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TITLE

Pharmacognostical and Analytical Standards of *Dioscorea Esculenta* Burkill.

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TITLE

Pharmacognostical and Analytical Standards of *Dioscorea Esculenta* Burkill.

ABSTRACT

Background: *Dioscoria* species is a *Kanda Shakha* (tuberous vegetable) useful as vegetable and grows abundantly around Udupi district, Karnataka, India. *Dioscorea esculenta* Burkill is one of the rare and costly tuber cultivated in very few areas of Udupi. Colloquially known as *Duk-Genasu* and *Tuppa-Genasu* the boiled tuber is used as a nutritional supplement for the children and is used in many forms of dishes. **Aim:** The study aimed at standardizing & authenticating the trial drug in accordance to international standards and quality control of Ayurveda drug. **Materials & Methods:** Pharmacognostical and analytical study including HPTLC finger printing of *Dioscorea esculenta* Burkill. was undertaken by making use of various parameters. **Results:** Physico-chemical tests revealed constants for routine analysis of *Dioscorea esculenta* Burkill. Preliminary phytochemical analysis of aqueous extract showed presence of carbohydrate, steroids, saponins, tannins, and coumarins. HPTLC finger print profile of ethanol extract of *Dioscorea esculenta* Burkill. showed maximum compounds under 254 nm frequency i.e. 13 compounds, while densitometry scan showed the maximum peaks at 366 nm i.e. 4 peaks. **Conclusion:** The present study carried out on *Dioscorea esculenta* Burkill. not only established the data that maybe utilized for identification, but also established the monographic data on purity and standard of the tuber sample.

Keywords: *Analytical, Dioscorea esculenta* Burkill., *Herbal Monographs, Pharmacopoeia, Quality Standards, Standardisation.*

Introduction

Vegetables play an important role in attaining the goal of Ayurveda. *Acharya* Charaka advised to collect locally available vegetables for preparing medicines and as diet. [1] He has described different types of vegetable under *Shakavarga* like *Patra*-leaf, *Kanda*-tuber and underground store parts, *Pushp*-flower, *Mula*-root. [2] Sushruta has described that, any substances belonging to the group of *Dhanya* (corns), *Mamsa* (meat), *Phala* (fruit) and *Saka* (vegetables) not mentioned here has to be identified and understood by its qualities, taste and features of the *Mahabhutas.*[3] Udupi district of Karnataka is considered to be *Anupa Desha* (marshy area) where different types of *Kanda Shakha* are in routine use. Most common and abundantly available among *Kanda Shakha* around Udupi is *Dioscorea* species. *Dioscorea esculenta* Burkill is one of the rare and costly tuber cultivated in very few areas of Udupi district. This tuber is believed to be a nutritional supplement for the children and is used in different food preparations. The vernacular name in Kannada is *Duk-Genasu* and *Tuppa-Genasu*. Pharmacognostical and analytical study including HPTLC finger printing was undertaken by making use of various parameters to standardize & authenticate in accordance to international standards and quality control of Ayurveda drug.

Materials and Methods

Collection of the sample:

The samples were collected from Udupi in October 2012, they were then washed thoroughly, cut into small pieces and shade dried, later they were pulverized into coarse and fine powders.

Macroscopy

The fresh tuber sample and powder of tuber were keenly observed under naked eyes to record the specific botanical characters.

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures were indicated by the scale-bars.[4]

Physicochemical – Standardization Parameters of *Dioscorea esculenta*. Burkill

Tuber powder was tested for loss on drying at 105°C, total ash, acid insoluble ash, ethanol soluble extractive, water soluble extractive were performed as per standard protocol.[5]

Preliminary phytochemical analysis

Preliminary phytochemical investigation was done to detect the presence of steroids, carbohydrates, tannin, saponins, and coumarins in aqueous and ethanol extract.[6]

HPTLC finger printing

One gram of powdered samples was dissolved in 10 ml of ethanol and kept for cold percolation for 24h and filtered. Five and ten µl of the above samples were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (10: 2.5: 0.5). The developed plates were visualized in UV 254, 366, under white light at 540 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded. [7]

Results

Macroscopic characters

Dried tubers were light in weight, shrivelled, sub-cylindrical, un-branched, dorsoventrally rounded measuring 3-8 cm in length and 1 to 3 cm in width. Surface rough, marked with root projection, root scar present, externally pale brown, internally pale whitish yellow, odour-indistinct, taste-sweet-pungent, and starchy. (Figure 1.1-1.2)

Microscopic characters

Diagrammatic TS of tuber is circular with irregular margin showing outer, narrow cork and inner wide parenchymatous ground tissue embedded with vascular bundles occupying almost the entire section of the tuber. (Figure 2)

Detailed TS shows 8 to 10 layers of outer thick-walled suberised cells of the cork and inner wide parenchymatous ground tissue, the peripheral 5 to 10 layer of the cells lying underneath the cork being circular to oval in shape, exhibiting pits and beaded-walled cells at places and embedded with bundles of acicular crystals of calcium oxalate and starch grains, the inner parenchymatous cells loaded with starch grains of various size and shape and vascular bundles consisting of xylem and phloem.

