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African Traditional Medicine — Medicinal plant standards — *Dioscorea bulbifera*

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Foreword

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This African Standard was prepared by the ARSO Technical Committee on African Traditional Medicine (ARSO/TC 82).

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Introduction

Dioscorea bulbifera is a tribal plant, which belongs to the family Dioscoreaceae assigned to the order dioscorales. It is native to tropical Asia and sub-Saharan Africa. Dioscorea bulbifera posses potentional therapeutic uses. It is found throughout India particularly in warmer places and is known as Yam or Air potato. Many tests prove phytochemically it contains flavnonoids, saponins, steroids, cardiac glycosides, terpenoids. (Mobo et al., 2013).

The tuber is edible when either boiled or cooked. One teaspoon of tuber powder and water taken in orally is a single dose cures for abdominal Traditionally *Dioscorea bulbifera* have been used to lower glycemic index, therefore it provides better protection against diabetes and obesity Fatima N. 2015).(

Dioscorea bulbifera bulbils are used in the treatment of piles, dysentery, syphilis, ulcers, cough, leprosy, diabetes, asthma, and cancer. It is a raw material for contraceptives, and it is one of the most consumed yam species especially in West Africa.

African Traditional Medicine — Medicinal Plant Standards — *Dioscorea bulbifera*

1 Scope

This Standard specifies requirements and related tests methods for *Dioscorea bulbifera L*) raw material to be used in consumer products including health, medicinal and cosmetic products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

AOAC Official Method 2008.02, Aflatoxins B_1, B_2, G_1, and G_2 and Ochratoxin A in ginseng and ginger by multi-toxin immunoaffinity column clean up and Liquid Chromatographic Quantitation

ARS 53, General principles of food hygiene — Code of practice

ARS 56, Pre-packaged foods — Labelling

ARS 950-2016, ATM - Terms and terminology

ARS 952:2016: Guidelines on Good Agricultural and Collection Practice (GACP) for Medicinal Plants

ARS 955-2016, African Traditional Medicine — Technical guidelines for safety, efficacy and quality of raw materials and herbal medicines

CODEX STAN 150, Standard for food grade salt

CODEX STAN 192, General standard for food additives

CODEX STAN 193, Codex general standard for contaminants and toxins in food and feed

CODEX STAN 1:1985; Rev. 8:2010, General Standard for the labelling of Pre-Packaged food

USP 30, NP 25, (2007), United States Pharmacopeia 30, National Formulary 25, 2: 1-1256

3 Terms and Definitions

In addition to ARS 950-2016, ATM - Terms and terminology, the following definitions shall apply for the purpose of this standard.

3.1
Disclaimer
a statement that you are not responsible for the effects or usage of the medicinal plant that has not gone through clinical trials

3.2
Clinical trials
a type of research that studies new tests and treatments and evaluates their effects on human health outcomes

3.3
Organic agriculture
production system that sustains the health of soils, ecosystems and people

NOTE It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. It combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved.

3.4
Organic farming
form of agriculture that relies on techniques such as crop rotation, green manure, compost and biological pest control

NOTE It uses fertilizers and pesticides but excludes or strictly limits the use of manufactured (synthetic) fertilizers, pesticides (which include herbicides, insecticides and fungicides), plant growth regulators such as hormones, livestock antibiotics, food additives, genetically modified organisms, human sewage sludge, and nanomaterial.

3.5
sustainable
The use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations.

3.6
wild crafting
The practice of harvesting plants from their natural or “wild” habitat, for food or medicinal purposes. It applies to uncultivated plants wherever they may be found and is not necessarily limited to wilderness areas. Ethical considerations are often involved, such as protecting endangered species and ensuring that the plant populations are harvested in a sustainable way

3.7
wild growing
wildcrafted
naturally grown
plants growing in their natural native habitat in an ecological area and applies to uncultivated plants wherever they may be found and is not necessarily limited to wilderness areas

4 Guidelines for production, harvesting and handling

4.1 General

4.1.1 The production of *Dioscorea bulbifera* raw material shall comply with applicable regulations e.g Good Agricultural and Collection Practices (GACP-ARS 952:2016).

4.1.2 Harvest containers shall be clean, dry and well ventilated. Containers that have previously been used to store other substances, e.g. agrochemicals, shall not be used. New bags shall be used which are labelled with the following information: Name of collected plant, collection area, collector name, date of collection, weight.

4.1.3 The raw material shall be free from extraneous matter, e.g. stones and twigs.

4.1.4 Harvesters shall wear clean gloves to prevent injury to skin due to the stinging of the leaves.

4.1.5 Workers shall wear hairnets, or any other suitable head cover, and gloves when the leaves are processed to maintain hygiene standards and avoid introduction of foreign matter.

4.1.6 The general and harvesting requirements as contemplated in this standard are applicable to organic, cultivated and wild growing medicinal plants and shall be in accordance with ARS 952:2016.

4.2 Collection and harvesting requirements

4.2.1 The bulbils of *Dioscorea bulbifera* shall be harvested within 180-200 days of sprouting. The mother corm shall be harvested after two to three years. In subtropical and tropical areas, mid-September to mid-October is the most suitable time. While in temperate zones October–November are the ideal months to harvest bulbils/ underground corms since the crop undergoes dormancy during dry season.

4.2.2 Harvesting or wild collection shall not take place in areas likely to be contaminated with heavy metals such as dump sites or mining areas (Olowu *et al*., 2015).

4.2.3 Rubber gloves shall be used during the harvesting process to prevent skin exposure to the contents of the stinging hairs of the plant.

4.2.4 Harvesters/collectors shall wear long-sleeves that go all the way down to the gloves as the inside of the wrists can be more sensitive than the hands.

4.2.5 The bulbils shall be plucked as and when they become fully grown, otherwise they are shed before the foliage develops.

4.2.6 Clean bags or containers shall be used to transport from the field to the drying location.

4.2.7 Inspections shall be done to avoid the inclusion of excessively damaged material.

4.3 Cultivation

4.3.1 The cultivation of *Dioscorea bulbifera* shall comply with the Good Agricultural and Collection Practices (GACP) elaborated in ARS 952:2016 in addition to the requirements outlined hereafter.

4.3.2 The planting beds should be tilled properly and made weed-free, and the soil shall be well-pulverized. A basal dose of 15–20 tonnes/hectare of FYM (farmyard manure) shall be applied to the soil at the time of pre-planting tillage.

4.3.3 Plant-to-plant spacing of 50 cm × 50cm shall be considered optimum for good growth and yield. This spacing gives an optimum crop stand of 40 000 plants per hectare. Plant spacing of 100 cm × 100 cm yields higher number of bulbils per plant; however, the average weight of bulbils as well as their number per unit area are much higher at the spacing of 50 cm × 50 cm.

4.3.4 The plant is preferred as a solo crop but needs staking support or host of shrubs and trees.

4.3.5 Only light irrigation to maintain humidity during dry season is recommended through sprinklers. Flood irrigation may result in waterlogging and should be avoided.

4.3.6 The species shall be easily propagated by underground corms or aerial bulbils. Small corms shall be cut into 2 - 4 sections, larger ones into 6 - 8 sections. Each section shall have 2 - 3 dormant buds. The cut tuber is often left in the sun for several hours to promote wound healing of the tuber and reduce the risk of fungal infection. The aerial bulbs are often divided into 2 or more equal sized pieces,

4.3.7 Direct planting of the corms or bulbils in the field is more effective than transplanting nursery-raised seedlings, hence it is the preferred method. The suitable time for planting corms or bulbils is April–May.

4.4 Handling and processing

4.4.1 When handling and processing dried or fresh material, gloves are recommended.

4.4.2 Care shall be taken to ensure that processed material does not become mouldy and it has to be free from other plants or plant parts.

4.4.3 The corms and bulbils can be stored in gunny or paper bags without causing any adverse effect on the rate of sprouting

4.4.4 Both bulbils and tubers are resistant to fungal infections and harvest wounds heal quickly; storage under dry, cool conditions, away from sunlight, appears to give moderate storage life.

5 Requirements

5.1 Quality requirements

5.1.1 Microbiological limits

The product shall comply with the microbiological limits given in Table 1 when tested in accordance with the test methods specified therein.

