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Macroscopic and microscopic features of diagnostic value for *Warburgia ugandensis* Sprague leaf and stem-bark herbal materials

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Warbugia ugandensis **is among the ten most utilized medicinal plants in East Africa. Stem-bark and leaves are used as remedies for malaria, stomachache, coughs and several skin diseases. Consequently, the plant is endangered because of uncontrolled harvest from the wild and lack of domestication. There is therefore fear of poor quality commercialized products due to lack of quality control mechanisms. The objective of this study was to investigate features of diagnostic value that could be used to confirm its authenticity and purity. Samples in the study were obtained from six different geographical locations in Kenya by random purposive sampling. Macroscopic and microscopic studies of the leaf and stem-bark were done based on a modified method from the American herbal pharmacopoeia. The study revealed over five macroscopic and organoleptic characteristics for *W. ugandensis* leaf and stem-bark including strong aromatic odor and bitter peppery taste. Major microscopic characteristics of the leaf included anomocytic stoma, oil glands and trichomes. Microscopy of stem-bark revealed scaly outgrowths and parenchyma cells in addition to clusters of simple starch granules. Macroscopic and microscopic features of diagnostic value identified can be used to evaluate the quality of *W. ugandensis* herbal materials especially for confirmation of purity and authenticity.**

Key words: Microscopic, macroscopic, Quality, *Warbugia ugandensis*, herbal.

INTRODUCTION

Warburgia ugandensis belongs to the order Canellales and family Canelliacea which is composed of nine species (Sue, 1995). It is a spreading evergreen tree 4.5-30 m tall and up to 70 cm in diameter (Bentje, 1994). Other common English names include East African green wood and East African greenheart (Kokwaro, 2009). This species occurs in lowland rainforests and upland dry evergreen forests. It is native to Democratic Republic of

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Congo, Ethiopia, Kenya, Malawi, South Africa, Swaziland, Tanzania and Uganda and exotic to India (Maundu and Tengnas, 2005).

W. ugandensis is among the ten most valued traditional remedies in Kenya (Kokwaro, 2009). Ethno medical information shows evidence of the plant treating many ailments including malaria, leishmaniasis, African trypanosomiasis, common cold, toothache, stomachache, cough, fever, rabies, muscle pains and several skin diseases (Okello D. and Kang Y (2019). Dried stem-bark and leaves are the parts commonly used and can be chewed, inhaled and/ or boiled in water to form a decoction (Dharani and Rukunga, 2010). With malaria and diarrheal diseases still ranking as major causes of higher mortality rates for all ages in the developing world (Jamison et al., 2006), the medicinal contribution of this plant is very valuable.

The major chemical constituents in *W. ugandensis* are sesquiterpenes of the drimane and colorotane skeletons (Mashimby et al., 1999). Pharmacological studies of compounds characterized in *W. ugandensis* have shown its anti-malarial activity (Muthaura et al., 2007), antimicrobial and cytotoxic activity (Lacroix and Prado, 2011), antifungal activity (Olila and Olwa, 2001), *in-vitro* antileishmanial activity (Ngure and Tonui, 2009), and toxicity to insects, nematodes and fish (Justicia et al., 2005). Because of its wide applications in herbal therapy, *W. ugandensis* is endangered due to uncontrolled harvesting of the stem-bark mostly from the wild and lack of cultivation (Kairu et al., 2013). The demand for *W. ugandensis* is so high that there is a possibility of adulterated or substituted products in the market. This is further complicated by lack of measures to ascertain the quality, especially authenticity and purity of medicinal products sold and claimed to contain *W. ugandensis*. Over the past decades, quality of herbal products has continued to be of concern in many countries (Straus, 2002). This is partly attributed to fake healers and poor-quality products in Africa due to economic and regulatory challenges (Pretorius, 1999). Moreover, the World Health Organization cites the use of poor quality, adulterated and counterfeit products as a major risk affecting the use of herbal medicines in member states (WHO, 2013). Adulterations, deterioration and false claims have previously been reported in herbal drugs (Onyambu et al., 2013). Though quality control of herbal materials can be achieved by use of many methods, macroscopy and microscopy are the first and simplest steps in the confirmation of authenticity and purity of herbal material (AHP, 2011). In spite of macroscopy and microscopy being valuable, rapid and cost-effective tools for quality assessment, they have not been utilized for quality evaluation of many herbal materials used widely in Africa including *W. ugandensis*. This study was therefore designed to use macroscopic and microscopic parameters to determine important features of diagnostic value in *W. ugandensis* herbal materials.

