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A study on the nutritional, phytochemical, antioxidant and antimicrobial properties of *Dioscoreaesculenta L.* (Cherukizhangu)

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Abstract
Dioscorea esculenta L. (Cherukizhaghu) is an underutilized tuber crop. Having its origin in the South Eastern parts of Asia, it is also prominent in the peninsular India and Sri Lanka. It was used as a traditional medicine against inflammations and stress. This is a scientific study aimed at estimating the antioxidant, antimicrobial, physicochemical, nutritional properties etc. of the tuber extracts. Estimation of Diosgenin, commercially used for the production of cortisone, progesterone and other steroids, was done. The study revealed significant antioxidant and antimicrobial properties and the presence of phytobetaines, tannins, terpenoids, saponin, flavonoids and alkaloids in the tuber extracts prepared in distilled water and ethanol. The quantification of starch, protein, crude fiber and ash suggested high nutritional potential. The investigation suggests the potential of *Diascorea esculenta L.* to be used as a highly nutritive and medicinal food crop.

Keywords: antioxidant, antimicrobial, *Diascorea esculenta*, diosgenin, nutritional

Introduction
Dioscorea esculenta, a prominent species in Dioscoreaceae family is a traditionally used edible tuber, native to South East Asia and can be found in India, Nigeria, China and some other parts of the world. It is a staple food in tropical countries, especially in most parts of West Africa. Decoctions made from the tuber have been traditionally used to cure ulcers, abscesses and boils. It has been used since ages in curing symptoms associated with menopause and several disorders of the genitals. The other ethnomedicinal uses of *D.esculenta* include remedies for fatigue, inflammation, stress and weak immunity. Reportedly the peelings of the yam possess properties against cancer and fungal infections. The major phytochemical constituents of the tuber are allantoin- a cell-proliferant; dioscin- a saponin and diosgenin- an aglycone used in the industrial production of steroid hormones. An active component in the yam-Diosgenin is historically used in the treatment of arthritis-like diseases and rheumatism (Krochmal *et al*., 1973; British Herbal Pharmacopoeia, 1983; Sofowora, 1993). It is also used against spasmodic cough, nausea, diarrhea etc. in pregnant ladies. The aim of the following investigation is to study the nutritional, antioxidant and antimicrobial properties of D. esculenta tuber and to estimate its phytochemical constitution.

Materials and Methods
Collection &Authentication of Plant Material
Tubers of *D.esculenta* were gathered from Chirakadavu, Kanjirapally, in the district of Kottayam of Kerala and was authenticated at Central Tuber Crop Research Institute (CTCRI), Thiruvananthapuram, Kerala. The specimen herbarium was annotated with the date of collection and locality. It was also labelled and numbered following the standards. The authenticated sample was washed, dried in shade, powdered and kept in air tight bottles for future studies.

Fig 1

Fig 2

Phytochemical Analysis
5g of the sample was weighed and passed into a Stoppard flask with 30 ml solvent (ethyl alcohol and double distilled water). The flask was shaken at regular intervals for the first

six hours and then after 24 hours. After that the extract was collected, straines/filtered and stored in sealed bottles.

Qualitative phytochemical analysis
Preliminary phytochemical analysis was carried out following the standard methods set forth by Brain & Turner (1975) [7] and Evans (1996) [14].

Method of Detection of Alkaloids
Mayer's test: Extracts were diluted separately in dilute HCL and filtered. Upon treatment of the filtrates with Mayer's reagent, a yellowish-cream precipitation indicates the presence of alkaloids.

Detection of Flavonoids
H_2SO_4test: A few drops of H_2SO_4 were added to the extracts. Development of an orange color represents the presence of flavonoids.

Detection of Steroids
Acetic anhydride (2ml) was added to 5ml of each extract to which 2 ml of H_2SO_4 was dripped. A change in color from violet to blue/ green is indicative of steroids in considerable concentration.

Method of Detection of Terpenoids
Salkowski s Test: 5 mg of leaves and flowers extract of D.esculenta was mixed in 2 ml chloroform and 3ml of con. H_2SO_4 form a layer. The development of a reddish brown color at interface is indicative of terpenoids.

