosphorus, sodium were 656, 481, 220, 160, and 63 mg/100g, respectively. The micro mineral concentrations for iron, zinc, copper, and manganese were 135, 7, 3 and 3 mg/100g, respectively (Table 1).

The mineral profile of *Q. undulata* confirmed calcium as the most abundant mineral element with 656 mg/100g. Ailero & Usman (2016) has concluded that calcium does not only take part in the development of teeth and bones but also regulates the nerve and muscle functions. Calcium also aids blood coagulation, enzyme activation, membrane permeability, and muscle contraction to name a few of its effects (Soetan & al. 2010). RDA for adults within the 19–50 years age bracket is 1000 mg, while the older adult age groups require 1200 mg (Odewale & Lawal 2018). The value obtained for calcium (656 mg/100g) in this study exceeds 613 mg/100g obtained for the *Crescentia cujete* leaves (Olaniyi & al. 2018). Magnesium is required for oxidative phosphorylation and activates pyruvic acid carboxylase and the condensing enzyme required for reactions in the citric acid cycle (Soetan & al. 2010). The amount of magnesium found in *Q. undulata* was 481 mg/100g, which exceeds 207 mg and 256 mg/100g obtained for the *C. cujete* leaves (Olaniyi & al. 2018) and *Diospyros mespiliformis* (Hassan & al. 2004). Phosphorus acts as a phosphate buffer aiding the homeostasis (the maintenance of fairly constant internal environment in the body). It binds with calcium to form calcium phosphate, which is needed for the development of teeth and bones (Odewale & Lawal 2018). Phosphorus has been also reported to be involved in the synthesis of such high-energy compounds as ATP, ADP, phosphoproteins and phospholipids (Soetan & al.2010). The result obtained for the *Q. undulata* leaves of 160 mg/100g is below 700mg/100g, while the recommended daily allowance for both men and women is 700 mg (Odewale & Lawal 2018).

Sodium is an important cation in the extracellular fluid, which helps maintain the membrane potentials, acid-base balance and the osmotic pressure of body fluids in the activation of muscle and nerve function (Soetan & al. 2010). Although the value of sodium of 63 mg/100g obtained for *Q. undulata* is higher than the 61 mg/100g obtained for the *Crescentia cujete* leaves, it is far below the WHO recommended limit for sodium intake of 2 g/day for adults, in order to reduce blood pressure and risk of cardiovascular diseases (WHO 2012). Iron functions in the proper myelination of the spinal cord and the white matter in the cerebellar folds of the brain. Kermanshah & al. (2003) have reported that iron is a vital trace element for haemoglobin formation, normal functioning of the central nervous system and in the carbohydrates, proteins and fat oxidation. It also plays an important role in the oxygen transfer in the body. Low iron content causes nose bleedings, gastrointestinal and myocardial infections. Iron deficiency results in anaemic conditions, with a decreased level of the red blood cells as a result of major shortage of iron content in the body (Mlitan 2014). The

Table 1. Mineral composition of *Quassia undulata* leaves.

Mineral element	Composition (mg/100g)
Calcium	656 ± 0.004
Magnesium	481 ± 0.004
Potassium	220 ± 0.020
Phosphorus	160 ± 0.030
Sodium	63 ± 0.003
Iron	135 ± 0.002
Zinc	7 ± 0.001
Copper	3 ± 0.001
Manganese	3 ± 0.001

Note: The data are presented as mean ± standard deviation of three replicates.

high value of iron 135 mg/100g obtained for the present study suggests its hematinic property, which may be employed as a remedy for iron deficiencies. Zinc is extremely important for numerous body functions but its deficiency is associated with mental impairment, emotional disorder and irritability (Watts 1997; Gupta &al. 2014). Copper plays an important role in cellular defence and protection of the mucous membrane (Ayoola & al. 2013), while manganese helps in iron absorption in the body (Olusanya 2008).The concentrations of 7.3 and 3 mg/100g detected in this study for zinc, copper and manganese, respectively, are comparable to the values (30, 3, 5 mg/100g) obtained for *Justicia secunda* leaves, respectively (Ogunbamowo & al. 2020).