MATERIALS AND METHODS

Collection and preparation of the plant material

Authentic *W. ugandensis* leaf and stem-bark was harvested from Kenyatta University Medicinal Plant Research Garden. The sample met requirements of a reference material which included use of a cultivated plant whose cultivar would be traced, identified by a certified botanist and deposited the voucher specimen in a recognized herbarium (WHO, 2003).

Five other samples from different geographical locations in Kenya as classified by Bentje (1994) and Najma (2002) were obtained by random purposive sampling. The specific locations included Mombasa, Trans-mara, Masimba, Kapsabet and Ngariama Kirinyaga. The samples were identified by a certified botanist and voucher specimens deposited at the East African Herbarium in the National Museums of Kenya, Nairobi (voucher specimen MO/002-008/2013). Leaves were cleaned and rinsed while the outer cork was removed from the stem-bark before being dried under shade. Samples were ground into fine powders using a disk mill, packaged into plastic containers and stored at room temperature. Whole and ground leaf and stem bark were studied; organoleptic characteristics were studied within 1 day of grinding.

Determination of macroscopic and organoleptic characteristics

Macroscopic examination was done by visual and physical examination of whole leaf, stem-bark and their processed powders. Color and shape were determined by examining the untreated sample under daylight, while size was determined by measurement with a graduated ruler. Surface characteristics including texture, fracture and appearance of the cut surface were determined using a hand lens. Determination of softness or hardness was done by touching while bending and rupturing was done to obtain information on brittleness and appearance of the fracture plane. Odor was determined by placing a small portion of the sample in the palm of the hand then slowly and repeatedly inhaling the air over the material. The taste/bitterness was determined by chewing a piece of crude drug in the mouth. At least four people carried out the assessments.

Determination of microscopic characteristics

Microscopy of the samples was done by first softening the dry leaf and stem-bark samples in water. Surface tissues of leaf and stem-bark were observed after rendering pieces of thin leaves and bark transparent by boiling them directly on a slide after adding chloral hydrate. More surface features in leaves were observed after bleaching the leaf by immersing it in sodium hypochlorite for 24 h.

Materials being examined were sectioned using free hand technique by cutting into suitable lengths using a razor blade. Lignified cells were examined after moistening the powder or section on a slide with a small volume of phloroglucinol and allowing it to stand for 2 min then adding 1 drop of hydrochloric acid. Iodine and methyl red were used to stain some tissues of diagnostic value. Powdered materials were prepared by placing 2 drops of fluids used such as water, glycerol, chloral hydrate on a glass slide and then transferring a small quantity of powder using a needle tip moistened with water. Removal of air bubbles was done by passing carefully over a small flame of a micro burner.

A light microscope, Leica BME 13395 H2X (SEO enterprises inc. Lakeland Florida, USA) was used for observations and microscope model-leica (DMILHC Bio) fitted with a camera (CANNON N-DC 150) used for photomicrography. Microscopic observations were done under objectives with a magnification of 4×, 10×, 40×, and 100× (oil immersion). Procedures carried out for the reference sample

Table 1. Macroscopic and organoleptic features for *Warburgia ugandensis* leaf, stem-bark and powders.

