Micromorphology and Antimicrobial Efficacy of Tubers of *Dioscorea bulbifera* L.

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Abstract

Dioscorea bulbifera L., which belongs to the family Dioscoraceae, is a well-known plant because of its unusual morphological characters and principal nutritional components. However, less report is available on the anatomy (micromorphology) and antimicrobial potency of the tubers of the species. In this study, tuber anatomy and antimicrobial activity of tuber extracts of *D. bulbifera* on six pathogenic microorganisms (*Bacillus subtilis, B. pumalis, Candida albicans*, *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*) were examined using light microscopy and agar well diffusion method, respectively. Anatomically, vascular bundles are amphivasal, oval or rounded, and scattered in cortex, and starch grains are abundant. In antimicrobial tests, acetone and ethanol extracts respectively showed 10 mm and 11 mm inhibition zone against all pathogens except *B. subtilis*, whereas no inhibition zone was appeared by distilled water extract against all pathogens. The findings of this study suggest that tuber anatomy can be applied as promising diagnostic characters for identification of the species, and acetone and ethanol extracts of *D. bulbifera* tubers can be employed for potential treatment of diseases caused by all pathogens, apart from *B. subtilis*, examined.

Key words: Dioscoraceae, anatomy, antimicrobial potency

Introduction

Dioscorea bulbifera L., also known as air potato, air yam, or bulbil-bearing yam, is a monocotyledonous plant and belongs to the family Dioscoraceae (Dassanayake 1995). Tubers of *D. bulbifera*, which were used as food, contain a higher nutritional value compared to other species of *Dioscorea* (Ezeabara & Anona 2018). The plant is believed to be native to both Asia and Africa (Martin 1974). In Myanmar, it was found in Chin, Kachin, Mandalay, Mon, Sagaing, and Shan State (Kress *et al.* 2003).

The anatomical studies on fresh materials of plants have played a crucial role in identification of plants (Endress *et al*. 2000). In regard to anatomy of *Dioscorea*, description made by Ayensu (1972) is considered as only comprehensive work. Nevertheless, a detailed interpretation of tuber anatomy at species level is not included in his work. Anatomy of *D. bulbifera* was also performed by Sonibare and Adeniran (2014), whereas this study solely focused on leaves. Anatomy of *D. bulbifera*, including tubers, was conducted by Raman *et al*. (2014).

Nature provides numerous wild edible-medicinal products, such as flowers, leaves, fruits, nuts, berries, stems, roots, and tubers (Geng *et al*. 2016; Keservani *et al*. 2016). About 70% of the world's population depends on traditional health care system to treat various diseases (WHO 2002). Ancient and modern literature indicated that tubers of *D. bulbifera* have therapeutic benefits such as nervous excitability, hysteria, senility, infertility, hemorrhoids, dysentery, diarrhea (Uniyal 1998).

The applications of plant extracts for control of diseases have shown the importance of natural chemicals as possible source of alternative fungicides and antibiotics (Okigbo & Mmeka 2008). The antibacterial activities of extracts of *D. bulbifera* tubers on *Enterobacter aeruginosa*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacteria* sp. and *Staphylococcus aureus* have been reported (Kuete *et al*. 2012).

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As noted above, several medicinal uses of *D. bulbifera* were reported in previous studies. However, available literature revealed that little is known about the antimicrobial potency of the tuber extracts of the species (Okigbo *et al*. 2009; Kuete *et al*. 2012). Indeed, *D. bulbifera* used in the previous studies including anatomy and antimicrobial tests were mainly collected from Africa, America, and Europe, none of these studies dealt with the plant materials collected from Southeast Asia. In the present study, anatomy and antimicrobial efficacy of tubers of *D. bulbifera* collected from Lashio, Northern Shan State, Myanmar were conducted.

This study aimed at providing detailed anatomical characteristics of tubers of *D. bulbifera* and investigating a potential antimicrobial application of tuber extracts of the species on some pathogenic microorganisms.

Materials and Methods

Plant Material

Dioscorea bulbifera L. examined in this study was collected from Lashio, Nothern Shan State, Myanmar (Fig. 1a). The plant was identified according to the taxonomic descriptions of Dassanayake (1995).

Figure 1 *Dioscorea bulbifera* L. showing leaf, inflorescences, and tuber (a) and close up view of fresh tubers (b).

Preparation of Tuber Extracts

Fresh tubers (Fig. 1b) harvested from plant specimens were washed thoroughly under tap water, followed by distilled water. The tubers were cut into small pieces and then air dried for 3 weeks and ground into fine powder. The powdered samples were extracted employing acetone, ethanol and distilled water for 7 days and then filtered. The filtrates were used for further studies.

Microbial Strains

A total of six pathogenic microorganisms viz., *Bacillus subtilis*, *B. pumalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and yeast-like fungus, *Candida albicans* obtained from DCPT, Insein Township, Yangon were used for *in vitro* antimicrobial tests.