Method of Detection of Phenols
10ml each of the extracts were treated with 2-4 drops of ferric chloride solution. Development of bluish black color is indicative of the presence of phenol.

Method to Detect Saponin
0.5ml of the extract was taken and shaken together with five ml distilled water. Appearance of froth shows that saponins are present in the sample.

Test for Tannins
A minimal volume of extract was mixed in water and kept in a hot water-bath. After filtration, ferric chloride was added to the filtrate. Formation of a green color (comparatively dark) shows substantial presence of tannins.

Test for Detecting the Presence of Carbohydrates
0.5ml each of the extracts were dissolved separately in five ml distilled water and then filtered. This mixture was then used to check the presence of carbohydrates.

Detection of Proteins and Amino acids
To 0.5 ml of extract, 0.5 ml 40% NaoH and 2 drops of 1% $CuSO_4$ solution was mixed. A violet color formation points to the presence of proteins in the sample.

Quantitative Analysis of Phytochemicals
Alkaloids:
One gram sample was taken in a 250 ml beaker and 10% acetic acid in ethanol (200 ml) was added and allowed to stand at rest for about 4 hours. After filtration, the extract was concentrated using a water bath to a quarter of the initial volume. NH_4OH (Concentrated) was added drop by drop till the completion of precipitation. The solution was then allowed to settle down and the precipitate was collected, washed with dilute NH_4OH and finally filtered. The residue that remains contains the alkaloid, which was later dried and weighed.

Flavonoids
1gram sample was repeatedly extracted with 100ml of 80% methyl alcohol (aqueous) at room temperature. The mixture was then filtered through a Whatman No1 filter paper to a 250ml beaker (pre weighed). The filtrate was moved to a water bath and left to evaporate. The residue was weighed.

Estimation of Total Phenols
The sample was boiled with 50 ml ether for at least 15 minutes. From this, 5 ml was pipetted into a flask and 10 ml water was added together with 2 ml NH_4OH and 5 ml amyl alcohol (concentrated). The samples were made up to 50 ml and kept to react for a minimum of 30 minutes for color development. The absorbance at 505 nm was measured.

Proximate Analysis
Moisture Content
The amount of moisture in 10 g material was determined by drying it in a drier at approximately 60˚C for 4 days. The sample was again weighed after 72 hours and the percentage of moisture (M %) was determined.

Estimation of total carbohydrate by Anthrone method
100 mg of homogenized sample was weighed out into a clean tube. 5ml HCl (2.5 N) was mixed and was let to react in boiling water-bath for 2-3 hours and later cooled down to room temperature. Powdered Sodium Carbonate was added slowly with constant shaking till the effervescence ceased. The mixture was transferred to a volumetric flask (100 ml) together with distilled water. A final volume of 100 ml was obtained with washings of the and mixed thoroughly. 10 ml of this solution was centrifuged and aliquotsof0, 0.2, 0.4, 0.6, 0.8 and 1 ml standard solution was pipetted out. These were transferred to 6 numbered test tubes and diluted to 1.0 ml final volume with distilled water by adding 1.0, 0.8, 0.6, 0.4, 0.2 and 0 ml in the respective tubes. The tubes were kept in boiling water bath for 10 minutes after 4.0 ml Anthrone reagent was added. The tubes were cooled rapidly under running water and the absorbance at 630 nm was noted. The standard graph was drawn by plotting the absorbance against concentration of standard solution.

Estimation of proteins by Bradford's colorimetric method
1.0g sample was weighed out and homogenized using mortar and pestle with 5ml phosphate buffer. The homogenate was decanted into a centrifuge tube. The residue was re-extracted 3-4 times with 5ml phosphate buffer and centrifuged at 5000RPM for twenty minutes. The supernatant was obtained in a volumetric flask of 50 ml. Re-extraction was done twice by mixing the pellet in buffer solution and by adding phosphate buffer the volume was made to 50 ml. 1.0ml aliquot was transferred to a centrifuge tube and 1ml 20% TCA was added, mixed and kept for 30 minutes. Centrifuged at 5000 RPM for 30 minutes was done and supernatant discarded. The pellet was washed with acetone twice and dissolved in 5ml 0.1N NaOH. 1.0 ml aliquot of this solution was taken for estimation. Aliquots of

standard solution of protein 0, 0.2, 0.4, 0.6, 0.8 and 1.0ml were pipetted and transferred to numbered test tubes and made up to 1.0ml with phosphate buffer. 5ml Bradford reagent was added in all the tubes. The absorbance at 595 nm was measured and the result determined using the standard curve.