Powder was Slightly yellowish brown, shows fragments of cork in surface and transversely cut view; plenty of starch grains scattered as such throughout and embedded inside the parenchymatous cells; longitudinally cut fragments of pitted vessels; pigment cells embedded with yellow content; collenchymatous tissue embedded with idioblast containing bundles of acicular crystals (raphides); and fragment of xylem elements of various size and shape. (Figure no. 3.0-3.15)

Figure 1. Macroscopy of *Dioscorea esculanta*

Fig. 1.1 Fresh tubers

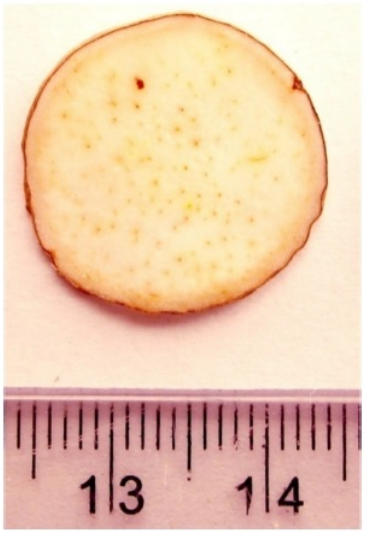


Fig. 1.2 Cut surface of tuber

Figure 2. Detailed microscopic features of tuber of *Dioscorea esculanta*

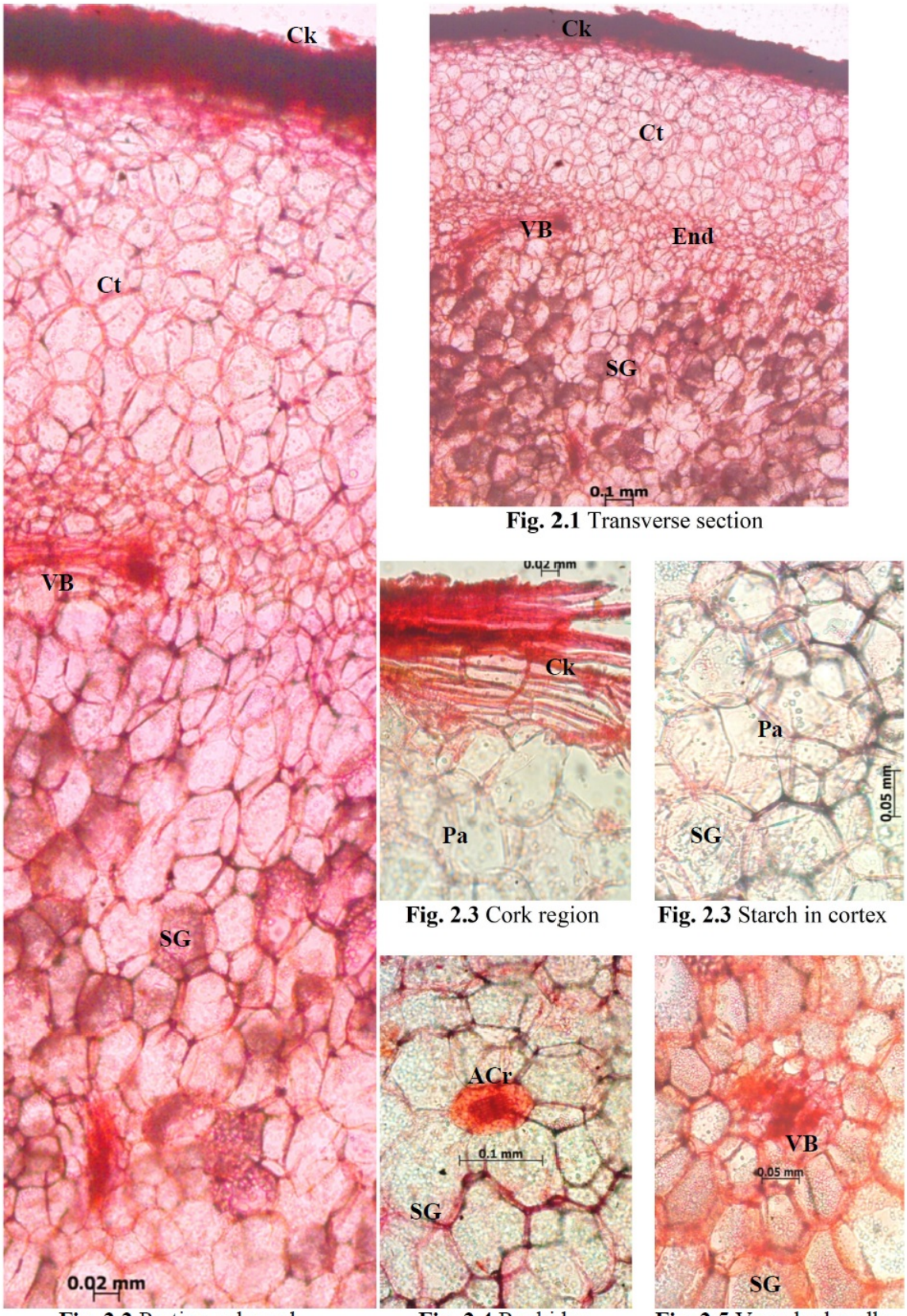


Fig. 2.2 Portion enlarged

Fig. 2.1 Transverse section

Fig. 2.3 Cork region

Fig. 2.3 Starch in cortex

Fig. 2.4 Raphides

Fig. 2.5 Vascular bundle

ACr, acicular crystals; **Ck**, cork; **Ct**, cortex; **End**, endodermis; **Pa**, parenchyma; **Ph**, phloem; **SG**, starch grains; **VB**, vascular bundle.

Figure 3. Detailed microscopic features of tuber powder of *Dioscorea esculanta*

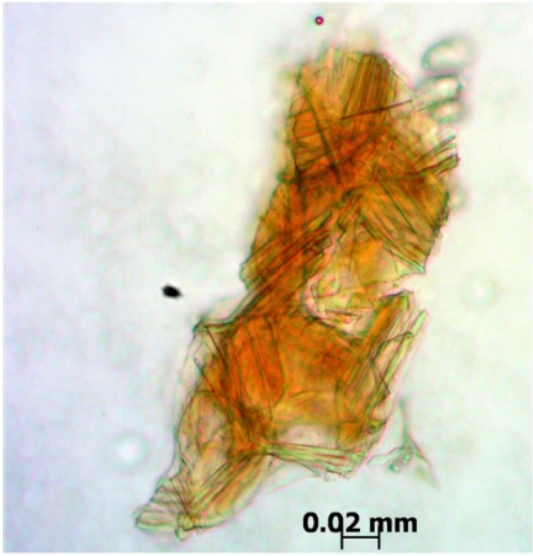
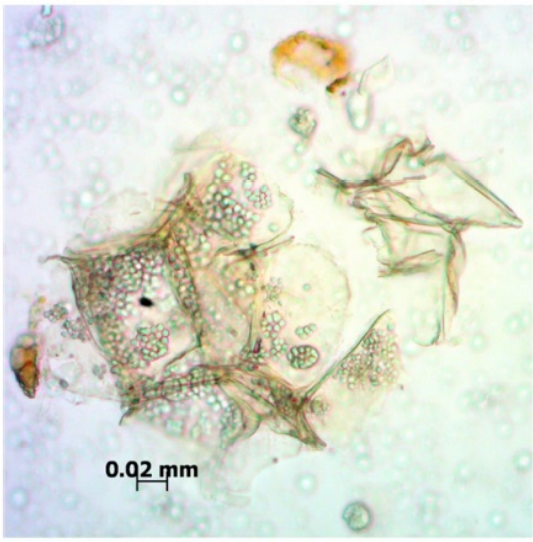
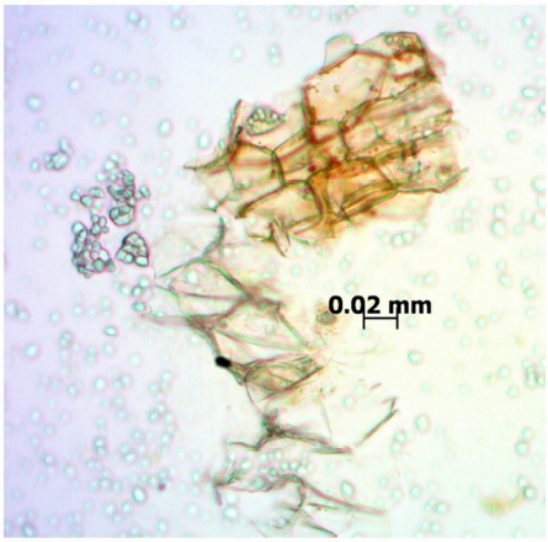
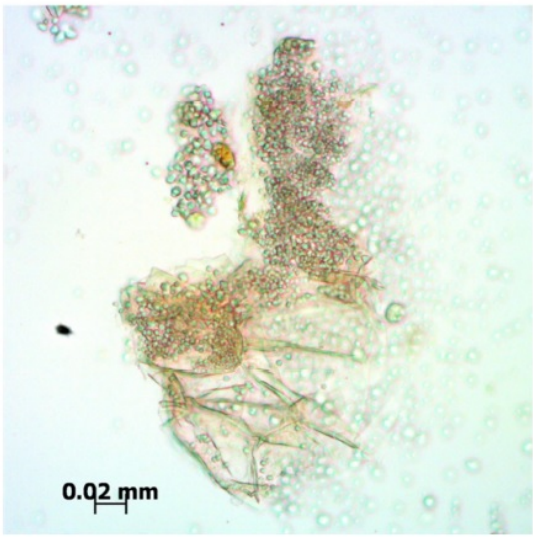


