



Phytochemical Screening and *in vitro* Antimicrobial Activity of Extracts from Tubers of Wild *Dioscorea* species

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Abstract

Genus *Dioscorea* of the family Dioscoreaceae, is a herbaceous annual vine plant. Some *Dioscorea* species are important food crops producing tubers and bulbils rich in medicinal value. Phytochemical screening was carried out and the *in vitro* antimicrobial activities of tuber extracts of *D. bulbifera* L., *D. alata* L., *D. esculenta* (Lour.) Burkill. and *D. pentaphylla* L. were assessed using standard techniques. Qualitative analysis for phytochemicals present in tuber extracts confirmed the presence of alkaloids, saponins, flavonoids, phenols, carbohydrates, proteins, glycosides and steroids. Tuber extracts of all the *Dioscorea* species exhibited inhibitory activity against all bacterial and fungal pathogens tested. The study demonstrated that tested *Dioscorea* species contains phytochemicals with antimicrobial properties and hence are potential medicinal dietary plant.

Key words: *Dioscorea* species, phytochemicals, antimicrobial activity, tuber

Introduction

Dioscorea is a monocot plant of the family Dioscoreaceae, the family of true yams. It is tropical twinning herb with underground and sometimes aerial tubers. It is popular due to its twinning characteristic of stem. It is an important biological and evolutionary link between Eudicots and Grasses (Mignouna, et al., 2009). Genus *Dioscorea* has more than 600 species in the world (The plant list, 2013) and about 50 in India (Mukherjee and Bhat, 2013).

The root tubers and bulbils are used as staple food in many parts of the world. *Dioscorea* is world's fourth most important crop after potatoes, casava and sweet potatoes. *Dioscorea* tubers are rich source of reserved starch and other nutrients like proteins, minerals, fibres and many secondary metabolites.

Tuber extracts of *Dioscorea* are rich in polyphenols, especially flavonoids; steroidal saponins, glycosides – diosgenin, dioscin; (Narula et al., 2007), resins, alkaloids, enzymes (Ghosh et al., 2011). The studies

also report the role of *Dioscorea* tubers for curing bronchitis, asthma, cancer, skin diseases, leprosy, urinary discharges, rheumatism etc. (Burnali Dutta, 2015).

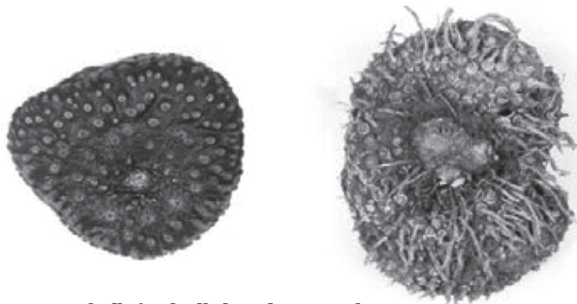
The aim and objective of this study was to examine the antimicrobial potential of phytochemicals present in the tubers of *D. bulbifera*, *D. alata*, *D. esculenta* and *D. pentaphylla*.

Materials and Methods

Collection of plant material and Preparation of sample

The tubers of *D. alata*, *D. esculenta*, *D. pentaphylla* and *D. bulbifera* were collected from Western Ghat Mountain Range of Maharashtra. The plants were identified based on DNA Barcoding using barcodes of life data system (Global Portal for Biodiversity) and NCBI (National Centre for Biotechnology Information) at Paul Hebert Centre for DNA Barcoding and Biodiversity Studies, Aurangabad. The tubers were washed and surface sterilized in 6% Sodium hypochloride solution. The tubers were sliced into pieces with a knife and then dried under

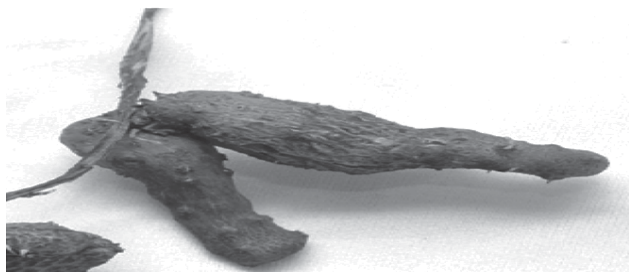
shade. Dried tuber pieces were ground in mortar with pestle, and then in an electric blender into powdered form. The powdered samples were stored in air tight containers at 4° C in the refrigerator, until used.