Microchemical analysis

Powder microscopic analysis indicated the presence of calcium carbonate as the only calcium compound in the tested sample (*Q. undulata* leaves). Other macro molecules such as starch granules, fat deposits, a mucilaginous compound, and lignin were absent (Table 2).

Epidermal sections

The qualitative foliar epidermal characteristics of *Quassia undulata* are summarized in Table 3. The epidermal cells on both abaxial and adaxial surfaces were generally irregular and polygonal, with straight anticlinal walls. Anomositic stomata were observed on the abaxial surface, while there were none on the adaxial surface. Trichomes were found on both surfaces, with glandular, non-glandular and straight trichomes on the abaxial surface and non-glandular and straight trichomes on the adaxial surface.

Table 2. Powder microscopic analysis of *Quassia undulata* leaves.

Components	Results
Starch	Absent
Fat	Absent
Mucilage	Absent
Calcium compound	Calcium carbonate
Lignified cells	Absent

Table 3. Qualitative foliar epidermal characteristics of *Quassia undulata* leaves.

Characters	Abaxial surface	Adaxial surface
Epidermal cell shape	Irregular, polygonal	Irregular, polygonal
Anticlinal wall	Straight	Straight
Stomata type	Anomositic	Absent
Trichomes	Glandular/ non-glandular/ straight	Non-glandular/ straight

The photomicrographs of *Quassia undulata* leaves revealed all structural components in the abaxial and adaxial sections both on transverse and epidermal surfaces shown in Plate 1, 2 and 3. In the transverse section, straight, glandular and non-glandular trichomes were seen attached to the thick cell wall on the abaxial and adaxial surfaces. Trichomes were obviously present both on the abaxial and adaxial surfaces of the epidermal layer though; more trichomes were found on the adaxial than on the abaxial surface (Plate 1 and 2). Numerous vascular bundles were found towards the middle region of the leaves (Plate 1). Anomositic stomata were present in the abaxial section only as shown in Plate 2. No stomata were found in the adaxial surface (Plate 3). Glandular trichomes indicated a role in secretion of specific phytochemicals, which could serve as a natural defence mechanism of the species against herbivores, insects and microbes (Seyed & Ali, 2014; Kortbeek & al. 2016). Presence of stomata on the abaxial surface is indicative of the hyposomatic nature of the plant under study (Abdulrahaman & Oladele 2008). The foliar micromorphological characters of medicinal plants, including *Q. undulata,* provide a comprehensive and thorough evidence for the proper identification of a wide range of species. These are some useful taxonomic characters that can help in minimising the misrepresentation and misidentification of medicinal plants.