Fig. 3.1. Obliquely cut cork

Fig. 3.2. Cork in surface view







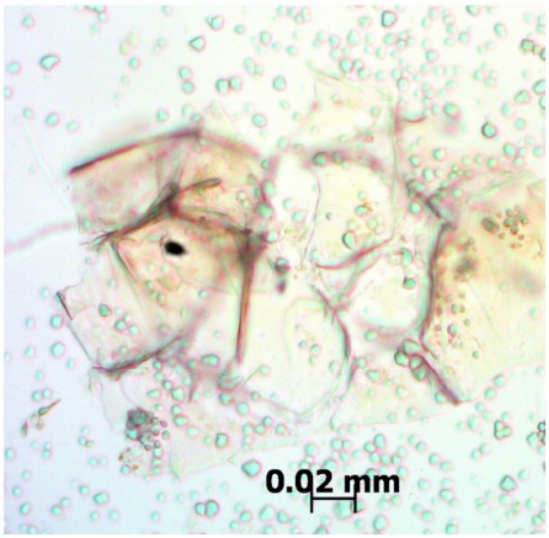


Fig. 3.3. Parenchyma cells with starch

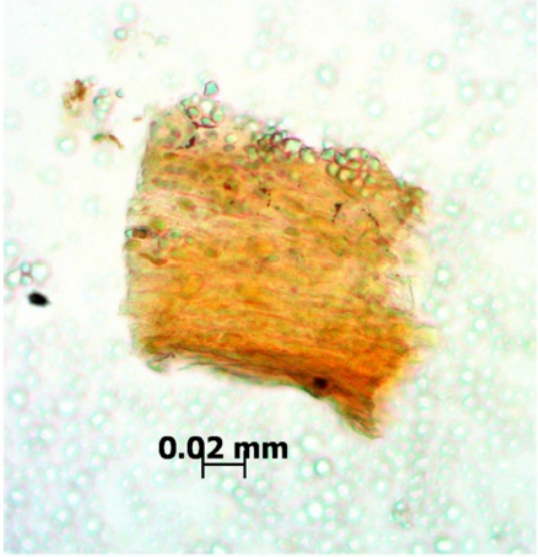


Fig. 3.4. Transversely cut phloem

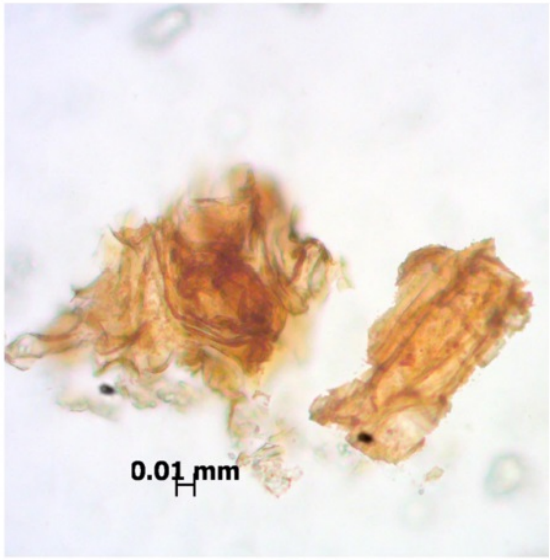


Fig. 3.5. Outer cork cells in surface view

Fig. 3.6. Inner cork cells in surface view

Fig. 3.7. Raphides

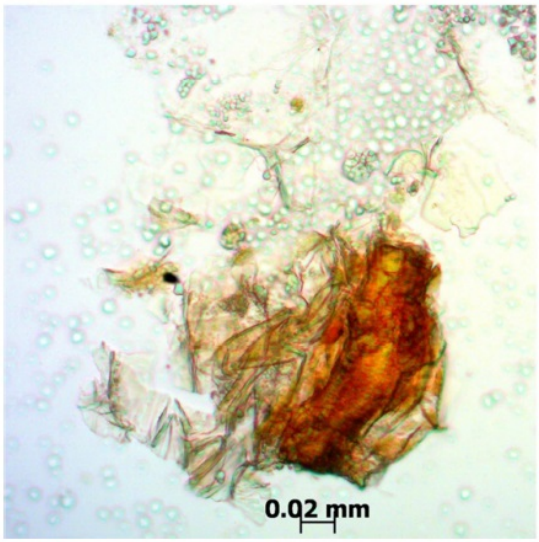


Fig. 3.8. Xylem elements

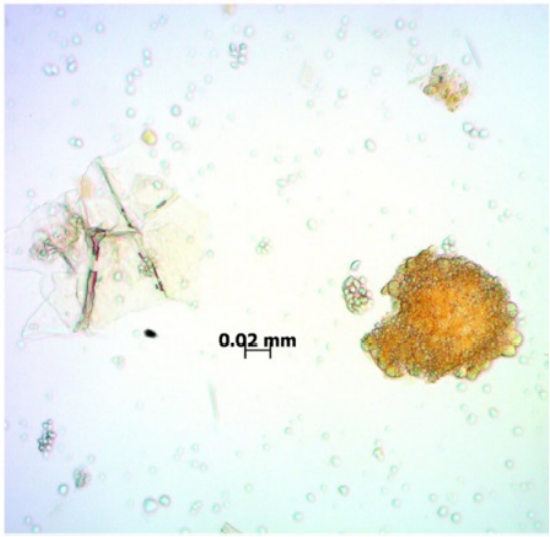


Fig. 3.9. Parenchyma with content

Fig. 3.10. Idioblasts with raphides

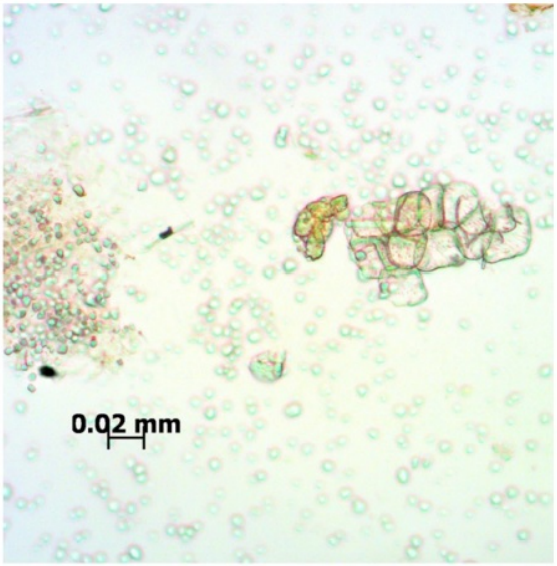


Fig. 3.11. Thick walled pitted cells

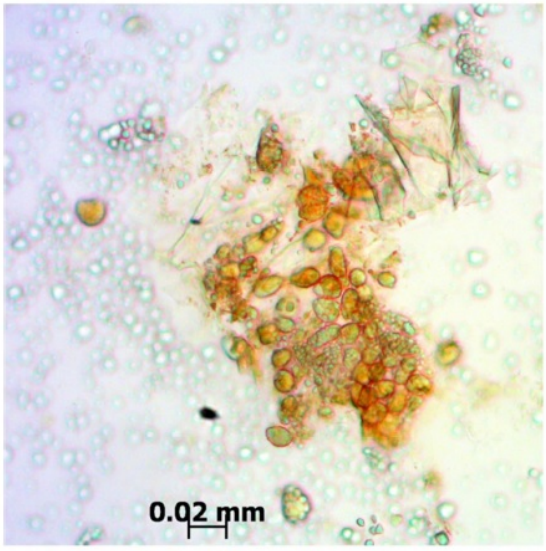


Fig. 3.12. Cells with colored content

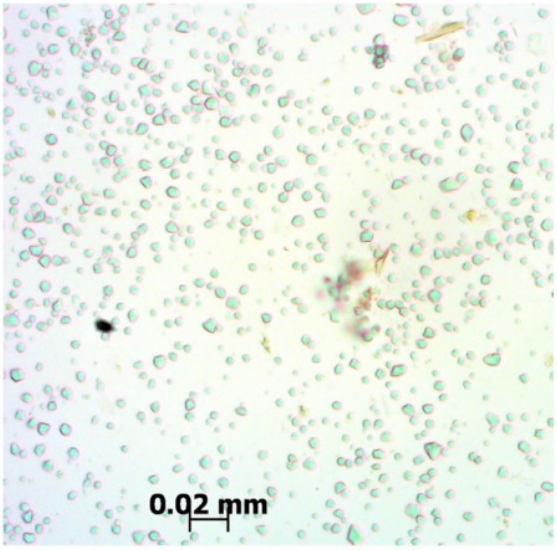


Fig. 3.13. Simple starch grains

Fig. 3.14. Thick walled pitted cells

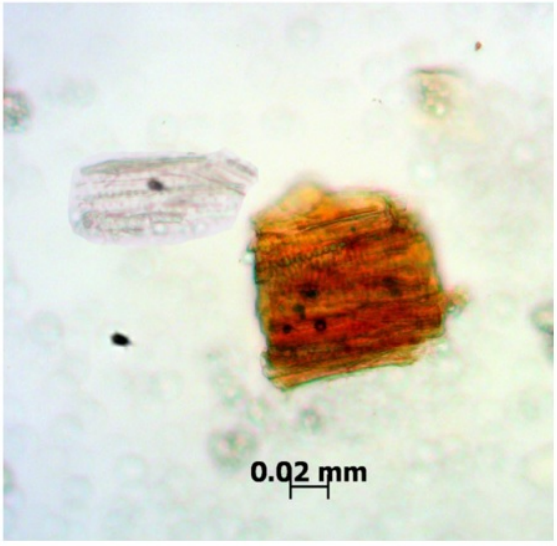


Fig. 3.15. Pitted vessels

Dioscorea esculenta was found to be free from foreign matter such as insects, moulds, animal faecal matter and other contaminants like sand, stone and extraneous materials. The loss on drying result was found to be 13.05 % w/w. Total ash value was 4.34 % w/w and showed 0.85 % w/w acid insoluble ash. The water and ethanol soluble extractive value was 15.82 and 2.16 % of w/w respectively. (Table 1).