Macroscopic property	Leaf	Leaf powder	Stem-bark	Stem-bark powder
Colour	Dark green when fresh, light to pale green when dry	Shiny to green	Pale green when fresh, cream white to brown when dry	Cream white to light brown
Size	Mature leaf; 110-150 mm length and 20-35 mm width	N/A	2-8mm thick	N/A
Shape	Lanceolate-oblong	N/A	Flat to curled when dry	N/A
Texture	Smooth	Fine	Smooth on small trunks, Slightly rough on older ones	Course
Fracture	Short	N/A	Short	N/A
Brittleness	Brittle when dry, breaking with a cracking sound when dry	N/A	Breaks without deformation with snapping sound when dry	N/A
Odour	Fruity, characteristic	Fruity, characteristic	Strong, fruity	Strong, fruity
Taste	Slightly bitter, peppery	Slightly bitter, peppery	Spicy after taste, peppery	Spicy after taste, peppery
Appearance of cut surface	Rough	N/A	Rough	N/A

a b c

Figure 1. Macroscopic features of (a) fresh (b) dried (c) powdered *W. ugandensis* leaf.

were repeated three times for other samples collected from different geographical zones for confirmation of consistency in the results, as recommended by AHP 2011.

RESULTS

Macroscopic and organoleptic characteristics of the leaf and stem-bark

The study established five major macroscopic and two organoleptic features of diagnostic value for *W. ugandensis* herbal materials as shown in Table 1. The diagnostic features may be applicable to whole parts such as leaf and stem-bark or their respective powders. Color, texture, odor and taste could form important tools for evaluating the quality of both whole and powdered materials while size, shape, fracture, brittleness and appearance of cut surface were only applied to whole materials.

The dark green color mostly seen for freshly harvested leaves changes to pale green when dried under shade. The color of powdered leaves is shiny green as shown in Figure 1. Similarly, the color of the stem-bark is pale green to cream white when fresh, changing to cream

Figure 2. Macroscopic features of (a) fresh (b) dried (c) powdered *W. ugandensis* stem-bark.