Table 1 — Microbiological limits for *Dioscorea bulbifera*

S/Nr	PARAMETERS	LIMITS	REF. TEST METHODS
A.	***Raw medicinal plant and herbal materials intended for further processing*** (including additional Decontamination by a physical or chemical process).		
1	Yeast and moulds, max. Per g	10^7	USP 30, NF 25
2	*E. coli*, max per g	absent	"
3	Shigella, per g	absent	"
B.	***Herbal materials that have been pre-treated*** (For herbal materials that have been pre-treated (e.g with boiling water as used for herbal teas and infusions) or that are used as topical dosage Zforms)		
4	Aerobic bacteria/g	10^5	USP 30, NF 25
5	Yeasts and moulds, max. Per g	10^4	"
6	*E. coli*, max. Per g	10^2	"
7	Enterobacteria and certain Gram- neg. Bact./g	10^3	"
8	Salmonella, per g	absent	"
9	Shigella, per g		
10	Clostridia, per g		
C	***Other herbal materials for internal use***		
11	Aerobic bacteria/g	10^3	
12	Yeasts and moulds, max. Per g	10^4	"
13	Escherichia coli, max. Per g	Absent	"
14	Salmonella, per g	"	"
15	Enterobacteria and certain Gram-neg. Bact./g	"	"
16	Clostridia, per g		
17	Shigella, per g		
D	***Herbal medicine to which boiling water is added before use***		
18	Aerobic bacteria/g	10^6	USP 30, NF 25
19	Yeasts and moulds, max per g	10^4	"
20	*Escherichia coli*, max per g	Absent	"
21	Salmonella, per g	"	"
22	Enterobacteria and certain Gram- neg. Bact/g	10^3	"
23	Clostridia, per g		
24	Shigella, per g		

5.1.2 Chemical, physical and mycotoxin requirements

The product shall comply with the Chemical, physical and mycotoxin requirements given in Table 2 when tested in accordance with the test methods specified therein.

Table 2 — Chemical, physical and mycotoxin requirements (WHO, 2007)**;** (Upton & AHP, 2012)

Chemical, physical and mycotoxin requirements

S/N	***Characteristics***	***Limits(seeds)***	***Ref. Test methods***
1	Lead (pb), max. mg/kg	20µg per gm	USP 30, NF 25 Pge 231
2	Cadmium (Cd), max. mg/kg	"	"
3	Arsenic (As), max. mg/kg	"	"
4	Chromium (Cr), max. mg/kg	"	"
5	Mercury (Hg), max. mg/kg	"	"
6	Copper (Cu), max. mg/kg	20µg per g	"
7	Total ash (aerial parts), max. %	4 – 8%	USP 30, NF 25
8	Total ash (roots), max. %	"	"
9	Acid – insoluble ash (leaves), max. %	2%	"
10	Acid – insoluble ash (roots), max. %	4 – 8%	USP 30, NF 25
11	Foreign matter, max. %	2 – 3 %	USP 30, NF 25
12	Total Aflatoxin (AFB_1 + AFB_2 +AFG_1 +AFG_2), ppb	2ppb	AOAC 2008.2
13	Aflatoxin B1 only, ppb	4ppb	"

The inputs and Methods are based on USP 30, NF 25 of May 1, 2007.
Aflatoxin Test Method is from AOAC 2008.2

5.1.3 Pesticide residues

For use of pesticides, reference shall be made to the CODEX list of approved pesticides for spices and their maximum residue limits (MRLs) (WHO, 2007).

5.1.4 Adulterants and adulterations

There shall be no adulterant.

5.1.5 Standard preparations

Examples of appropriate dosage preparations, frequencies of use and directions for use are provided in Annex J.

6 Packaging

In addition to the requirements in ARS 56, Pre-packaged foods — Labelling, the following shall apply:

6.1 Packaging

6.1 The *Dioscorea bulbifera* raw material shall be packed in acceptable containers that will withstand normal usage and transportation and prevent any risk of contamination and significant losses.
6.2 Raw material sensitive to light shall be packed in containers that are light-resistant
6.3 Liquid raw material shall be packed in leak-proof containers.
6.4 List of ingredients should be declared
6.5 Powdered raw material shall be packed in plastic bags and placed inside an airtight container.

7 Labelling

In addition to the provisions of ARS 56, Pre-packaged foods - Labelling and Codex Alimentarius Commission (CODEX STAN 1-1985; Rev-8-2010) on General Standard for the labelling of Pre-Packaged food, the following shall be inscribed on the package:

a) name of the product (type of raw material);

b) species

c) date and method of collection (i.e., wild or cultivated);

d) geographical origin of the species;

e) classification or grade, e.g., pharmaceutical grade or food grade;

f) name and address of the manufacturer, packer, co-packer, distributor, importer, exporter or vendor;

g) code or batch number;

h) net weight of product in grams;

i) date of packaging and best before date shall not be in code for the benefit of consumers;

j) producing country or country of origin;

k) mark of quality;

l) other rregulatory marking as appropriate;

m) name of additive, if present; and

n) disclaimer of the product will be added if there is no evidence of clinical trials.

NOTE The symbol for organic certification may be exhibited on products that have organic certification.

Annex A
(informative)

Determination of foreign matter

A.1 Herbal drugs should be free from moulds, insects and other animal contamination.

A.2 Foreign matter is material consisting of any or all of the following:

(1) *Foreign organs*: matter coming from the source plant but not defined as the drug,

(2) *Foreign elements*: matter not coming from the source plant and either of vegetable or mineral origin.

A.3 Determination of foreign matter

Weigh 100 g to 500 g of the substance to be examined, or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. Examine for foreign matter by inspection with the unaided eye or by use of a lens (6X). Separate foreign matter and weigh it and calculate the percentage present.

Annex B
(informative)

Sampling procedures

B.1 General

The following sampling procedure shall be applied in determining whether a lot submitted for inspection and testing complies with the relevant requirements of this standard. The sample so drawn shall be deemed to represent the lot.

B.2 Definitions

B.2.1
batch
defined quantity of material produced from a single process or, in the case of a continuous production process, the material produced in a defined cycle of manufacture that is intended or purported to be homogeneous in character and quality

NOTE Small batches may be combined and blended together to form a uniform batch.

B.2.2
defective
raw material that fails in one or more respects to comply with the requirements of this standard

B.2.3
lot
the quantity of *Dioscorea bulbifera* raw material that bears the same batch identification, from one manufacturer, and submitted at any one time for inspection and testing

B.3 Sample for inspection

B.3.1 The leaves or calyces shall be visually inspected for damage, discolouring or disease. Infected leaves shall be discarded.

B.4 Sample for testing

B.4.1 Sampling products

B.4.1.1 Take 3 g of powdered sample from the top, middle and bottom of each container per batch of product.

B.4.1.2 Before drawing a liquid sample, thoroughly mix the contents of the relevant container. Take 3 mL of liquid sample from the top, middle and bottom of each container per batch of product.

B.4.1.3 Transfer into a clean, dry, air-tight container clearly marked with the sample identification, batch number and date of sampling.

B.4.1.4 Take a composite of each sampling container and mix a sufficient amount for all the required tests.

B.4.1.5 Store the remainder of the samples for retention purposes.

B.5 Compliance with this standard

The lot shall be deemed to comply with the requirements of this standard if, after inspection and testing of the samples taken in accordance with B.1, B.3 and B.4, no defective is found.

Annex C
(normative)

Determination of loss on drying

C.1 Apparatus and glassware

C.1.1 **Drying oven**, capable of operating between 100 °C ± 1 °C and 110 °C ± 1 °C.

C.1.2 **Balance**, accurate up to three decimals.

C.1.3 **Desiccator**.

C.1.4 **Sample holder**.

C.2 Procedure

C.2.1 Determine the mass of the sample holder.

C.2.2 Accurately weigh approximately 20 g ± 0.1 g of sample into the holder.

C.2.3 Dry in an oven at 105 °C ± 1 °C for 24 h.

C.2.4 Cool in a desiccator for 30 min to 60 min.

C.2.5 Determine the mass of the holder with the sample to the third decimal without delay.

C.3 Calculation

Calculate the loss on drying using the following formula:

$$L = \frac{B - C}{B - A} \times 100$$

where

L is the loss on drying as a mass fraction, expressed as a percentage;

B is the mass of the sample holder and sample before drying, in grams;

C is the mass of the sample holder and sample after drying, in grams;

A is the mass of the sample holder, in grams.

Annex D
(normative)

Determination of ash values

D.1 Apparatus and glassware

D.1.1 **Crucible** (Platinum or silica).

D.1.2 **Balance**, accurate up to three decimals.

D.1.3 **Muffle furnace**, capable of operating between 400 °C ± 1 °C and 800 °C ± 1 °C.