Table no. 1: Results of standardization parameters for *Dioscorea esculanta*

Parameter	Result in %
Loss on Drying	**13.05**
Total Ash	**4.34**
Acid Insoluble Ash	**0.85**
Alcohol Soluble extractive value	**2.160**
Water Soluble extractive value	**15.82**

The qualitative analysis carried out showed the presence of carbohydrates in water extract as well as ethanolic extract. Carbohydrates were found positive by using Molisch's test, Fehling's test and Benedict's test in water extract whereas in case of ethanolic extract, it was positive by using Fehling's test and Benedict's test. Steroids were present in water extract. Steroids were found positive in Liebermann-Buchard test and Salkowski test. The test conducted to detect saponins present in test samples became positive in water extracts. Saponins were found positive with $NaHCO_3$ on shaking with water. There was the presence of tannins in water extract which was found positive by using $FeCl_3$. Coumarins were found positive in water extract when analysed using 2N NaOH. The test using tin and thionyl chloride was negative which was indicative of absence of triterpenoids in both the extractives (Table no. 2 and 3).

Table 2. Results of preliminary phytochemical tests

Sl No	Tests	Colour if positive	*Dioscoria esculanta*
1	**Alkaloids**		
	Dragendrof's test	Orange precipitate	Orange Red colour Solution
	Wagners test	Red precipitate	Orange Red colour Solution
	Mayers test	Dull white precipitate	Light yellow colour Solution
	Hagers test	Yellow precipitate	Yellow colour Solution
2	**Steroids**		
	Liebermann-buchard test	Bluish green	Reddish Brown colour
	Salkowski test	Bluish red to cherry red	Cherry Red colour
3	**Carbohydrate**		
	Molish test	Violet ring	Violet colour at junction
	Fehlings test	Brick red precipitate	red precipitate
	Benedicts test	Red precipitate	red precipitate
4	**Tannin**		
	With $FeCl_3$	Dark blue or green or brown	Brown precipitate
5	**Flavanoids**		
	Shinoda's test	Red to pink	Yellow colour
6	**Saponins**		
	With $NaHCO_3$	Stable froth	Stable froth formed
7	**Triterpenoids**		
	Tin and thionyl chloride test	Pink	Dark Red colour
8	**Coumarins**		
	With 2 N NaOH	Yellow	Yellow colour
9	**Phenols**		
	With alcoholic ferric chloride	Blue to blue black, brown	Brown colour
10	**Carboxylic acid**		
	With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
11	**Resin**		
	With aqueous acetone	Turbidity	No turbidity
12	**Quinone**		
	5% NaOH	Pink/purple/ red	Yellow colour

Table no. 3: Results of preliminary phytochemical tests for *Dioscorea esculanta*