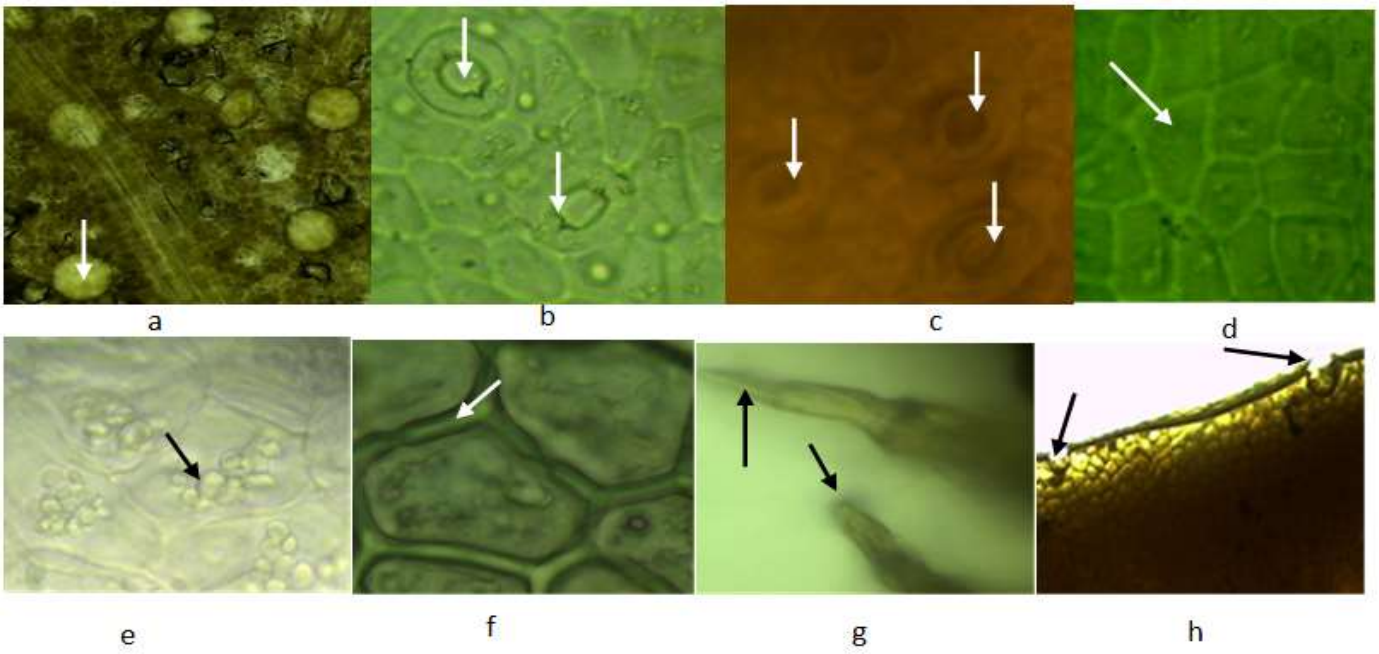

Figure 3. Surface features of diagnostic value for the leaf (Magnification x400): (a) Dotted oil glands, (b) Anomocytic stomata on the upper side of leaf, (c) Scattered anomocytic stomata on the lower side of leaf, (d) Sinuous anticlinal epidermal cell walls with primary walls, (e) Starch granules, (f) thick walled polygonal cells at lower leaf epidermis, (g) Uniseriate non-glandular trichome with warted cuticle in young leaves, (h) older leaf with scars left by broken off trichomes.

white to light brown upon drying. The color of powdered stem-bark remains cream white to brown as shown in Figure 2.

Microscopic characteristics of the leaf, stem-bark and powdered materials

The study established at least eight microscopic surface features of diagnostic value in *W. ugandensis* whole leave as shown in Figure 3. Both lower and upper parts of the leaf have oil glands appearing as translucent spherical dots spreading on both leaf surfaces. The anomocytic stoma is more abundant in the lower part of the leaf. This kind of stomata is irregular celled hence surrounded by cells similar to the other epidermal cells of the leaf. The simple starch granules seen on the leaf appear as clusters, shiny to grayish when the leaf is treated with sodium hypochlorite. The granules appear blue black when stained with iodine. Trichomes seen in