Dioscorea bulbifera bulbil and root tuber



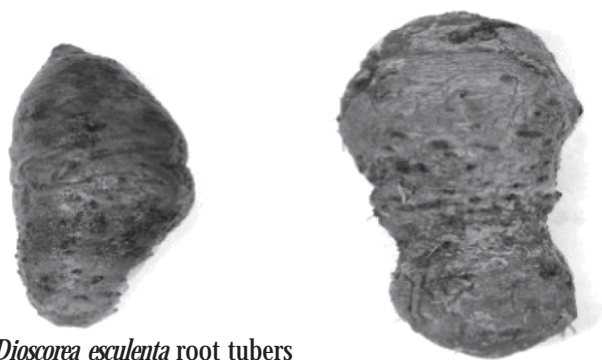
Dioscorea alata root tuber



Dioscorea alata aerial tuber



Dioscorea pentaphylla root tuber



Dioscorea esculenta root tubers

Preparation of plant extracts

The plant extracts were prepared by Ayurvedic Pharmacopoeia method. Ten gram of powder was soaked in 100 ml of distilled water, methanol and hexane separately. The mixtures were stirred for 3-4 hrs on a rotary shaker and allowed to stand for 24 hrs. Mixtures were filtered by using Whatman filter paper. The extracts were collected and evaporated to dryness by using boiling water bath. The dried aqueous, methanol and hexane extracts were collected separately (The Ayurvedic Pharmacopoeia of India, 2008).

Qualitative Phytochemical analysis

The crude powder, aqueous and methanol extracts were screened for the presence of alkaloids, saponins, flavonoids, tannins, phenols, carbohydrates, proteins, amino acids, lipids, glycosides and steroids using standard techniques.

Antimicrobial activity

Procurement of culture

The pure cultures of the microorganisms were obtained from the National Collection of Industrial Microorganisms (NSIM), NCL, Pune. The bacterial cultures of *Streptomyces albus*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus faecalis* and the fungi used for testing were *Candida albicans* and *Aspergillus niger*.

Disc diffusion assay by Kirby Bauer method

All the extracts were subjected to antimicrobial assay using disc diffusion assay by Kirby Bauer technique. The nutrient plates were prepared by pouring 20 ml Mueller Hinton agar and Potato Dextrose agar in sterile petri plates for bacteria and fungi respectively and allowed to solidify. Twenty four hours old bacterial cultures and 48 hours fungal cultures were inoculated by using cotton

swabs. The cotton swabs were dragged across the agar surface in a zigzag pattern for uniform inoculation. The sterilized filter paper discs of 5 mm were dipped into individual extracts and placed on the swabbed agar plates before incubation period of 48 hours at 37°C for bacteria and 72 hrs at 30°C for fungi. The plates were observed for zones of growth inhibition, and the diameter of these zones was measured in mm. The effect of the extract on fungi and bacteria was compared with the standard antibiotics viz., Fluconazole and Streptomycin for fungi and bacteria respectively.

Result and Discussion

The phytochemical composition of the extracts given in the Table 1 indicates that *Dioscorea* species shows presence of alkaloids, saponins, flavonoids, phenols, carbohydrates, glycosides and steroids. Yams have received importance from many generations due to their potency in fertility in males due to presence of steroid – diosgenin. Diosgenin is precursor for synthesis of corticosteroids and hormones which increases fertility in males (Crabbe, 1979).

Table 1. Phytochemical screening of four species of *Dioscorea*

Tuber Extract	Chemical constituent	Dioscorea species					
		<i>D. alata</i>		<i>D. bulbifera</i>		<i>D. esculenta</i>	<i>D. pentaphylla</i>
		Root tuber	Aerial tuber	Root tuber	Aerial tuber	Root tuber	Root tuber
Methanol Extract	Alkaloids	+	+	+	+	+	+
	Saponins	-	-	-	-	-	-
	Flavonoids	+	+	+	+	+	+
	Tannins	+	+	+	+	+	+
	Phenol	+	+	+	+	+	+
	Carbohydrates	-	-	-	-	-	-
	Proteins	-	-	+	+	-	-
	Amino acids	-	-	-	-	-	-
	Lipids	-	+	-	-	-	-
	Glycosides	-	-	-	-	-	+
Steroids	+	+	+	+	-	-	
Aqueous Extract	Alkaloids	+	+	+	+	+	+
	Saponins	+	+	-	-	-	-
	Flavonoids	+	+	-	-	+	+
	Tannins	+	+	-	-	+	+
	Phenol	+	+	-	-	+	+
	Carbohydrates	-	-	-	-	-	-
	Proteins	-	-	-	-	-	-
	Amino acids	-	-	-	-	-	-
	Lipids	-	-	-	-	-	-
	Glycosides	-	-	-	-	+	+
Steroids	+	+	+	+	-	-	
Crude Powder	Alkaloids	+	+	+	+	+	+
	Saponins	+	+	+	+	+	+
	Flavonoids	+	+	+	+	+	+
	Tannins	+	+	+	+	-	-
	Phenol	+	+	+	+	-	-
	Carbohydrates	+	+	+	+	+	+
	Proteins	-	-	-	-	-	-
	Amino acids	-	-	-	-	-	-
	Lipids	-	-	-	-	-	-
	Glycosides	-	-	-	-	+	-
Steroids	+	+	+	+	-	-	