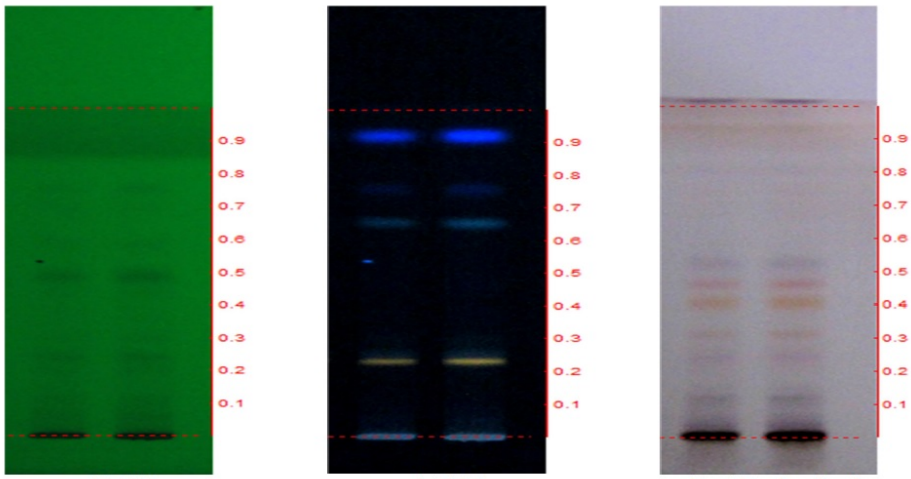
Test	Inference
Alkaloid	–
Carbohydrate	+
Carboxylic acid	–
Coumarins	+
Flavanoids	–
Phenol	–
Quinone	–
Resins	–
Steroid	+
Saponins	+
Tannin	+
Terpenoid	–

Table no. 4. R_f value of Alcohol extract of *Dioscoria esculanta* (12 µl)

At 254nm	At 366nm	Post Derivatisation
0.07(Light Green)	-	0.07(Light Violet)
0.12(Light Green)	-	0.12(Light Violet)
-	0.24(Yellow)	-
0.25(Light Green)	-	0.25(Light Pink)
-	0.26(Light Violet)	-
0.31(Light Green)	-	0.31(Light Brown)
-	-	0.42(Light Brown)
-	-	0.47(Light Pink)
0.51(Green)	-	-
-	-	0.54(Light Violet)
0.59(Light Green)	-	-
-	0.66(Violet)	-
0.77(Light Green)	0.77(Light Violet)	-
-	-	0.81(Light Pink)
-	0.94(Violet)	-

The HPTLC finger print profile of alcohol extract has been obtained with suitable solvent system. At 254 nm and under white light the alcohol extract showed 13 spots with different R_f values. Whereas at 366 nm and after derivatisation with Formic acid it has shown 9 spots of same R_f values. On densitometric scan of the alcohol extract, there were 13 peaks under 254 nm, 14 peaks at 366 nm, and 4 peaks under white light (Table no. 4 and Figure 4-6).

Figure 4. TLC photodocumentation of Alcohol extract of *Dioscoria esculanta*



At 254nm At 366nm Post Derivatisation

Track 1- 8 µl; Track 2 –12 µl

Solvent system – Toluene: Ethyl acetate: Formic Acid (8:2:0.1)