Micromorphological Observations

Free hand sections of fresh tubers were cut using razor blade. Of these, good quality thin sections were employed for the preparation of slides according to the published protocol (Johansen 1940) with a few modifications. In brief, thin sections were treated with a few tiny pieces of chloral hydrate crystals and stained with

safranin solution for 1 min. Excess stain was washed with an aliquot of distilled water. Afterwards, the sections were kept in dilute glycerin solution and mounted temporarily on a clean glass slides under a cover slip. Maceration of fresh tubers was also made by boiling them in equal volume of 50% of acetic acid solution and 50% of hydrogen peroxide solution. Slides prepared using thin sections or macerates were observed under a light microscope and their photomicrographs were recorded.

Antimicrobial Tests

Agar well diffusion method was employed for antimicrobial test (Murray *et al*. 1995). The 0.1% of particular bacterial suspension was added into a test tube containing 20 ml of sterilized agar medium. The tube is shaken vigorously and poured the medium into a petridish. The prepared agar plates were settled out with 7 mm agar well. The 0.2% of plant extract was added into each well and incubated at 37°C for 24 hours. The formation of clear zone around the well during 24 hours incubation had shown that the samples had antimicrobial activity.

Results

Micromorphology of Tuber of *Dioscorea bulbifera* L.

In transverse section (T.S), the tubers studied are circular in outline, 5000–6000 μm in diameter and distinguished into dermal, ground and vascular tissue systems.

Dermal Tissue System (Fig. 2): Periderm composed of phellem or cork, the meristematic phellogen or cork cambium and phellodrem or secondary cortex. Phellogen produced phellem or cork cells towards the outside and phelloderm towards the center; cells of the periderm radially arranged, parenchymatous, rectangular in shape, tangentially flattened, thin-walled. Phellogen 1- or 2-layered. Phellem stratified, 5- to 8-layered, the layer 10– 200 μm thick, cells 50–80 μm in tangential diameter, 10–20 μm in radial diameter. Phelloderm 4- to 6-layered.

Ground Tissue System (Fig. 2): Composed of cortex parenchymatous tissue and sclereids, cells polygonal, 20–80 μm in length, 20–90 μm in width, starch grains abundant, intercellular spaces small, acicular or bundles of raphides present.

Vascular Tissue System (Fig. 3): Vascular bundle scattered in cortex, amphivasal, oval or rounded; phloem 4- to 6-layered, the layers 30–50 μm thick, cells 2.5–10 μm in length, 5–10 μm in width, phloem composed of sieve-tube and companion cells, xylem arranged in circular ring, continuous or discontinuous, 2- to 3-layered, the layers 60–70 μm thick, cells polygonal, 10–30 μm in length, 10–30 μm in width, xylem composed of vessel elements, tracheids, fibers and xylem parenchyma. Vessel elements thick-walled, lateral walls straight, end walls oblique or transverse, thickening spiral, perforation plate simple, cells about 500 μm in length, 20–30 μm in width; tracheids elongate, lateral walls straight, end walls acute, thickening spiral, cells about 450 μm in length, about 20 μm in width; fiber long, lumen narrow, lateral walls straight, end walls acute, cells 130–200 μm in length, about 2.5 μm in width.

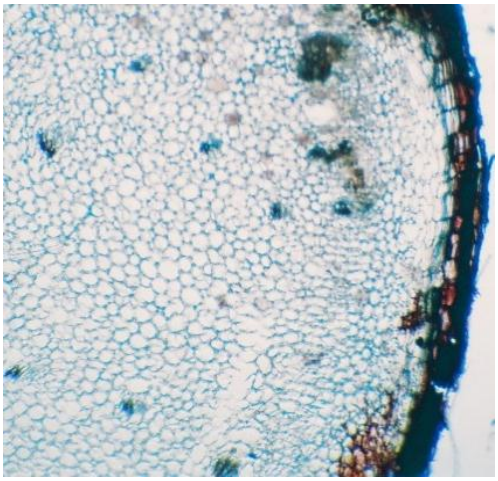

Figure 2 T.S of tuber of *D. bulbifera* L. showing periderm and vascular bundles.

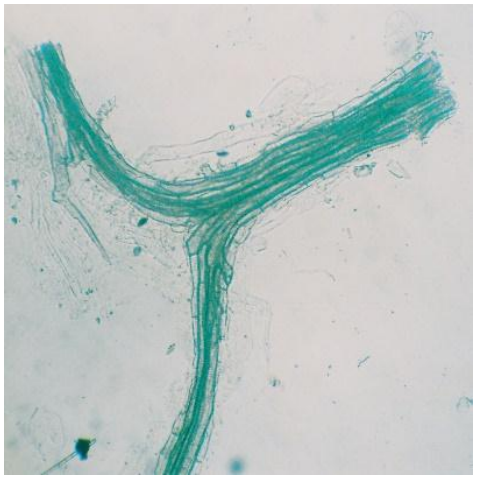

Figure 3 Macerated components of tuber of *D. bulbifera* L. showing vessel elements.

Antimicrobial Activities

In this experiment (Fig. 4) (Table 1), all test microorganisms with exception of *Bacillus subtilis* were susceptible to the acetone and methanol extracts, whereas none was susceptible to the distilled water extract. Acetone and methanol extracts revealed zone of inhibition of 10 and 11 mm, respectively against *B. pumalis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Control experiment did not inhibit the growth of test microorganisms (Data not shown here).