D.2 Reagents

D.2.1 **Hydrochloric acid** (70g/L)

D.2.2 **Distilled water.**

D.3 Determination of Ash

D.3.1 Total ash

Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator and weigh. Unless otherwise prescribed, evenly distribute 2 g - 4 g of the substance or the powdered vegetable drug to be examined in the crucible. Dry at 100 °C to 105 °C for 1 h and ignite to constant mass in a muffle furnace at 600 °C ± 25 °C, allowing the crucible to cool in a desiccator after each ignition.

Flames should not be produced at any time during the procedure. If after prolonged ignition the ash still contains black particles, take up with hot water, filter through an ashless filter paper and ignite the residue and the filter paper. Combine the filtrate with the ash, carefully evaporate to dryness and ignite to constant mass.

Allow the residue to cool in a suitable desiccator for 30 minutes, and then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

D.3.2 Acid-insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter-paper and wash with hot water until the filtrate is neutral.

Transfer the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

D.3.3 Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C.

Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material.

Annex E
(Normative)
E.1 Raw material presentations

6.1 Fresh or dried bulbils, rhizomes

Figure 1: Fresh leaves of *Dioscorea bulbifera*

6.2 Young aerial tuber (bulbil)

Figure 2: Young aerial tuber (bulbil)

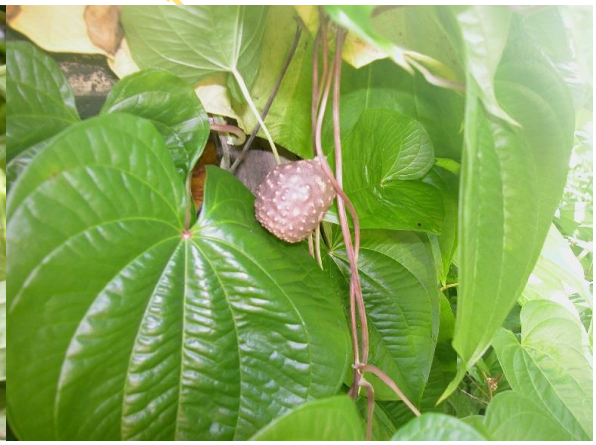


Figure 3: Mature bulbil

6.4 Air yam

Figure 4: Air yam

E.1.1 Organoleptic properties

***Dioscorea bulbifera* Root**
Aroma: Not verified.
Taste: Bitter

***Dioscorea bulbifera* Leaf**
Aroma: Not verified.
Taste: Bitter.

***Dioscorea bulbifera* Stem**
Aroma: Potato-like flavour.
Taste: Not verified

E.1.2 Macroscopic characteristics

***Dioscorea bulbifera* Root**
Adventitious fibrous dense strands, rough and soft, externally cream to yellowish and cream to whitish internally.

***Dioscorea bulbifera* Leaf**
Simple, large broad cordate or ovate-suborbicular of 5-30 cm length, alternate, dark green color, divided longitudinally into lobes with characteristic simple leaf venation that emerges from leaf base, acuminate apex, undulate margins, glabrous on both surfaces.

***Dioscorea bulbifera* Stem**
Herbaceous perennial aerial vine, twining climber with height of up to 20 m and 0.5 m thickness, twines counter-clockwise, round to slightly angled in cross section, greenish surface.

E.1.3 Microscopic characteristics

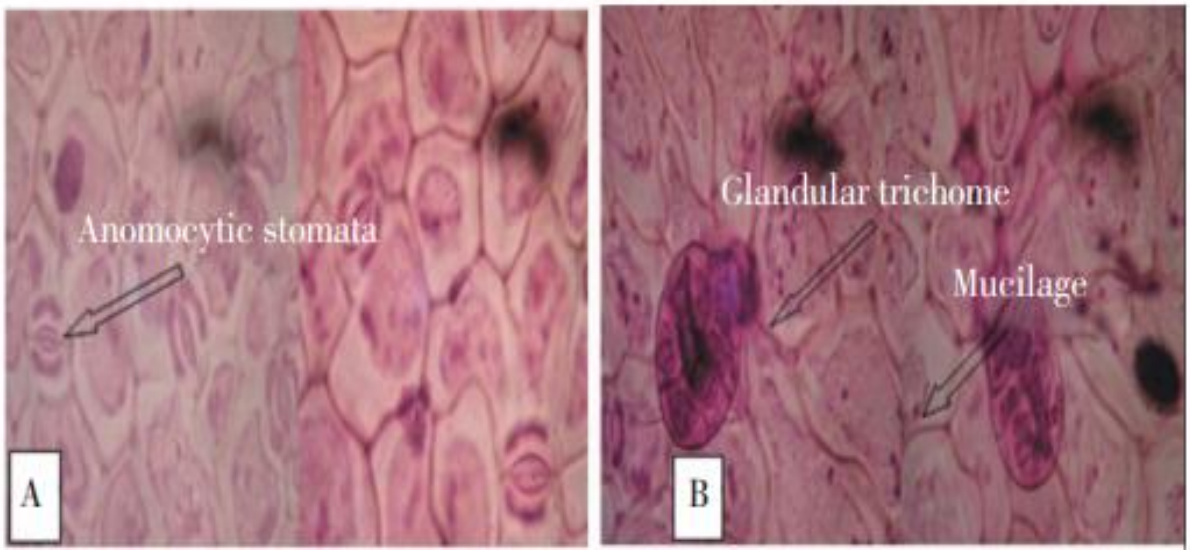


Figure 5: Foliar Microscopic features of abaxial epidermis of *Dioscorea bulbifera* (400x)

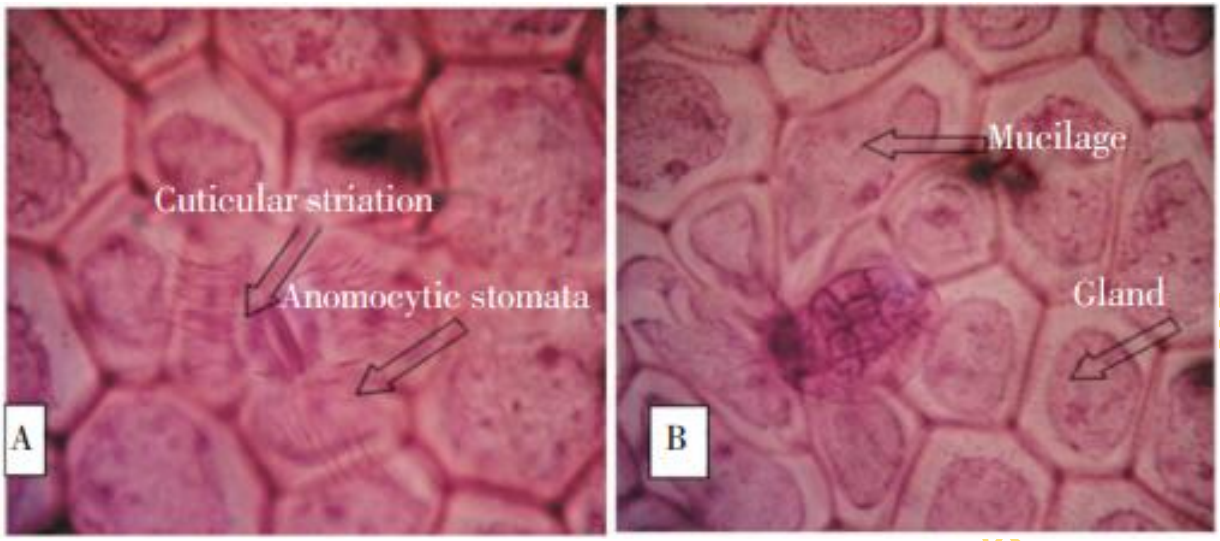
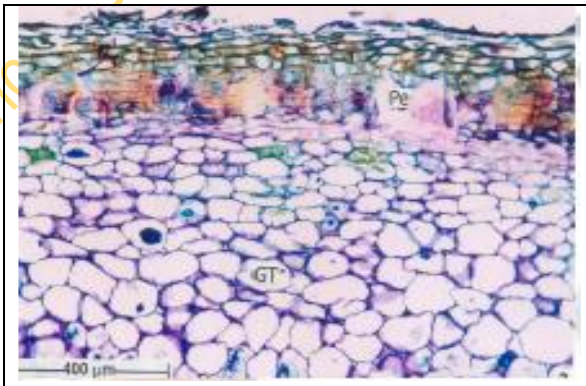