Figure 5. HPTLC photo documentation at 12 µl

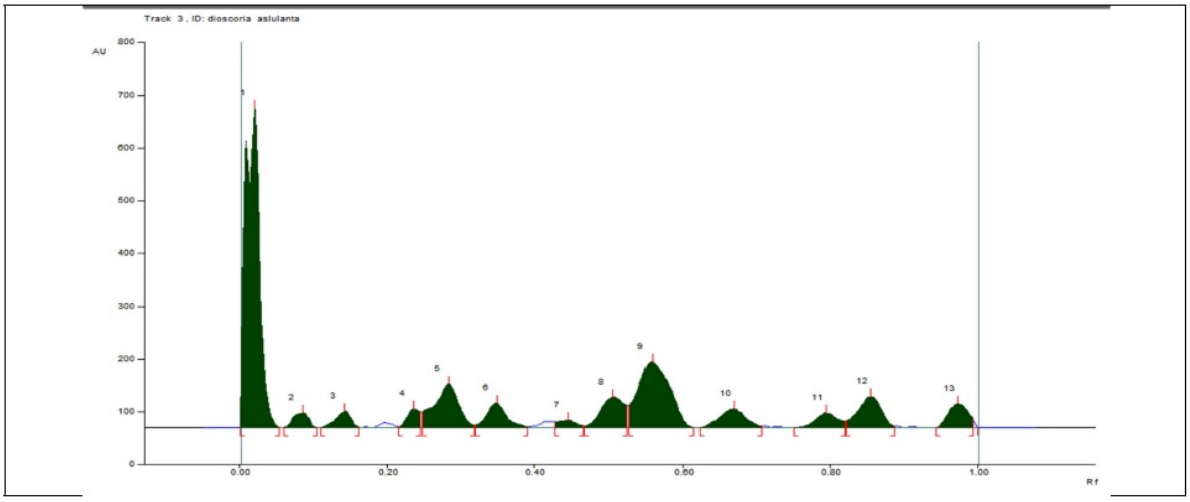
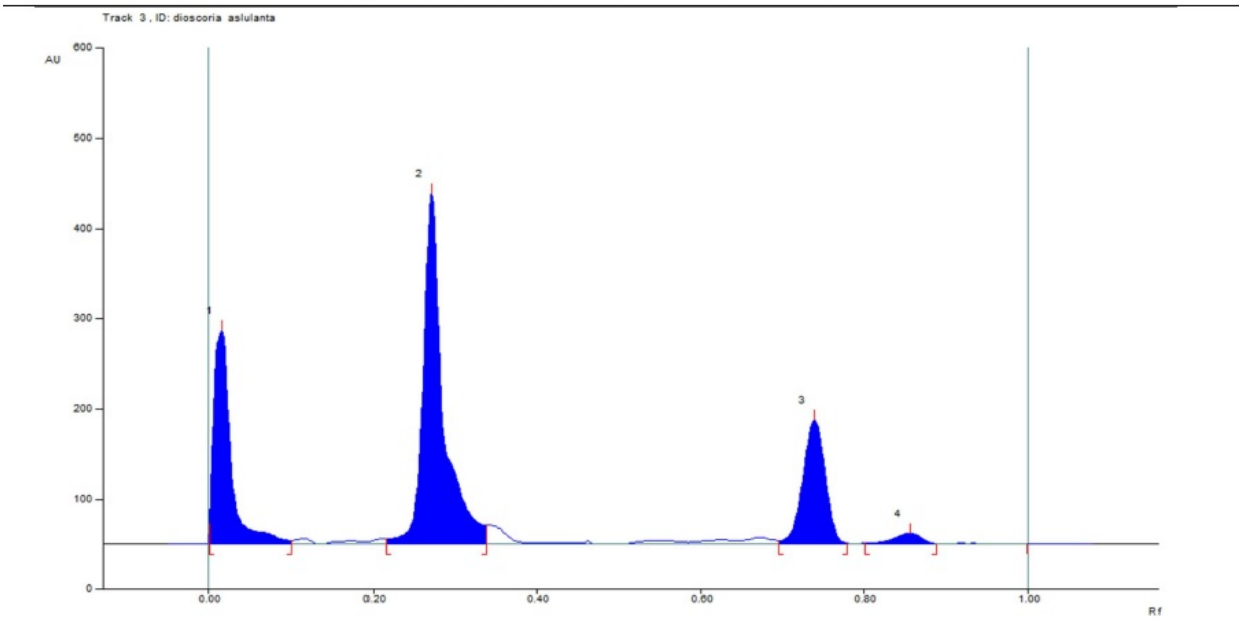


Fig 6.a *Dioscoria esculanta* at 254nm



Discussion

Study of traditionally used and commonly available plants will be an added benefit to the existing knowledge. Folklore medicine is the knowledge transmitted informally as general knowledge and practiced or applied by anyone in the culture having prior experience. Conducting researches in a systematic way are the best method for the proper application of the folk medicines with evidence, incorporating this view the present study was carried out. Pharmacognostical as well as analytical study of *Dioscorea esculenta* were the primary steps to establish its quality control and authentication as per WHO guidelines.

The *Dioscoria bulbifera* Linn. is considered as source of *Varahikanda* which is *Rasayana* (Rejuvenator) and *Vrishya* (Vrilificant). *Acharya* Bhavaprakash mentioned many *Aluka's* (colocasia) and in which *Maadhvaluka* (lesser yam) is considered as *Dioscoria esculenta* Burkill. by P.V. Sharma. [4] Further the nutritious quality attributed to the *Dioscorea esculenta* can be correlated with similar species having *Rasayana* quality. Further studies on the same line can reveal the effectiveness of *Dioscorea esculenta*.

Conclusion

Phytochemical study reveals the observation conducted using alcoholic extract of *Dioscorea esculenta* which showed the presence of Carbohydrate, Coumarins, Steroid, Saponin and Tanin in alcoholic extract. HPTLC finger print profile of alcohol extract *Dioscorea esculenta* showed maximum compounds under 254 nm frequency i.e. 13 compounds. On densitometric scan of the *Dioscorea esculenta* alcohol extract, it showed the maximum peaks at 366 nm i.e. 4 peaks. This study carried out on *Dioscorea esculenta* not only established the data that maybe utilized for identification, but also established the purity and standard of the leaf sample. Monographic data obtained by pharmacognostical and analytical study on this herbal medicine may be used as reference standard in future works.

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