Key : + present, - absent

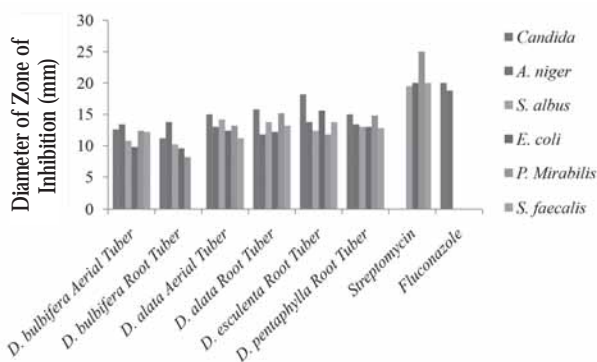


Fig. 1. Comparative analysis of inhibitory effect of Methanol extracts and standard antibiotics against selected pathogens

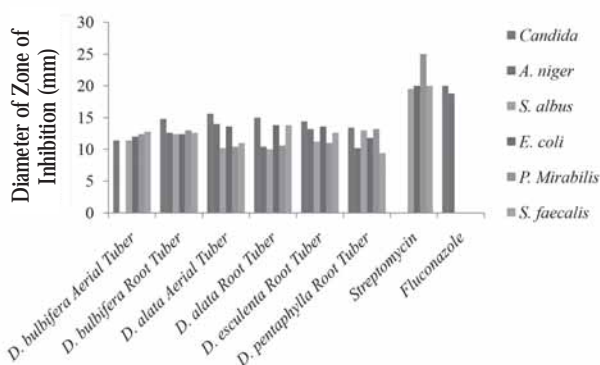


Fig. 2. Comparative analysis of inhibitory effect of Hexane extracts and standard antibiotics against selected pathogens

The biological functions of flavonoids apart from antioxidant properties are protection against allergies, free radicals and platelet aggregation, inflammation, viruses, ulcers and tumours (Barakat et al., 1993). The presence of alkaloids in the tubers of *Dioscorea* species indicate that these tubers cannot be eaten raw. The tubers should be cooked thoroughly before consumption to avoid itching and toxicity. The presence of phenols in the extract indicate that these tubers could act as anti clotting, antioxidant, anti inflammatory and hormone modulator (Singh et al., 2007). Glycosides were found in all species of *Dioscorea* which helps to lower the blood pressure (Nyarko et al., 1990) while (Adedapo, et al., 2009) reported that Saponins are of great importance due to their relationship with compounds like glycosides, steroids, sex hormones and Vitamin D.

In the antimicrobial tests of tuber extracts of *Dioscorea* species, the methanol and hexane extracts exhibited inhibitory activity against all test bacterial and fungal

pathogens which are associated with various diseases in humans (Fig. 1). This indicates that compounds with antibacterial and antifungal actions are present in the root and aerial tubers of all species of *Dioscorea*. The inhibitory activity against these bacterial and fungal pathogens may be due to presence of alkaloids, saponins, flavonoids, phenols, tannins, glycosides and steroids in the tubers. Alkaloids, flavonoids, tannins, phenols and saponins are secondary metabolites that serve as defence mechanism against invasion of microorganisms (Bonjar et al., 2004). Tannins prevent growth and proliferation of microorganisms by binding to proline rich proteins which interfere with the process of cell protein synthesis (Shimada, 2006). Flavonoids are synthesized by plants to fight against pathogenic microorganisms (Dixon *et al.*, 1983). More amount of lipophilic flavonoids may disrupt microbial membranes (Tsuchiya et al., 1996). Antimicrobial activity of saponins is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz et al., 1996). Glycosides are natural drugs with potential of antimicrobial activity (Sameerah et al., 2013) whereas nitrogen containing steroids are more effective against microorganisms (Kull et al., 1953 and Tarbet et al., 1953). Flavonoids and steroids possess antimicrobial activities (Silvia et al., 2003).

Conclusion

The presence of wide range of phytochemicals like alkaloids, saponins, flavonoids, phenols, tannins, glycosides and steroids in all the species of *Dioscorea* confirms that these are potential source of medicinal dietary crops. The present study supports the ethnomedicinal use of *Dioscorea* tubers against different bacterial and fungal pathogens tested for antimicrobial activity. Further research can be done for isolation and purification of drugs for the treatment of various diseases.

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