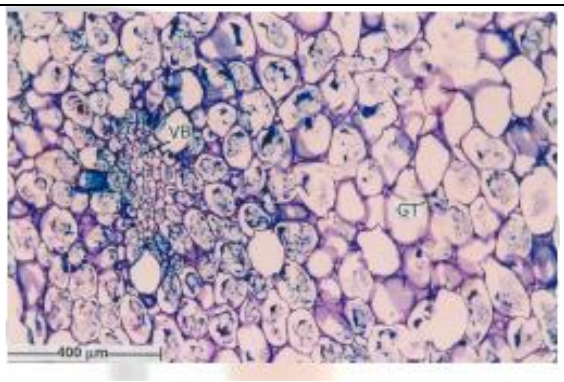
Figure 6: Foliar Microscopic features of adaxial epidermis of *Dioscorea bulbifera* (400x)

S/N	Parameter		Length (µm)
1.	Adaxial surface	Epidermal cell length	49.0±5.25
		Epidermal cell width	40.5±2.73
		Stomata length	19.0±0.67
		Stomata length	6.0±0.41
		Trichomes length	56.0±1.19
		Trichomes width	37.5±1.29
2.	Abaxial surface	Epidermal cell length	50.5±5.93
		Epidermal cell width	36.25±2.51
		Stomata length	17.50±0.83
		Stomata length	55.75±0.38
		Trichomes length	47.25±1.56
		Trichomes width	31.5±0.85
			Description
3.	Diagnostic feature	Cell wall pattern	Smooth
		Epidermal cell shape	Polygonal
		Trichome type	Glandular unicellular trichome with multicellular head
		Stomata type	More of anomocytic than anisocytic
		Mass of mucilage	Evenly and wide spread within epidermal cell

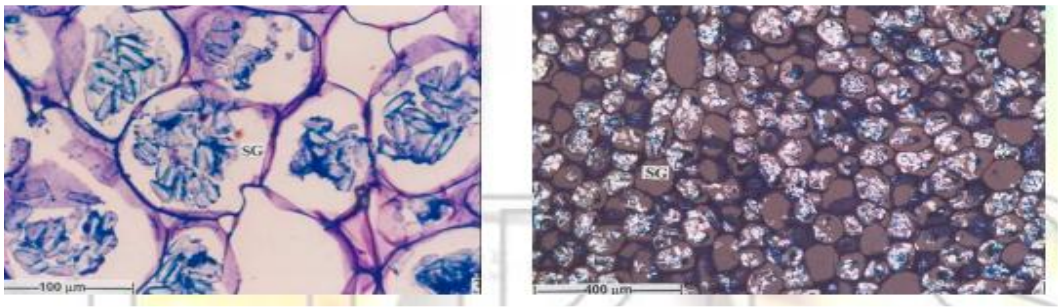
Data are expressed as mean± SEM, n=10. Diagnostic features of *Dioscorea bulbifera* Linn. foliar epidermis; abaxial and adaxial were carried out using independent student t-test. *: P<0.05.



Epidermal cells



Vascular bundle tissue



Bulb tissues showing starch grains

(**Subasini** *et al.,* 2013)

Figure 7: Ground tissues showing epidermal cells, vascular bundle cells and bulb cells with starch grain

E.1.4 Chemical constituents

Flavonoids and Phenolic Acids: Ethyl acetate soluble fraction of 75 % ethanol extract of the rhizomes of *Dioscorea bulbifera* was shown to consist of both flavonol aglycones, namely kaempferol-3,5-dimethyl ether, caryatin, (+)-catechin and flavonol glycosides, namely, quercetin-3-*O*-galactopyranoside, myricetin-3-*O*-galactopyranoside, myricetin-3-*O*-glucopyranoside (Gao *et al.,* 2002). Ethanolic (80%) extract was reported for efficient extraction of epicatechin, isovanillic acid, vanillic acid and myricetin (Tang *et al.,* 2006).

Terpenoids: 8-epidiosbulbin E acetate (EEA) was isolated from aqueous methanolic extract of bulbs from India (Shriram *et al.,* 2006). Diosbulbin B is a demethyl diterpenoid found as a main constituent of chloroform fraction (Figure 2) (Gao *et al.,* 2007). Similarly, *D. bulbifera* from China contains diosbulbin A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P as major phytochemicals (Tang *et al.,* 2014; Wang *et al.,* 2009). Methanolic extract of bulbils from Cameroon are found to be rich in clerodane diterpenoids, bafoudiosbulbins A, B, C, F and G (Teponno *et al.,* 2006). Phytoalexin called demethylbatatasin IV and norditerpenoid diosbulbin D were isolated from bulbils from Nigeria and tubers from Bangladesh, respectively (Adesanya *et al.,* 1989).

Steroids: Steroidal sapogenins named diosbulbisins A, B, C, D, spirostane glycosides named diosbulbisides A, B and cholestane glycoside named diosbulbiside C were found to be predominant in 95 % ethanolic extract of rhizomes of China (Liu *et al.,* 2009). Flowers of *D. bulbifera* from Cameroon has wide range of steroidal saponins namely, dioscoreanosides A, B, C, D, E, F, G, H, I, J and K (Tapondjou *et al.,* 2013).

Carotenoids: Carotenoids such as lutein, zeaxanthin, neoxanthins are also prevalent in *D. bulbifera* (Khare *et al.,* 2007).

Phenanthrenes: 2,7-dihydroxy- 4-methoxyphenanthrene, 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene, 1,6-dihydroxy-2,5,7- trimethoxyphenanthrene (Galani and Patel, 2017)

Phytosterols: Daucosterol, *β*-sitosterol, 3-O-*β*-D-glucopyranosyl- *β* -sitosterol, stigmasterol (Galani and Patel, 2017)

Fatty acids: Palmatic acid, succinic acid, shikimic acid, tetracosanoic acid, 1-(tetracosanoyl)- glycerol, *trans*-tetracosanylferulate, mono-arachidin, C22 ω-hydroxy fatty acid, 3-hydroxy-5-methoxybenzoic acid, batatasin III, behenic acid, ethyl ester of undecanoic acid, Z-1,9-dodecadiene (C H), *n*-hexadecanoic acid, ethyl ester of eicosanoic acid (Galani and Patel, 2017)

Tannins : Catechin, protocatechuic acid, (+) epicatechin, (-) epicatechin (Galani and Patel, 2017)

Volatile oils: Vanillic acid, isovanillic acid (Galani and Patel, 2017)

E.1.5 Radioactive residues

The WHO guidelines emphasize that the health risk, in general, due to radioactive contamination from naturally occurring radio nuclides is not a real concern, but those arising from major nuclear accidents may be serious and depend on the specific radionuclide, the level of contamination, and the quantity of the contaminant consumed. Taking into account the quantity of herbal medicine normally consumed by an individual, they are unlikely to be a health risk. Therefore, at present, no limits are proposed for radioactive contamination (WHO, 2011).

E.1.6 Loss on drying

Loss on drying shall be determined in accordance with the test method in Annex C in line with recognized pharmacopoeias (BPCO, 2012; CE, 2005). The following requirements shall be met:

Material type	Requirements
Whole or cut dried bulbils	- %, max
Whole or cut dried tubers	- %, max

E.1.7 Total ash content

The ash content of flour sample of *Dioscorea bulbifera* was 3.35 ± 0.15% (Nwachukwu and Okoroafor 2019).
Wet matter; peel ash was 3.35±0.08%, tissue as was 1.5 ± 0.02%.
Dry matter; peel ash was 6.15 ± 0.64%, tissue as was 2.77±0.165 (Abara, A. E. 2011)
When determined in accordance with Annex G, the total ash content shall be not more than 2.67 %.
Ash content of flours for three cultivars ranged from 2.58 – 2.67% (Ojinnaka *et al*., 2017)

E.1.8 Acid-insoluble ash content 2.3 %

E.1.9 Water-soluble ash content 1.5%

E.1.10 Moisture content

Moisture content of growing bulbis was 61.93+ 0.25%, for dried powdered bulbis moisture content was 8.7±0.1%(Nwachukwu and Okoroafor., 2019).