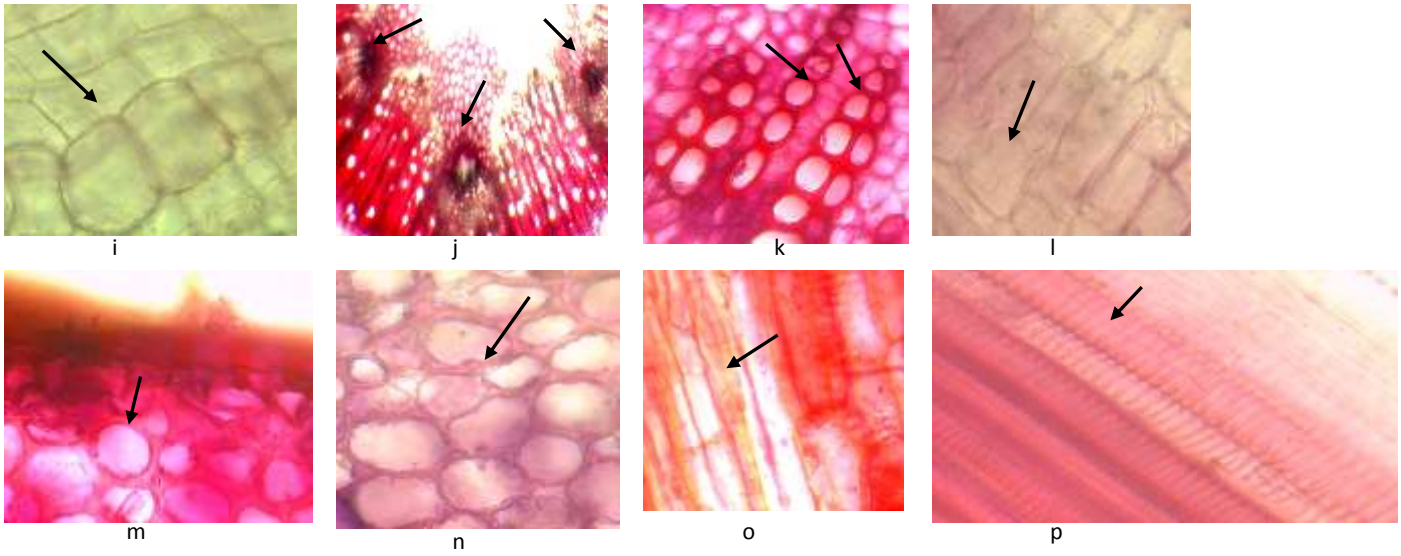

Figure 4. Transverse sections (TS) of leaf cells (Magnification x400): (i) parenchyma cells, (j) vascular bundles arranged in ring form with centered pith, (k) xylem vessels, (l) spongy mesophyll, (m) collenchyma cells, (n) isodiametric collenchyma cell walls, (o) elongated collenchyma cell walls, (p) Palisade cells.

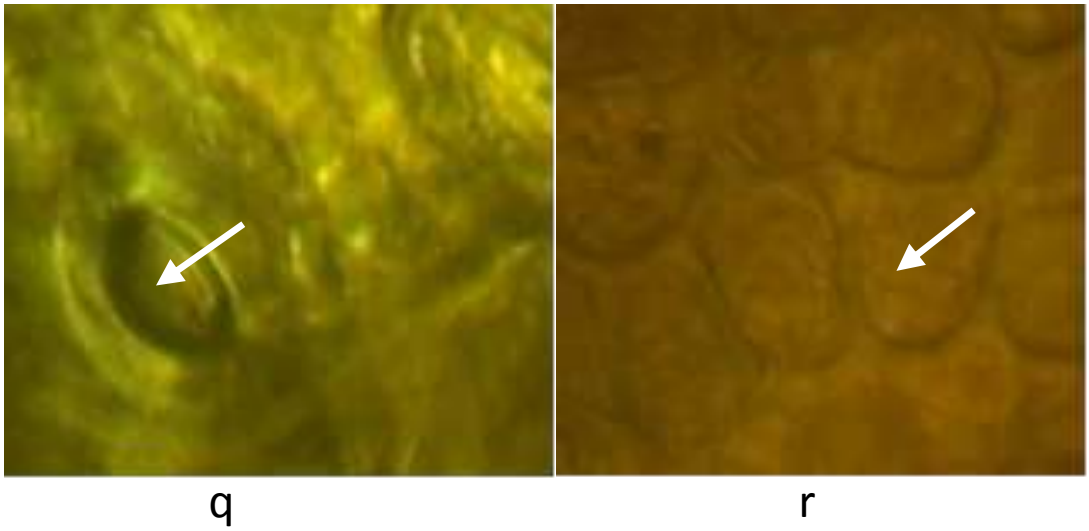

Figure 5. Powdered fragments (Magnification x400) (q) anomocytic stomata in leaf powders fragment, (r) thick polygonal epidermal cells in leaf fragments.

the study are uniseriate nonglandular with warted cuticle and were mostly on the margins of younger fresh leaves. However, in older leaf margins, scars of fallen off trichomes were seen. The sinuous anticlinal cell walls with primary walls appear in both leaf surfaces.

The study further established eight tissues that would be useful in the diagnosis of *W. ugandensis* whole leaf as shown in Figure 4. The parenchyma cells on the leaf are rectangular shaped, thin walled and closely packed while the collenchyma cells had thickened primary wall and more or less spherical. The leaf had palisade cells rectangular in shape, closely arranged in planes parallel to each other.