Estimation of ash

The samples were analyzed for total ash using the AOAC procedures. Samples were dried for the estimation and for determining total ash content. About 3 g of the sample was weighed out into a ceramic crucible and heated in a muffle furnace at 550°C for about half an hour and let to cool in desiccators.

Estimation of fiber

2g of dried tissue was weighed out and boiled in 200 ml 1.25% H_2SO_4 for 30 minutes and filtered using muslin cloth and later washed in boiling water till the filtrate become non-acidic. Then it was boiled in 200 ml of 1.25% w/v NaOH for 30 minutes and again filtered. After that the sample was washed in 25 ml of boiled 1.25% H_2SO_4 first and then three times in distilled water and at last with 25 ml absolute alcohol. Then the residue was taken in a pre-weighed dish and dried for 120 minutes at 130±2˚C. The dry weight was measured and the residue ignited for half an hour at 660±15˚C. At the end, the residue was cooled in a dissector and weighed again.

Antioxidant Assay

Antioxidant properties of tuber extracts were estimated by the procedure of Oyayizu et.al, 1986. Different concentrations of the extract was prepared using ethanol and water.

The sample was made up to 1 ml and 2.5ml with Phosphate buffer (0.2M, pH6.6). 2.5ml of1% Potassium Ferric Cyanide was added and the mixture was then incubated at a temperature of 50^0C for 20 minutes. After removing from incubation, 2.5 ml 10% Trichloro acetic acid was mixed and centrifuged at 3000 rpm for about 10 minutes. From supernatant, 2.5 ml was removed and mixed with 2.5 ml of water (distilled) and 0.5ml $FeCl_3$ (0.1%). Blank was prepared and the absorbance was measured at 700nm.

Antimicrobial Assay

Antibacterial Assay

The antibacterial effectiveness of the extracts were estimated by using five gram negative bacterial strains *viz*., *Klebsiella pneumonia, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, and Streptococcus pyogens* and two gram positive bacteria namely *Staphylococcus aureus* and *Bacillus cereus*. The bacterial suspension was seeded on a sterile molten LB medium with a sterile swab and disc diffusion method was employed for the assay. Incubated at 37^0 for 18 hours was done. The ZOI (Zone of Inhibition) was determined by measuring the diameter of the inhibition zone around the disc (in millimeters) including the disc diameter. It was measured in three different fixed directions in all the replica and the average values found out.

Antifungal Assay

The *D.esculenta* tuber is known to contain an alkaloid Dioscin which is proved to have antifungal properties. The effect of the tuber extracts in inhibiting *Malassezia furfur* was investigated by disc diffusion assay. Fungal suspension was swabbed using a sterile loop on to the medium in the petri plates and prepared discs saturated with plant extract were carefully placed over each of the four quadrat. The plates were put for incubation at 37^0C for 18 hours to determine fungal activity.

Estimation of Diosgenin

Diosgenin is a phytosteroid sapogenin extracted from the *Diascorea esculenta* tubers, reported to have tremendous medical applications. Dry and powdered sample (5g) was mixed in a solution of sodium acetate (0.2 mg) in 10 ml water. The mixture was set to rest for 24 hours and then hydrolyzed with 5% HCl (20 ml) for 14 hours. The hydrolysate mass was washed and filtered with water untill free from acid. After that the sample was dried and extracted with hexane with the help of a Soxhlet apparatus for 4 hours.

Upon concentrating hexane extract, crude Diosgenin was obtained in the form of a slightly yellow solid.

Diosgenin obtained was crystallized by adding 95% ethyl alcohol to produce colorless needle like crystals (diosgenin).

A stock solution of diosgenin (1,000 µg ml^{-1}) was prepared with acetonitrile. To each tube with different concentrations of diosgenin (standard) (100–1,000 µg ml^{-1}), 2 ml of acetonitrile and 1 ml of 70–72 % perchloric acid was mixed.