Annex F
(informative)

Plant description

F Plant description

F.1 Scientific name with author

Dioscorea bulbifera Linn

F.2 Synonyms

Dioscorea anthropophagorum A.Chev., *Dioscorea bulbifera* var. anthropophagorum (A.Chev.) Summerh, *Dioscorea bulbifera* var. crispata (Roxb.) Prain, *Dioscorea bulbifera* var. elongata (F.M.Bailey) Prain & Burkill, *Dioscorea bulbifera* var. pulchella (Roxb.) Prain, *Dioscorea bulbifera* var. sativa Prain, *Dioscorea bulbifera* var. suavia Prain & Burkill, *Dioscorea bulbifera* var. vera Prain & Burkill , *Dioscorea crispata* Roxb, *Dioscorea heterophylla* Roxb, *Dioscorea hoffa* Cordem, *Dioscorea hofika* Jum. & H.Perrier, *Dioscorea korrorensis* R.Knuth, *Dioscorea latifolia* Benth, *Dioscorea longipetiolata* Baudon, *Dioscorea perrieri* R.Knuth, *Dioscorea pulchella* Roxb, *Dioscorea rogersii* Prain & Burkill, *Dioscorea sativa* f. domestica Makino, *Dioscorea sativa* var. elongata F.M.Bailey, *Dioscorea sativa* var. rotunda F.M.Bailey, *Dioscorea sylvestris* De Wild, *Dioscorea tamifolia* Salisb, *Dioscorea tenuiflora* Schltdl,

F.3 Family

Dioscoreaceae

F.4 Common names

Air Yam, Air Potato, Bitter Yam, Aerial Yam, Potato Yam, Aerial yam, Cheeky yam, Bulbil-bearing yam

F.5 Vernacular names

Nigeria: Igbo- Adu, Hausa-doyan-iska, Yoruba-Esuru, Igala-Okuta-echi, Cameroon: Mbo/Bakossi- alloh

F.6 Botanical description

Dioscorea bulbifera is a herbaceous perennial twining vine and can climb up to 30 m tall. **Leaves**: The leaves are large, green, hairless, untoothed, alternate, palmately veined from the leaf base, long-petioled, and broadly heart-shaped 7 to 22cm long and 7 to 8cm wide.

Stem: The slender, twining, hairless, green to purple-flecked stems climb to the left (clockwise) up to several meters long, are round to slightly angled in cross section, and have no spines.

Flowers: The plants are dioecious with male and female flowers on separate plants, however these plants rarely bloom. When present, the tiny flowers are in slender, pendent spikes or panicles at the leaf axils. The female flowers are followed by seed capsules that are only winged on the basal side. Capsule is drooping, oblong, and with 3 membranous wings.

Tubers: The potato-like bulbis (aerial tubers) which are rounded, and up to 13 cm in diameter are formed on the stems at the leaf axils. The plants sometimes also have small underground tubers. Even when the plants are not producing seeds, they can be propagated by both the bulbils and tubers.

Seeds: (1.2-1.6 by 0.5 cm) are trilocular, dark brown and partially winged. (Martin, 1974; Lim, 2016).4.7

F.7 Origin and distribution

The plant is native to Africa, Asia and Australia. It is cultivated in western and Southern parts of Cameroon, Nigeria and other parts of West Africa.

F.8 Plant part used

The plant part used for medicinal purpose is the aerial tuber (bulbis), Tubers and Roots.

Annex G
(informative)

Identification of *Dioscorea* raw materials

NOTE This annex applies to raw materials derived from whole or cut dried leaves and/or calyces of *Dioscorea bulbifera* prepared in powder form. This annex references the British Pharmacopoeia 2012 (BPCO, 2012)

Test solution:

Reference solution:

Plate: *TLC silica gel plate* (5-40 µm) [or *TLC silica gel plate* (2-10 µm)].

Mobile phase:

Application: 10 µL [or 4 µL] as bands of 10 mm [or 8 mm].

Development: Over a path of 8 cm [or 6 cm].

Drying: In air.

Detection:

Results:

Annex H
(normative)

Determination of Diosgenin content – Liquid chromatography

Test solution:

Reference solution:

Precolumn:
— *size*: *l* = 4 mm, Ø = 4 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography* (5 µm).

Column:
— *size*: *l* = 0.125 m, Ø = 4 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography* (5 µm);

— *temperature*: 25 °C.

Mobile phase:
— *mobile phase A*: nanopure *water* adjusted to pH 2.0 with *dilute phosphoric acid*;

— *mobile phase B*: *methanol*;

Time (min)	Mobile phase A (% V/V)	Mobile phase B (% V/V)

Flow rate:

Detection: Spectrophotometer at ??? nm.

Injection: 20 µL.

Relative retention:

Annex I
(normative)

Determination of ash insoluble in hydrochloric acid

Ash insoluble in hydrochloric acid is the residue obtained after extracting the sulfated or total ash with hydrochloric acid, calculated with reference to 100 g of drug.

To the crucible containing the residue from the determination of sulfated or total ash, add 15 mL of *water* and 10 mL of *hydrochloric acid,* cover with a watch-glass, boil the mixture gently for 10 min and allow to cool. Filter through an ashless filter, wash the residue with hot *water* until the filtrate is neutral, dry, ignite to dull redness, allow to cool in a desiccator and weigh. Reheat until the difference between 2 consecutive weighings is not more than 1 mg.

Annex J
(normative)

Pharmacological properties and applications of *D. bulbifera*

J.1 Major ethnopharmacological uses

The extracts of *Dioscorea bulbifera* are reported to exhibit antitumor, antioxidant, anorexiant, antihyperlipidemic, analgesic, anti- inflammatory, plasmid curing, antidiabetic and antihyperglycemic effects (Shririam *et al.,* 2008; Ahmed *et al.,* 2009; Nguelefack *et al.,* 2011) and immunomodulatory function (Ghosh et al., 2013). . There are also literature supports on its medicinal use as purgative, anthelmintic, diuretic, deflatulent, rejuvenating tonic and aphrodisiac. The tubers can also be used for treatment in scrofula, haematological disorders, diabetic disorders, worm infestations, haemorrhoids, skin disorders, general debility as well as polyurea. The tubers are used for treatment of leprosy and tumours in Bangladesh while in Chinese medicine they are used as a remedy for sore throat and for struma (Ghosh *et al.,* 2015). In Zimbabwe it is applied on cuts and sores as infusion, while in Cameroon and Madagascar it is used for treatment of abscesses, boils and wound infections [Ghosh *et al.,* 2015]. It has also got its wide applications in piles, ulcers, pain and inflammation in Indian traditional medicine [Ghosh *et al.,* 2015]. Crushed tubers and decoction are emulsified into oil to treat infected ulcers and sinus (Subasini *et al.,* 2013). It is profusely used in both Indian and Chinese traditional medicine to cure gastric cancer and carcinoma of rectum and goitre (Ghosh *et al.,* 2015). Dried yam is known to dissolve toxins, cures carbuncles, scrofula and purulent infections.It is used for the prevention of early miscarriage, hernia, reliving the pain associated with child birth (Kumar et al., 2017).. It is considered as remedy against dysentery and diarrhoea in addition to syphilis in Java and Brazil. It is used against dog bites, snake bites and food poisoning in China (Williams, 2013). It is also reported to have remedial potential against conjunctivitis, leucoderma, dyspepsia, urinary discharges, jaundice, diabetes, asthma, bronchitis, strangay and vata biliousness (Ghosh *et al.,* 2015)

J.2 Other relevant uses

In Nigeria, it is used as anti-diabetic . The bulbils are normally cooked and eaten in a manner similar to other starchy root crops, though many African forms require detoxification by soaking in water or prolonged boiling before they are safe to consume. A few are very succulent and may be eaten raw. The flavour is reported to be inferior to that of most common yams and some are bitter. However, these yams have some popularity because of the convenient size of the bulbils for kitchen use
The bulbils and tubers are occasionally used for the production of flour. In Indonesia a fish poison is made from the bulbils of toxic varieties, and in Africa poisonous varieties may be planted among safe varieties to discourage thieves. In India a paste from the tuber is used as a cure for snakebite and in Jamaica for treatment of scorpion and centipede stings.

J.3 Pharmacological properties

J.3.1 Antimicrobial activity

J.3.1.1 Antibacterial activity

In a study, the acetone extract, ethyl acetate extract, 95% ethanol extract and methanol extract of *D. bulbifera* each was shown to exhibit moderate antibacterial activity against bacteria isolated from animals and poultries. The acetone extract showed the most significant anti-bacterial effect when compared to the other extracts [Okigbo *et al.,* 2009]. In a related study, the aqueous extract of *D. bulbifera* showed strong anti-bacterial activity against *E. coli* while the ethanol extract was found to be potent against *S. aureus* and *Candida albicans* when tested using disc-diffusion method of antimicrobial assay [Kuete *et al.,* 2012].

In another study, the successive extracts of bulbils of *D. bulbifera* was investigated for *in vitro* antimicrobial activity. The petroleum ether and chloroform extracts were found to exhibit significant activity against *A. fumigatus* and *R. nigricans* among the tested fractions. The petroleum ether and water extracts were also found to show good activity against *K. pneumoniae* while the chloroform extract showed poor activity against *S. aureus* [Ghosh *et al.,* 2012].