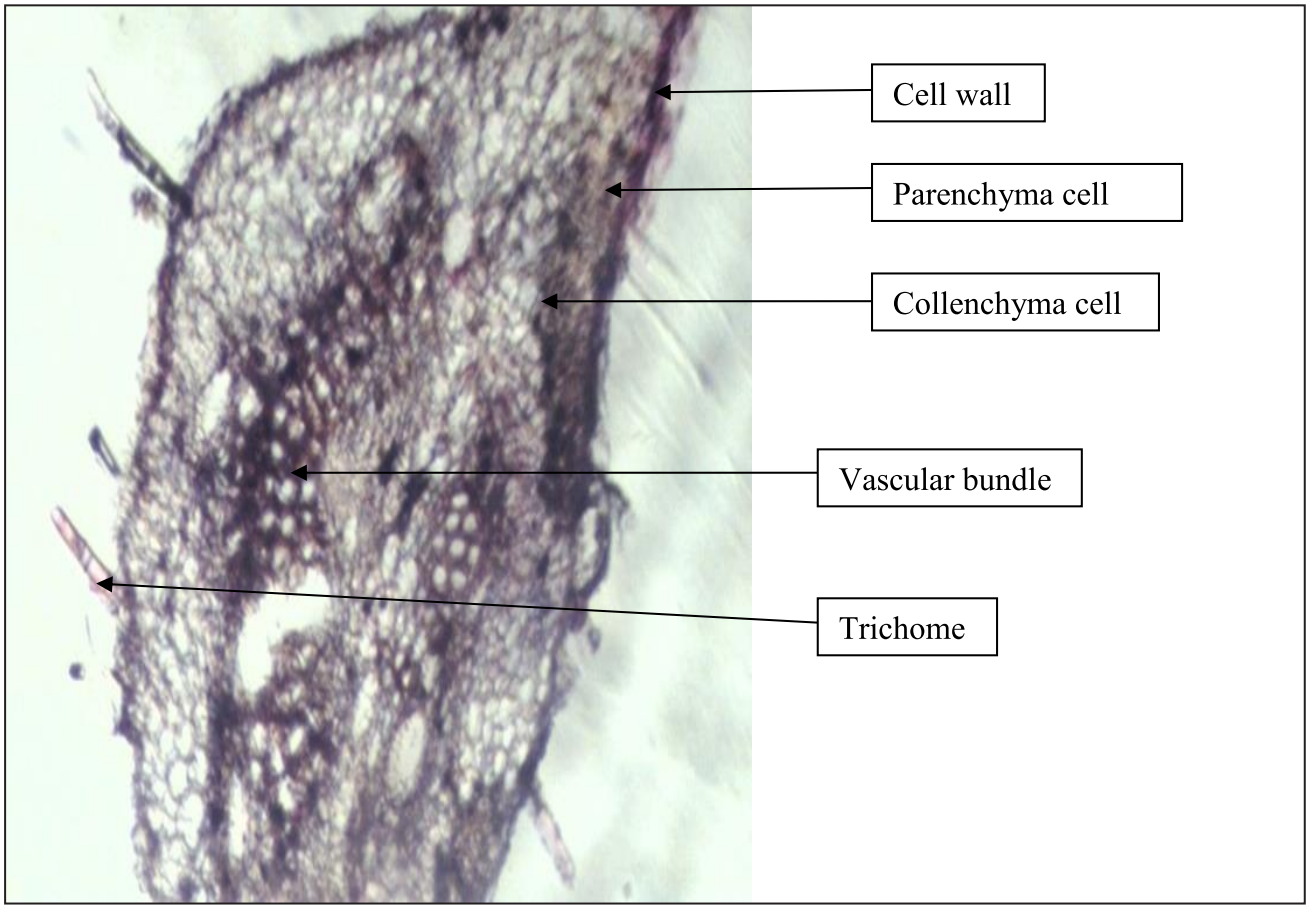
Quantitative foliar epidermal characteristics of the *Quassia undulata* leaves

The quantitative data obtained in this study indicated that epidermal cells and stomata cells found on the adaxial and abaxial surfaces of the *Quassia undulata* leaves varied greatly in terms of size and density (Table 4). The length of epidermal cells was 79.65–57.59 μm and the width was 44.26–30.28 μm on the

Table 4. Quantitative microscopic leaf characteristics of the *Quassia undulata* leaves.

Characters	Adaxial surface	Mean ±SE	Abaxial surface	Mean ±SE
Cell length (μm)	79.65–57.59	70.65 ±7.77	50.33–23.39	38.95 ±9.88
Cell width (μm)	44.26–30.28	37.01 ±4.81	36.91–22.89	28.18 ±4.33
Stomatal length (μm)	Nil	Nil	36.76–25.41	31.32 ±3.51
Stomatal width (μm)	Nil	Nil	20.84–11.23	17.05 ±3.43
Stomatal density	Nil	Nil	32.00–16.0	21.83 ±7.57

Note: The values are presented within the maximum – minimum range above, mean ± standard error beneath.



Plates 1. Photomicrographs of transverse section of *Quassia undulata* leaf x10.