For estimation, 0.5 ml Diosgenin was treated with 2 ml of acetonitrile and 1 ml of perchloric acid(70–72%). The tubes were incubated at room temperature until a stable yellow chromogen developed. The concentration of yellow colored chromogen was measured by spectrophotometer at 430 nm and a standard calibration curve was made (Peiqin *et al*. 2012 with slight modification).

Results and Discussion

Results from the aforementioned estimations and assays are listed below:

Phytochemical Analysis

Qualitative Phytochemical Analysis

Table 1

Components	Ethanol	Water
Alkaloid	+	+
Flavanoid	+	+
Phenol	+	+
Tannin	-	+
Carbohydrate	+	+
Steroid	+	+
Protein	+	+
Terpenoids	+	-
Saponins	+	+
Phytobetaines	+	+

Quantitative Phytochemical Analysis

Table 2

Components	Quantity per g
Alkaloids	0.36 mg
Flavonoids	0.128 mg
phenolics	0.1125 mg

Proximate Analysis

Analysis of the proximate components of *Dioscorea escluenta* showed that it contains all important contents in varying concentration as illustrated below

Moisture content

Moisture content of the tubers influence the textural quality and keeping quality of the tubers. The chance of spoilage always increases with the increase in moisture content.

M% = (Fresh wt - Dry wt /Fresh wt) x 100

M% = (117.076 – 91.504 / 117.076) ×100=21.84%

Ash

Ash content of the tuber represents its mineral composition and amount of ash depends on the soil it grows, moisture content, time of harvest.

$$\text{Ash content (g/100 g sample)} = \frac{\text{weight of the ash}}{\text{Weight of sample}} * 100$$

Ash content present in *dioscorea escluenta* was 5.67%

Fibre

$$\text{Crude fibre \% by weight} = \frac{\text{weight of the fibre}}{\text{Weight of sample}} * 100$$

The amount of fiber present is 7.57%.

Protein

Table 3

Sample	OD at 595nm	Concentration of protein in mg/1g of sample
Dioscorea esculenta, L	0.188	45

Diosgenin

Diosgenin is the most active component in Dioscorea species.

It is similar to cholesterol structurally. The amount of diosgenin present in 5g of sample is 230µg

Table 4

Sample	OD at 430 nm	Concentration of Diosgenin (µg/5g of sample)
Dioscorea esculenta, L	0.350	230µg

Antioxidant Activity

Antioxidant activity estimation was done by reducing power assay. The results show the potent use of *Dioscorea escluenta* as an antioxidant and anti-cancerous agent.

Table 5

Concentration (in ethanol)	O.D at 700nm
50	0.109
100	0.218
150	0.365
200	0.452
250	0.528

Antibacterial assay

The antibacterial activity against Gram-negative and Gram-positive bacteria was tested.

The zone of inhibition of the microbial colony are depicted in the tables below.

Ethanolic extracts proved to be most potent bactericidal agent against all the strains studied, in comparison to water.

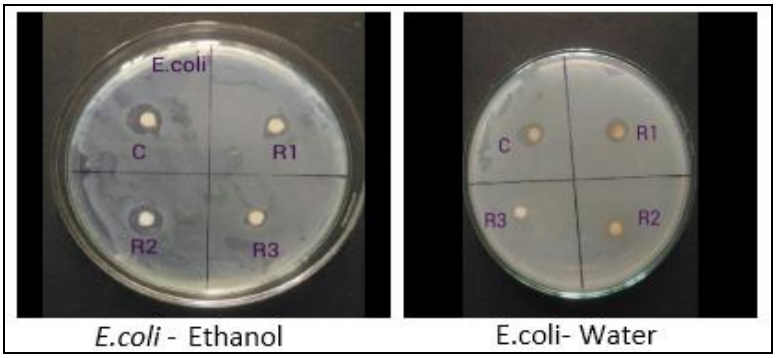
The largest zone of inhibition was observed in ethanolic extract against *E.coli* (1.07 cm) and minimum zone of inhibition by water extract against *Klebsiella* (0.25 cm).