The methanol extract, fractions and six compounds isolated from the bulbils of *D. bulbifera*, namely bafoudiosbulbins A, B, C, F, G and 2,7-dihydroxy-4-methoxyphenanthrene, were tested against gram-negative bacteria involving multidrug resistant (MDR) phenotypes expressing active efflux pumps

using microplate alamar blue assay and the broth microdilution methods. The crude extract, fractions and bafoudiosbulbins C exhibited potent activity against most of the tested microorganisms belonging to MDR phenotypes such as *E. coli* AG102, *P. aeruginosa* PA124, *E. aerogenes* CM64, *K. pneumoniae* KP55 and KP63 [Teponno *et al.,* 2006]. In a similar study, two clerodane diterpenoids, Bafoudiosbulbins A and Bafoudiosbulbins B were also shown to exhibit significant activities against *P. aeruginosa, S. typhi, S. paratyphi A* and *S. paratyphi B* [Shriram *et al.,* 2008] while 8-epidiosbulbin E acetate was shown to exhibit broad-spectrum plasmid-curing activity against MDR bacteria, including Vancomycin-resistant enterococci (VRE). This compound (8-epidiosbulbin E acetate) also cured antibiotic resistance plasmids from clinical isolates, including *Enterococcus faecalis, E. coli, Shigella sonnei, P. aeruginosa* and *Bacillus subtilis* with 12-48% curing efficiency [Tang *et al.,* 2006]. The Plasmid curing by 8-epidiosbulbin E acetate resulted in effective reversal of bacterial resistance to multiple antibiotics in an *E. coli* strain that was resistant to a whole range of antibiotics namely, gentamicin, kanamycin, neomycin, streptomycin, tetracycline, novobiocin, ciprofloxacin, cefoperazone, oxacillin, ceftazidin, co-trimazine, imipenem, cefalexin, cefotaxime, oxytetracycline, cloxacillin, doxycycline, levofloxacin, ofloxacin, gatifloxacin, moxifloxacin, norfloxacin, cefpirome and cotrimoxazole

J.3.1.2 Antiviral activity

The alcohol extract of *D. bulbifera* when tested in the concentration range of 0.017–0.034 mg/ml was shown to kill DNA virus while inhibiting the transcription of RNA virus in direct or indirect inhibitory experiments. Its antiviral activities on HIV and the hepatitis B Viruses were illustrated by Chaniad, 2016. Of all the tested fractions of the ethanol extracts of *D. bulbifera*, the butanol and ethyl acetate fractions were found to show significant inhibition on Coxsackie B I–VI virus, but their effects on herpes simplex virus I were nearly the same. The decoction of *D.* bulbifera, however, had no inhibitory effect on various types of viruses [Cao *et al.,* 1957]. Seven compounds isolated from the ethyl acetate and water fractions of *Dioscorea bulbifera* bulbils were found to exhibit anti-HIV-1 integrase activity. Of all the compounds Myricetin, a flavonol, exhibited the most potent anti-HIV-1 integrase activity (IC_{50} value of 3.15 mM) followed by 2,4,6,7-tetrahydroxy- 9,10-dihydrophenanthrene (IC_{50} value of 14.20 mM), quercetin-3-O-*β*-D-glucopyranoside (IC50 value of 19.39 mM) and quercetin-3-O-*β*-D-galactopyranoside (IC50 value of 21.80 mM). The study also investigated the potential interactions of the active compounds with the IN active site and found that these compounds interacted with Asp64, Thr66, His67, Glu92, Asp116, Gln148, Glu152, Asn155, and Lys159, which are involved in both the 3'-processing and strand transfer reactions of integrase enzyme [Ghosh *et al.,* 2013]

J.3.1.2 Antifungal activity

The decoction of *D. bulbifera* (1:3 ratio of *D. bulbifera* to water) was shown to exhibit different degrees of inhibitory effects on a variety of skin fungi, such as *Trichophyton violaceum*, *T. concentricum* and *T. schoenleinii* [Adeniran *et al.,* 2013]. It has been proved that the isolated dihydrodioscorine from *D. bulbifera* at 0.1% concentration could inhibit the growth of some plant pathogenic fungi [Wang et al., 2009].

J.3.1.2 Antiparasitic activity

A study has confirmed *in vitro* anthelmintic activity of methanol extracts of the flesh and peel of *D. bulbifera* bulbils. The extracts were found to efficiently show anthelmintic activity against *Pheritima posthuma* and *Fasciola gigantic.* In the *in vitro* study, when treated with 100 mg/ml with peel extracts, earthworms were paralysed in 5.6 min and death was observed in 10 min, while treatment with 100 mg/ml with flesh extract led to paralysis in 8.4 min and death in 13.8 min. Similarly, liver flukes were paralyzed after 10.2 min and died after 15.81 min when exposed to 100 mg/ml of peel extract. The plausible mechanism was proposed to be the binding of phenolic and tannin compounds, which were found present in abundance in the extracts, to glycoprotein present on the cuticle together with saponin mediated alteration of permeability and pore formation in the membrane of the parasite leading to paralysis and death [Adeniran, 2013].

J.3.2 Antipyretic, antinociceptive and anti-inflammatory activities

In China, *D. bulbifera* is used to treat inflammation associated dispersal of “lumps”, hernia, sprain, injury, testicular inflammations, while an ointment prepared by incorporation of bulbils into palm oil are used in Congo and Gabon to relieve rheumatic pain and breast problems respectively [Williams, 2013]. To provide scientific evidence to these, the aqueous and methanol extracts (300 and 600 mg/kg, p.o.) from the dry bulbils of *Dioscorea bulbifera* L. var sativa were evaluated in a study against pain induced by acetic acid, formalin, pressure and against inflammation induced by carrageenan, histamine, serotonin and formalin in experimental animals. The results from this study showed that the extracts exhibited potent analgesic and anti-inflammatory activities which may be due to inhibition of inflammatory mediators such as histamine, serotonin and prostaglandins [Omodamiro, 2015]. Similarly, in case of formalin- induced paw licking test, intraplantar injection of formalin (2.5%) into the right hind paw of adult Wistar rats (weighing 180–200 g), generated a classical biphasic nociceptive response that was significantly inhibited by aqueous and methanol extracts as compared to indomethacin. In another study, the ethanol extract of *Dioscorea bulbifera* leaf (in a dose range of 500 to 15 mg/kg p.o.) was reported to exhibit strong antiinflammatory activity in egg albumin induced rat paw oedema model [Tan *et al.,* 2003]. Diosbulbin B from *D. bulbifera* also showed inhibitory effects on both acute and subacute inflammation [Nguelefack et al., 2010]. Methanol extract of the bulb of *Dioscorea bulbifera* var sativa at the dose of 250 and 500 mg/kg, p.o. was tested for the effects in mechanical hypernociception induced by intraplantar injection of complete Freund’s adjuvant (CFA), lipopolysaccharides (LPS) or prostaglandin-E2 (PGE2), as well as in partial ligation sciatic nerve (PLSN), nociception induced by capsaicin and thermal hyperalgesia induced by intraplantar injection of CFA. The therapeutic effects of *Dioscorea bulbifera* on PGE2-induced hyperalgesia were also evaluated in the absence and in the presence of L-NAME, an inhibitor of nitric oxide synthase (NOS) and glibenclamide, an inhibitor of ATP-ضsensitive potassium channels. The results of these studies showed that the extract exhibited significant antinociceptive effects in persistent pain induced by CFA and on neuropathic pain induced by PLSN. The extracts also significantly inhibited acute LPS-induced pain and PGE2 induced pain. The results further showed that the mechanism of antinociceptive activities of *D. bulbifera* in both inflammatory and neuropathic pain may be by the activation of the NO-cGMP-ATP-sensitive potassium channels pathway [Liu *et al.,* 2008]. Methanol extract of *D. bulbifera* also inhibited the nitric oxide production and iNOS mRNA expression of LPS-induced macrophages *in vitro*, which may be one of the mechanisms of its anti-inflammatory action

J.3.3 Antioxidant activity

Free radicals are key mediators for emergence, progression and the associated pathology of many degenerative diseases including diabetes, cancer and even AIDS [Manjula, 2015]. Antioxidants, namely, epicatechin, isovanillic acid, vanillic acid, myricetin are important bioactive principles in *D. bulbifera* that are responsible for protection against cardiovascular diseases and styptic activities [Tang *et al.,* 2006].