Fragments of cells such as epidermal cells and stomata appear in leaf powders as shown in Figure 5. The study further established three surface features of diagnostic value for the stembark as shown in Figure 6. Longitudinal section sections of the stembark established three features of diagnostic value as shown in Figure 7 while transverse sections of the stembark also established two key features of diagnostic value as shown in Figure 8. Further studies of the stembark powders showed

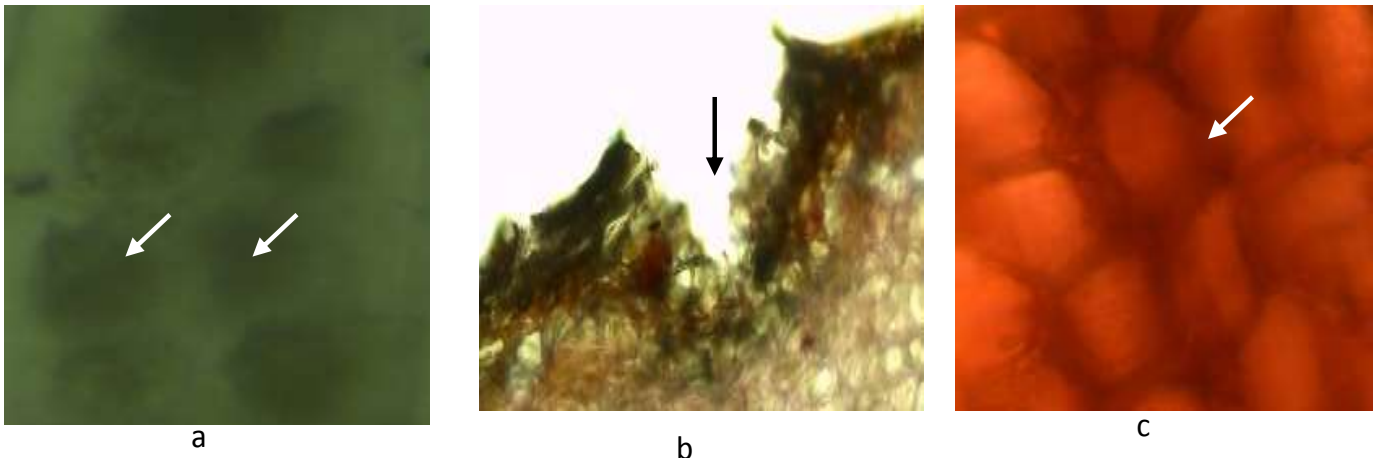

Figure 6. Surface features of diagnostic value for the stem-bark (Magnification x400): (a) Scaly outer bark, (b) Lenticels at bark surface, (c) Parenchymatous cells.