Table 6

No	Gram negative	Water (cm)					Ethanol (cm)				
		C	R1	R2	R3	Avg	C	R1	R2	R3	Avg
1.	*E.coli*	0.333	0.556	0.558	0.567	0.560	0.835	0.846	1.122	1.264	1.07
2.	*Protease*	0.44	0.56	0.567	0.668	0.598	0.825	0.99	1.018	1.056	1.02
3.	*Klebsiella*	0.22	0.260	0.256	0.243	0.253	0.536	0.581	0.725	0.793	0.69

Table 7

No	Gram Positive	Water (cm)					Ethanol (cm)				
		C	R1	R2	R3	Avg	C	R1	R2	R3	Avg
1.	*Bacillus*	0.408	0.274	0.282	0.240	0.265	0.504	0.287	0.289	0.230	0.268
2.	*Staphylococcus*	0.50	0.507	0.516	0.602	0.541	0.456	0.899	0.827	1.103	0.943



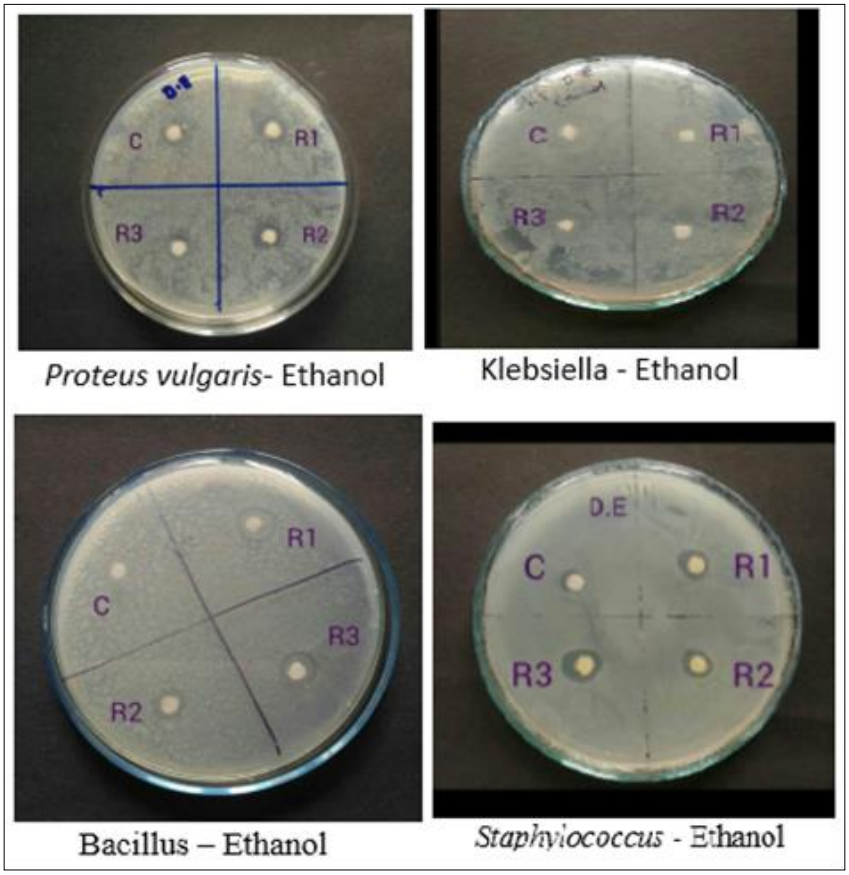


Fig 3

Antifungal activity of *Dioscorea esculenta, L*

Table 8

No	Fungai	Water (cm)					Ethanol (cm)				
		C	R1	R2	R3	Avg	C	R1	R2	R3	Avg
1.	*Malassezia furfur*	0.311	0.457	0.501	0.517	0.491	0.655	0.83	0.84	0.78	0.816

From the present study it is inferred that underutilized tubers such as *Dioscorea esculenta L.* has a wide range of properties.The amount of starch is also higher so it can be used as a food crop in our daily life.

Diosgenin an important component which has anticancer property is also evaluated. It can be used as a precursor for the production of steroids and can be used as a natural source of diosgenin. Products from *Dioscorea esculenta, L* have much pharmaceutical and industrial advantages.

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