Extracts from tubers of *D. bulbifera* collected from Nepal showed potent antioxidant activity when screened on DPPH radical scavenging, ferrous ion chelating, reducing power, and total antioxidant activity models. The extracts were found to contain high oxalic acid (67 ± 9 mg/100g), citric acid (282 ± 24 mg/100g), malic acid (266 ± 20 mg/100g), succinic acid (2510 ± 108 mg/100g) and polyphenols (166 ± 10 mg/100g). These chemical constituents may be responsible for its pronounced antioxidant activity [Bhandari and Kawabata, 2004]. *D. bulbifera* from Guangzhou of China also showed a very high phenolic content (59.43 mg GAE/g), and further exhibited potent antioxidant property in terms of ABTS·+ radical scavenging activity (708.73 µmol Trolox/g) as well as ferric reducing antioxidant power (FRAP) assay (856.92 µmol Fe2+/g) [Song et al., 2010]. A bibenzyl compound, 2,5,2',5'-tetrahydroxy-3'-methoxybibenzyl and diobulbinone isolated from the ethyl acetate fraction of hydro alcoholic extract of *D. bulbifera* from China, were investigated for antioxidant activity using Trolox-equivalent antioxidant capacity by FRAP and DPPH radical scavenging models. The new bibenzyl 7 was found to show Trolox-equivalent antioxidant capacity of 0.52 ± 0.01 at a concentration of 1 mM and an EC_{50} value of 2.57 ± 0.06 mM for DPPH radical scavenging. The new diarylheptanone, diobulbinone A, however, did not show significant antioxidant activity at the same concentration by either of the methods [Liu *et al.,* 2011].

In a study, dried bulbs of *D. bulbifera* from India were extracted in 70% (v/v) ethanol in distilled water and further sequentially fractionated into petroleum ether, ethyl acetate and methanol fractions. The methanolic fraction which had the highest phenolic content (145.44 ± 3.29 µg/mL) showed very potent antioxidant activity. The percentage scavenging activity of the methanolic fraction against DPPH, hydroxyl, superoxide anion radical and nitric oxide was found to be 84.94 ± 0.62 %, 76.11 ± 1.26 %, 59.75 ± 0.98 % and 57.59 ± 0.64 %, respectively. This fraction also scavenged pulse radiolysis generated ABTS·+ radical with a second order rate constant of 1.72×10^{6}, respectively. The ethyl acetate extract also showed high percent scavenging activity of free radicals owing to its high phenolic content

(98 ± 1.17 µg/mL) and 94.05 % diosgenin [Ghosh *et al.,* 2013]. Ethanol extract of tubers of *D. bulbifera* also showed antioxidant activity in enzymatic assays (glutathione peroxidase, catalase, superoxide dismutase, glucose-6- phosphate dehydrogenase and glucose-s-transferase) and nonenzymatic assay (reduced glutathione, vitamin C and vitamin E) [Zhang et al., 2009]. Copper nanoparticles synthesized by *D. bulbifera* tuber extract also showed significant scavenging activity against DPPH, nitric oxide and superoxide radicals respectively [Mbiantcha *et al.,* 2011].
Hydroalcoholic extract of *D. bulbifera* tubers (doses of 100, 200 and 400 mg/kg) reversed the indomethacin induced gastric ulcers associated free radical changes and showed antioxidant activity by inducing a significant increase of peroxidase and catalase and a reduction in glutathione peroxidase, reduced glutathione, and lipid peroxidation level in tissues [Balasubramanian *et al.,* 2012].

J.3.4 Antitumor and Cancer chemo-preventive activity

Phytochemical constituents from *D. bulbifera* extracted using organic solvents of little polarity significantly inhibited the growth of tumour and prolonged the survival of tumour-bearing mice and human liver cancer, colon cancer and other tumour cells [Kuete *et al.,* 2012]. *D. bulbifera* decoction also inhibited cell growth in the human squamous cell carcinoma cell line SiHa, in the human cervical cancer cells Hela, and in the human hepatoma cells HepG2, in a dose and time-dependent manner [Tapondjou *et al.,* 2013]. In another study the anti-tumour-promoting effect of 75 % ethanol extracts of the rhizomes of *Dioscorea bulbifera* L. was reported in the neoplastic transformation assay of mouse epidermal JB6 cell lines. In this study the phytochemicals present in the extract were shown to exhibit potent antitumor promoting properties. Among the flavonols, kaempferol-3,5-dimethyl ether (IC_{50}= 0.64 µg/mL) exhibited strongest inhibition followed by caryatin (IC_{50} = 3.0 µg/mL), myricetin (IC_{50} = 3.7 µg/mL) and (+)-catechin (IC_{50} = 13.1 µg/mL) against tumour promotion in JB6 (Cl 22 and Cl 41) cells induced by a promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In contrast to the aglycones, the flavonol glycosides, quercetin-3-*O*-galactopyranoside, myricetin-3-*O*-galactopyranoside, myricetin-3-*O*-glucopyranoside showed a considerably reduced activity due to the presence of sugar moieties [Gao *et al.,* 2002].

The results of antitumor activity of water extract (fraction A), ethanol extract (fraction B), ethyl acetate extract (fraction C), non-ethyl acetate extract (fraction D) and isolated diosbulbin B showed that fractions B and C both decreased tumour weight in S180 and H22 tumour cells bearing mice, while fractions A and D had no such effect. Furthermore, fraction C altered the weight of spleen and thymus, and the amounts of total leukocytes, lymphocytes and neutrophils in tumour-bearing mice. Diosbulbin B was found to be the major antitumor bioactive component in the extracts and demonstrated antitumor effects in the dose-dependent manner at the doses of 2 to 16 mg/kg without significant toxicity *in vivo* [Ghosh *et al.,* 2014]. Further studies revealed moderate inhibitory effect of ethyl-*O*-β-D- fructo-pyranoside (IC_{50} > 30 µg/mL) and butyl-*O*-β-D-fructopyranoside (IC**50** > 30 µg/mL) from *D. bulbifera* on JB6 neoplastic transformation [Gao *et al.* 2007]. In another study, norditerpene compound, 8-epidiosbulbin E acetate failed to show any cytotoxicity against variety of human cancer cells, namely MCF-7 (breast cancer), SiHa (cervical cancer) and A431 (epidermal carcinoma), ensuring its potential to be used in non-cancer drug discovery programmes [Shriram et al., 2008].
Immune system modulation might be a mechanism for the antitumor effects of *D. bulbifera* rhizome, as reported in S180 and H22 tumor cells bearing mice [Chen *et al.,* 2013]. Alcoholic extracts (70%, 80% and 90% alcohol) of *D. bulbifera* were also found to inhibit the proliferation of human gastric cancer cell line SGC-7901 [Zhao *et al.,* 2012]. *D. bulbifera* extracts also significantly decrease the expression of SW579 Survivin mRNA and protein in human thyroid cancer cells and also induce apoptosis of cancer cells [Tapondjou *et al.,* 2013; Cui *et al.,* 2016].

J.3.5 Antidiabetic activity

D. bulbifera has been used traditionally to lower glycemic index in Diabetes mellitus. One of the major constituents, diosgenin has also been shown in a study to ameliorate diabetic neuropathy [Williams, 2013]. In a study, aqueous extract of *D. bulbifera* tubers administered for 3 weeks to glucose primed and streptozotocin induced diabetes Wister rats at 250, 500 and 1000 mg/kg doses showed significant antihyperglycemic activity [Okon and Ofeni, 2013]. Ethanolic extract of *Dioscorea bulbifera* was studied against alloxan-induced diabetic rats. Intraperitoneal administration of a single dose of 380.0, 760.0 and 1140.0 mg/kg body weight of the extract were found to exhibit significant reduction in the blood glucose levels of the albino rats [Ghosh *et al.,* 2014].

The actual mechanism of action by which *D. bulbifera* exerts the antidiabetic effect was furnished in a report which confirmed the inhibition of two key enzymes, α-amylase and α-glucosidase. In this study it was shown that among petroleum ether, ethyl acetate, methanol and 70% ethanol (v/v) extracts of bulbs of D. bulbifera, the ethyl acetate extract showed highest inhibition up to 72.06 ± 0.51% and 82.64 ± 2.32% against α-amylase and α-glucosidase respectively. Diosgenin, which was isolated from the extract also showed an α-amylase and α-glucosidase inhibition up to 70.94 ± 1.24% and 81.71 ± 3.39%, respectively [Ghosh et al., 2015]. The mechanism was established to be uncompetitive mode of binding to α-amylase as confirmed by decrease in both Km and Vm values. Further, hydrogen bonding between carboxyl group of Asp300 and hydrophobic interactions between Tyr62, Trp58, Trp59, Val163, His305 and Gln63 residues of α-amylase was indicated by molecular docking. Similarly, two catalytic residues (Asp352 and Glu411) from α-glucosidase were found to be the target of interaction with diosgenin [Ghosh et al., 2014]. Copper nanoparticles synthesized by *D. bulbifera* tuber extract also showed significant inhibition against α-glucosidase and murine intestinal glucosidase [Mbiantcha *et al.,* 2011].