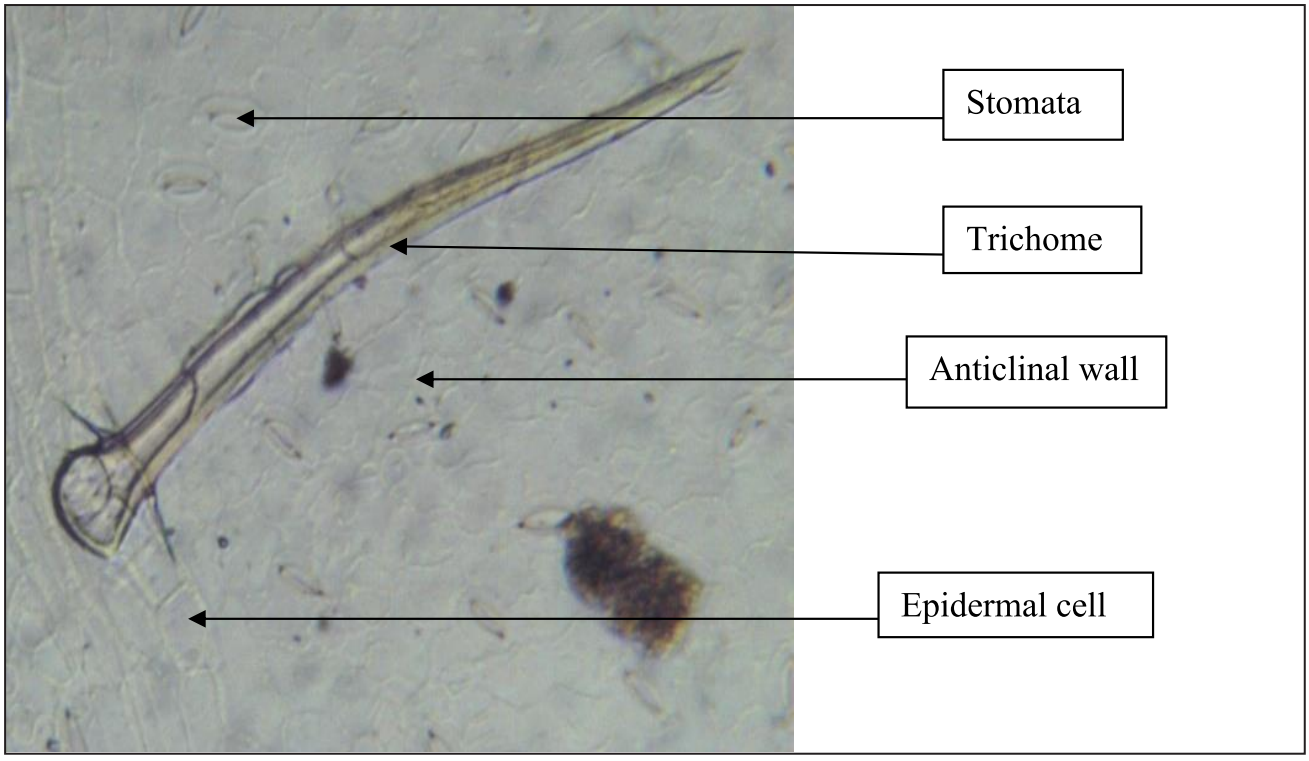


Plate 2. Photomicrographs of Adaxial surfaces of epidermal layer of *Quassia undulata* leaf x10.