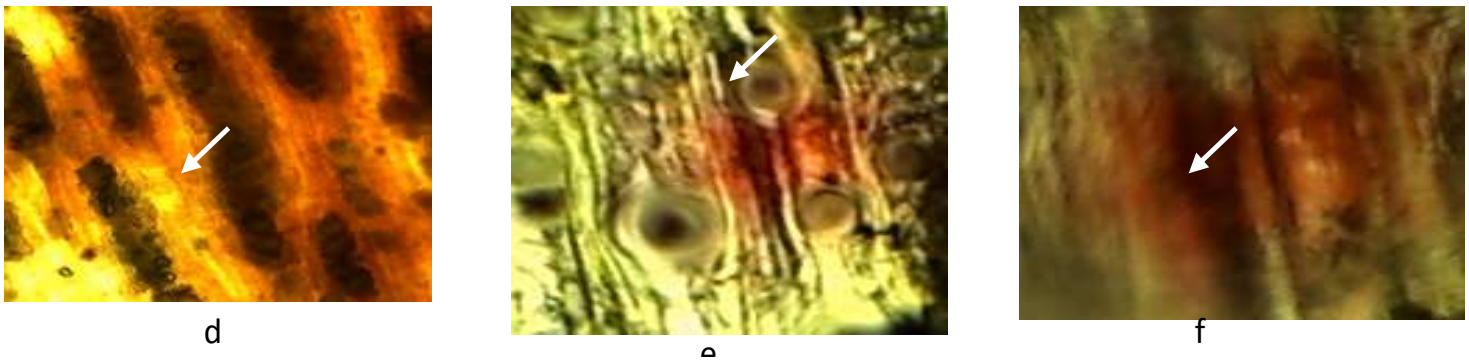

Figure 7. Longitudinal section of stembark (d) Rod-shaped arrangement of simple starch granules at inner side of stem-bark, (e) Stem-bark fibers with lenticels (f) stem-bark with lignified cells.

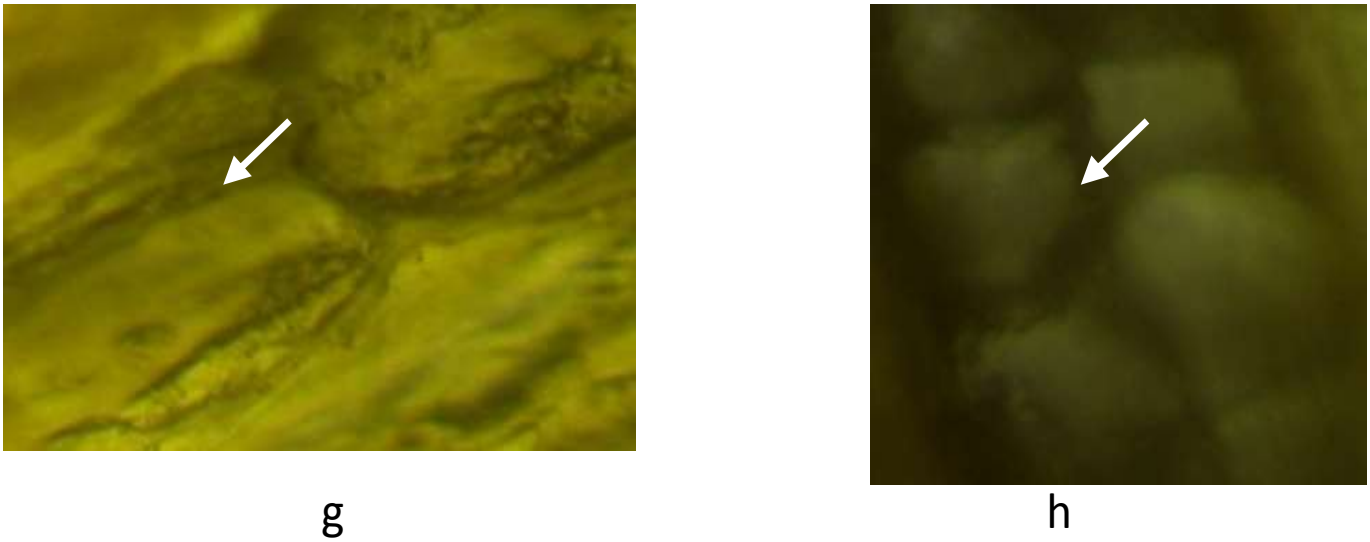

Figure 8. Transverse sections of the stembark (g) Rectangular shaped Parenchyma cells, (h) Thick cell walled parenchyma cells, (i).