Table 1 Antimicrobial activities of various solvent extracts of tuber of *Dioscorea bulbifera* L.

Solvent	Test organisms					
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*B. pumalis*	*Candida albicans*	*E. coli*
Acetone	–	10 mm (+)	10 mm (+)	10 mm (+)	10 mm (+)	10 mm (+)
Ethanol	–	11 mm (+)	11 mm (+)	11 mm (+)	11 mm (+)	11 mm (+)
Water	–	–	–	–	–	–

Agar well = 7 mm; 7 mm–11 mm (+); 12 mm–16mm (++); 17 mm above (+++)

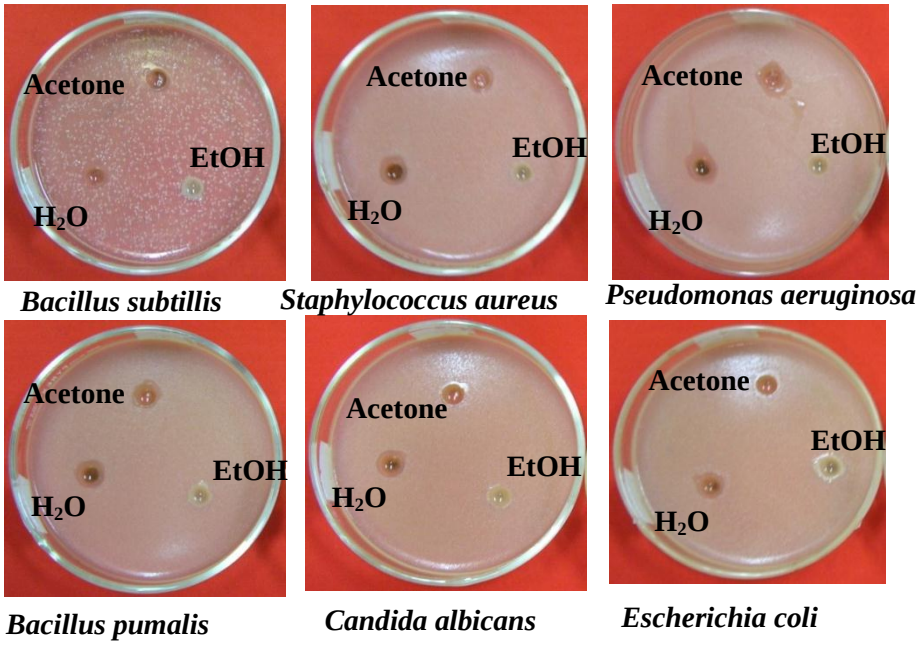

Figure 4 Treatment of different extracts of *D. bulbifera* L. tuber on test organisms.

Discussion and Conclusion

The anatomical characters and antimicrobial potential of acetone, ethanol, and distilled water extracts of *Dioscorea bulbifera* L. tubers were investigated in this study.

T.S of tubers revealed that dermal tissue is comprised of phellem, phellogen and phelloderm. Ground tissue is composed of cortex, parenchymatous and contains small intercellular spaces and raphides; cells are filled with starch grains. Vascular bundles are amphivasal, scattered in the cortex. The above mentioned characters except bundle type are consistent with the previous report (Raman 2014), in which collateral type was proposed. To clarify the bundle type, an extensive anatomical study on tubers collected from several geographical regions is inevitable.

The increasing incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant-derived substances (Savoia 2012). Accordingly, antimicrobial efficacy on six pathogens was detected with acetone, ethanol, and distilled water extracts of tubers of *D. bulbifera*.

B. subtilis could not be killed by any kind of the crude extracts of *D. bulbifera* tubers; suggesting that to fight against the infection caused by *B. subtilis*, none of these extracts may be applied. Once detection was continued, the results had been found as follow. Acetone and ethanol extracts showed their activities to fight against *B. pumalis, C. albicans, E. coli, P. aeruginosa* and *S. aureus*, and zone of inhibition appeared is 10 mm for acetone and 11 mm for ethanol, respectively; implying that each extract equally inhibited the growth of the pathogens tested. Okigbo *et al*. (2009) proposed that *C. albicans, E. coli, and S. aureus* were sensitive to the ethanol extract of *D. bulbifera* L. tuber. Furthermore, Kuete *et al*. (2012) proposed that methanol extract of tuber of *D. bulbifera* had antimicrobial activity against *E. aerogenes, E. coli, Klebsiella pneumonia*, and *P. aeruginosa*. Therefore, tuber extracts of the species may be regarded as a potential source of antimicrobial drugs.

In conclusion, the anatomical features of the tubers of *D. bulbifera* observed in the present work can be applied as potential diagnostic characters in taxonomic identification of the species. At the same time, tuber extracts of *D. bulbifera* using acetone and ethanol as solvents indicate that they have potential for the treatment of the diseases caused by the pathogenic strains, but *B. subtilis*, used in this study. The present finding is hence encouraging a plant of interesting antimicrobial activity.

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