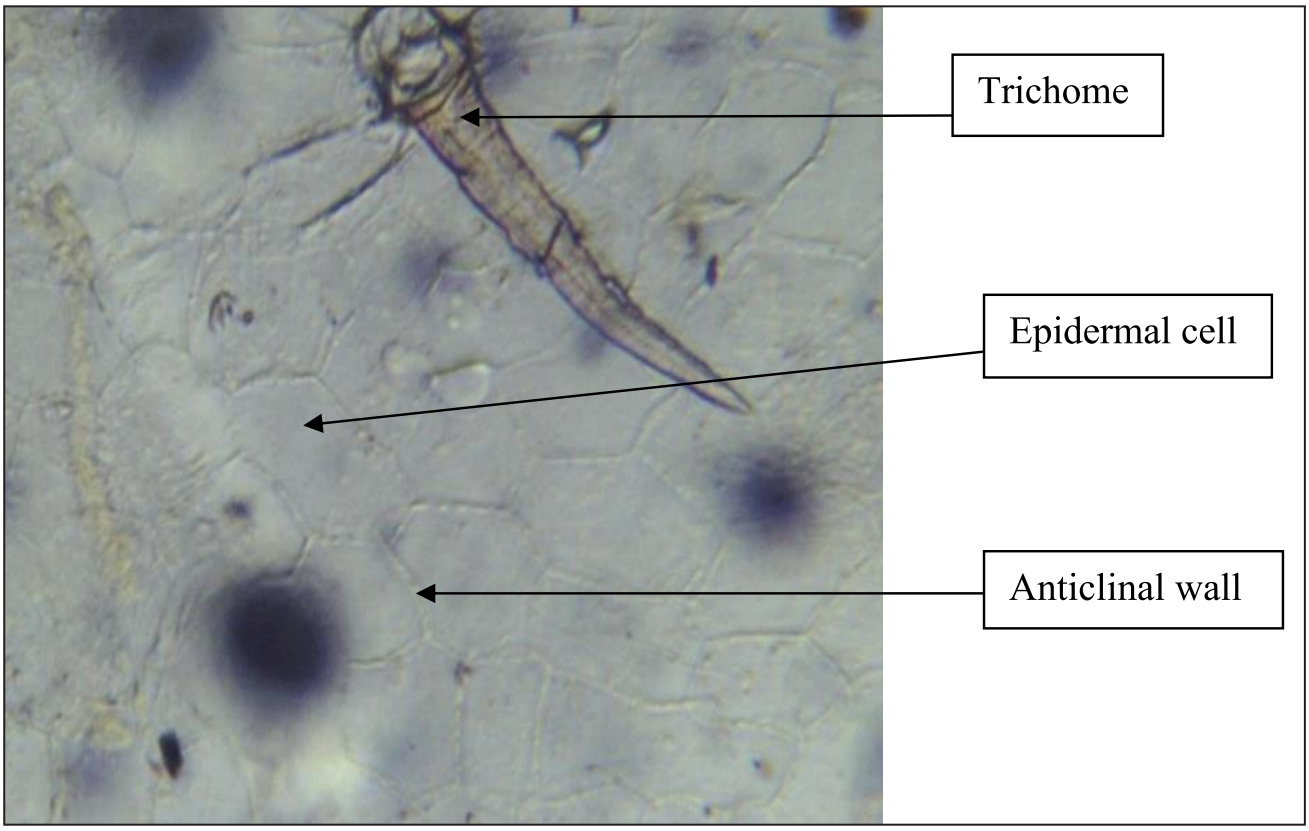


Plate 3. Photomicrographs of Abaxial surfaces of epidermal layer of *Quassia undulata* leaf x10.

abaxial surface. Whereas, the length of epidermal cells on the adaxial surface was 50.33–23.39 μm and the width was 36.91–22.89 μm, respectively. The average stomatal size on the abaxial surface was 36.76–25.41 μm (length) and 20.84–11.23 μm (width) and stomatal density was 32.00–16.00.

Conclusion

In the present study, *Quassia undulata* has been found to contain macro and micro-minerals that are beneficial to humans and animals. Isolation and usage of such bioactive compounds in orthodox medicine will have to go a long way in providing treatment for some ailments. The unique micromorphological characters, such as the irregular and polygonal epidermal cell pattern; glandular, non-glandular and straight trichomes; anomositic stomata and anticlinal wall patterns provide baseline information on the species' uniqueness among other members of its genus. This will assist the easy identification, authentication and standardization of the species for its medicinal use.

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