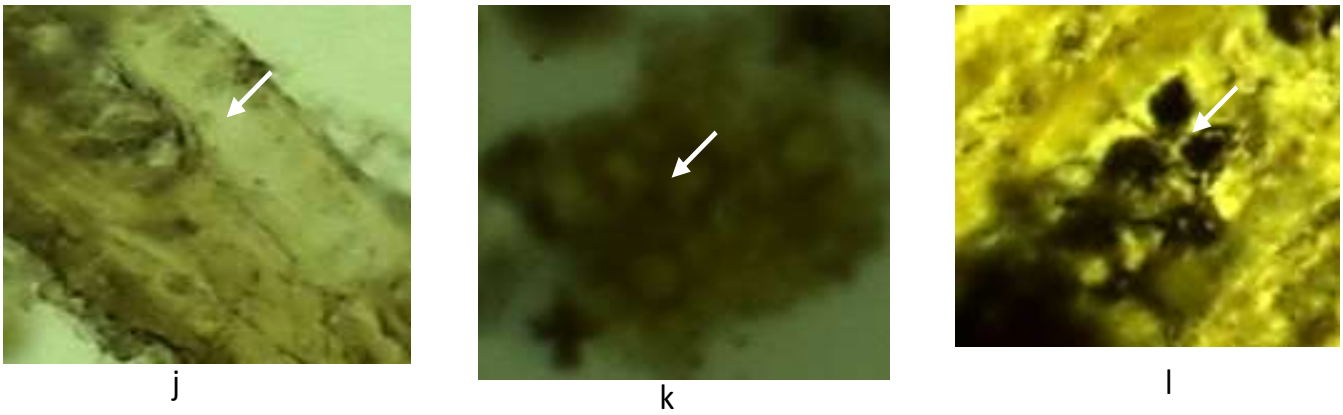

Figure 9. Powdered cell fragments from the stembark (j) parenchyma cell fragments, (k) collenchyma cell fragments, (l) starch granules in bark cell fragments.

fragments of cells in Figure 9.

DISCUSSION

Macroscopy and microscopy have been utilized to establish major characteristics of diagnostic value for *W. ugandensis* herbal materials in this study. The findings agree with some morphological description for *W. ugandensis* leaf and stem-bark given by ICRAF (1992) and Katende (1995). The histological and morphological characteristics described in the study would be used for qualitative evaluation of organized crude drugs in entire and powder forms. The various cellular tissues detected such as trichomes, stoma and starch granules are some of the important parameters which play a role in identification of crude drugs (Evans, 2009).

Microscopy has been used for the standardization of many herbal drugs including *Zanthoxylum armatum (*Kamalesh et al*.,* 2013), *Ficus* species (Koilpillai et al., 2010), *Aloe vera, Radix astragali* and *Alium cepa* in WHO monographs (WHO, 1999). Specific features used for quality control include, the parasitic stomata for Senna leaves and the lignified trichomes in *Nux vomica* (WHO, 1998). The microscopic characteristics described in this study form the first ever reported histological studies for *W. ugandensis* herbal materials. The World Health Organization and the American Herbal Pharmacopoeia Recommend Microscopy as the quickest, most affordable way of establishing the purity and authenticity of herbal materials (WHO, 2007). However, the importance of having an authentic reference material against which organoleptic and macroscopic properties can be compared with others whose quality is being evaluated is emphasized (WHO, 1998). The study findings could therefore be used as reference because the major attributes of a reference material in pharmacognostic studies were considered.

Conclusion

This study confirms the important role played by classical methods in quality control of herbal medicines. Macroscopic and microscopic studies revealed the major organoleptic properties, tissues and cells of diagnostic value for *W. ugandensis* leaf, stem-bark and respective powders thereby a first step in quality control. The simplicity and affordability of the techniques utilized makes the data obtained routinely useful as reference in the standardization of commercialized products and the entire production process for *W. ugandensis* herbal materials. Moreover, the technique is sustainable considering that the equipments needed are fewer, available, affordable and easy to use anywhere in the production chain and market surveillance. Microscopy and macroscopy can therefore be of value in quality control of *W. ugandensis* plant drugs. A study is underway to determine its other quality evaluation parameters including microbial, physicochemical and phytochemical and upon completion, a *W. ugandensis* monograph will have been formulated.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the study.

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