J.3.6 Effect on lipid metabolism

Significant antidyslipidemic effects were observed in C57BL/6J mice fed for 4 weeks with aqueous extract of *D. bulbifera* tubers at doses of 250, 500 and 1000 mg/kg [Okon and Ofeni, 2013].

J.3.7 Diuretic activity

Ethanol extract of *Dioscorea bulbifera* leaf administered to rats at 500 mg/kg, 250 mg/kg, 125mg/kg, 62.5mg/kg, 31.25mg/kg, 15mg/kg p.o. exhibited profound diuretic activity [Tan *et al.,* 2003].

J.3.8 Gastroprotective activity

In a study, a hydroalcoholic extract of *D. bulbifera* tubers administered at doses of 100, 200 and 400 mg/kg was reported to exhibit gastroprotective effect against indomethacin-induced gastric ulcers in rats [Balasubramanian *et al.,* 2012].

J.3.9 Effect on immune systems

The effect of 15-day oral administration of decoction of *D. bulbifera* at doses of1000, 490, 240 g/kg body weight on immune systems were investigated in a study. Results showed that at high doses the decoction of *D. bulbifera* significantly suppressed the phagocytosis activity of mononuclear macrophages. However, at medium doses enhanced activities of natural killer cells, the antibody quantity of B cells and the quantity and proliferation of spleen T lymphocytes were observed. This experiment indicated that high doses of *D. bulbifera* could suppress the immune function in mice, while medium doses could improve the immune function [Cui *et al.,* 2016]. Polysaccharides from *Dioscorea bulbifera* administered at doses of 100 or 150mg/kg lowered peripheral blood T-cell subpopulation CD4+/CD8+ ratio. The *Dioscorea bulbifera* polysaccharides + Cyclophosphamide combination also attenuated Cyclophosphamide effect in lifting CD4+/CD8+ ratio [Hu *et al.,* 2005].

J.3.10 Neuropharmacological activity

Hydroalcoholic extract of tuber of *Dioscorea bulbifera* at doses of 100 and 300 mg/kg, p.o. was investigated for the central nervous depressant action. The results showed that the treatments significantly reduced spontaneous motor activity, rectal temperature and prolonged the pentobarbitone induced hypnosis in mice. There was however, no effect on motor co-ordination as determined by the rota rod test and this confirmed central action rather than peripheral action of extract. Further extract treatments also showed anxiolytic activity in plus maze test and head-dip test [Tang *et al.,* 2006].

J.3.11 Cardioprotective activity

Myricetin, epicatechin, isovanilic acid and vanillic acid have been shown to be important bioactive components in *D. bulbifera* that protect against cardiovascular diseases [Galani and Patel, 2017]. In a study, administration of 70% ethanolic extract of *D. bulbifera* to rats at doses of 150 mg/ kg of body weight for 30 days resulted in significantly improved ventricular performance in terms of aortic flow, left ventricular developed pressure (LVDP) and the first derivative of developed pressure (LVmax dp/ dt) of

D. bulbifera treated rats during post-ischemic reperfusion. *D. bulbifera* also significantly reduced the size of myocardial infarction by 20 ± 2.64% as compared to the control group. Administration of *D. bulbifera* resulted to a decreased number of apoptotic cardiomyocytes by 16.89 ± 1.7% thus revealing that *D. bulbifera* had anti-apoptotic activity. The study also examined the modulation of pro- and anti-apoptotic proteins by *D. bulbifera*. The observed upregulation of procaspase 3 and downregulation of cleaved caspase 3 coupled with prevention of loss of phase II enzyme HO-1 suggested that *D. bulbifera* extract ameliorates rat myocardial ischemia and reperfusion injury with an associated reduction in apoptotic cell death [Karuppiah *et al.,* 2016]. The isolated steroidal saponin Diosgenin, was shown to exhibit cardioprotective action against Hypoxia-reoxygenation Injury in H9c2 cardiomyoblast cells as evidenced from the improved cell survival after hypoxia-reoxygenation injury, decreased release of lactate dehydrogenase, during cell death, upregulated the pro-survival molecules like B-cell lymphoma 2 (Bcl-2), heme oxygenase-1 and the phosphorylation of ATK (at serine 473); and at the same time down regulated pro-death molecules like Bax [Li, 2003]

J.3.12 Effects on Thyroid Glands

D. bulbifera has been employed in the treatment of subacute thyroiditis with good success [Nam *et al.,* 2006]. In a study, the administration of extracts of *D. bulbifera* (0.75 or 1.5 g/kg) to Sprague-Dawley (SD) rats treated with sodium levothyroxine (160 lg/kg, 5 days) resulted in decrease of thyroxine (T4) concentration and triiodothyronine (T3)-uptake level. The results suggested that *D. bulbifera* decreased excess thyroid hormone and increased metabolism, resulting in improvement of the hyperthyroid state [Jindal *et al.,* 1969].

J.3.13 Anorexiant Activity

Anorexient activity of *Dioscorea bulbifera* Linn has also been reported [Song *et al.,* 1983].

J.3.14. Anthelmentic Activity

Methanolic extracts of the flesh and peel of the bulbils of D. bulbifera, showed in vitro anthelmintic activity on Fasciola gigantica and Pheritima posthuma at concentrations ranging from 10 to 100 mg/ml (Patel and Galani, 2017)

J.4 Safety data

J.4.1 Ethnic use safety

J.4.2 Single dose toxicity

J.4.3 Reproductive toxicity

J.4.4 Cytotoxicity

Using the “hepatotoxic equivalent combinatorial markers (HECMs)” for a hepatotoxic HM, *Dioscorea bulbifera* tuber (DBT), two diterpenoid lactones, 8-epidiosbulbin E acetate (EEA) and diosbulbin B (DIOB), were discovered as the most hepatotoxicity-related markers. The chemical combination of EEA and DIOB, reflecting the whole hepatotoxicity of original DBT extract with considerable confidential interval, was verified as HECMs for DBT.(Wei Shi., *et al.,* 2018).
Hepatotoxicity is a very important limitation to the multiplicity uses of the plant.

J.5 Key (proposed) usage

J.5.1 Therapeutic indications

J.5.2 Dosage/posology

J.5.4 Contraindications

J.5.5 Special warnings and precautions for use

J.5.7 Interactions

J.5.8 Pregnancy and lactation

J.5.9 Adverse effects

J.5.10 Overdose

J.5.11 Evaluation of efficacy

Traditional and pharmacological evidence proved through tests.

J.6 Safety and interaction classification

J.7 Regulatory information

British Pharmacopoeia 2012 (BPCO, 2012)

WHO Monograph on Selected Medicinal Plants Volume 2, 2002

European Pharmacopoeia 7.0 (2008)

USP30-NF25, The United States Pharmacopeia 30-National Formulary 25 (USPC, 2006)

J.8 Trade information

J.9 Possible developments

Annex K
(informative)

Example of appropriate dosage preparations, frequencies of use and directions of *Dioscorea bulbifera*

Dosage forms used in tradition: One spoonful of root powder, tuber paste is given with Curcuma aromatic, root paste given with milk (IBSD, BSI).

Contraindications
Dioscorea contains chemical like the drug digoxin. Consuming this plant along with digoxin might increase the effect of digoxin and increase the risk of side effect.

14. Precautions
Do not eat raw plant as medicine. It contains chemicals like digoxin (lanoxin), which causes irregular heartbeat. Certain chemical of the plant causes seizures. Female should be taken care while consuming this plant it will affect fertility.

Experimental pharmacology
Studies conducted for the effect of polysaccharides on cervical cancer. The oral treatment of polysaccharides (DBLP) at concentration of 100-150mg/kg in U14 cervical tumour bearing mice treated with Cyclophosphamide (CTX) (25 mg/kg).
DBLP alone also inhibited tumour (25.6% at 100 mg/kg or 37.6% at 150 mg/kg), CTX+DBLP combination produced tumour inhibition rates of 5.6 -9% higher than CTX alone. (Cui et al., 2016).

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