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## *In vitro* Antimicrobial Activity of Garhwal Himalaya Medicinal Plant *Dioscorea deltoidea* Tuber

Subhash Chandra, Sarla Saklani, Abhay P. Mishra

### Abstract

The antimicrobial activity of the all extracts of *Dioscorea deltoidea* were studied against ten (gram negative and gram positive bacteria) and three fungal stain. The results showed that the minimum inhibitory concentration (MIC) of *Dioscorea deltoidea* tubers extract was 50 µg/ml against *P. aeruginosa*. The ethanolic tubers extract of *Dioscorea deltoidea* showed significant activity 19±1 mm, 17±1 mm and 15±1 mm against *S. aureus*, *P. aeruginosa* and *E. coli* against food poisoning bacteria and phytochemical screening for the presence of glycosides, flavonoids, phenols, resin and tannins.

**Keywords:** Antibacterial, Antifungal and Phytochemical screening.

### 1. Introduction

India has great wealth of medicinal plants and their traditional uses. The use of traditional medicinal plants as a source for relief from illness. Herbal medicine is the oldest form of health care known to mankind. Herbs have been used by all cultures throughout the history and they constitute an integral part of the development of modern civilization. Medicinal and aromatic plants and their derived are rich in antibacterial compounds which could be an alternate way to combat bacterial diseases even against some bacteria which are becoming resistant to certain synthetic medicines. *Dioscorea deltoidea* belongs to the family of dioscoreaceae which is commonly known as Tedu in Uttarakhand. *Dioscorea deltoidea* tubers have ginger like shaped and occurs in a range of habitats from rocky areas to (semi) shady woodland with acidic or alkaline soils. *Dioscorea deltoidea* tubers are used as a food product and also used for the treatment of different diseases such as digestive disorder, diarrhea, irritability, abdominal pain, wounds burns, chronic liver pain and anemia<sup>[1]</sup>. *Dioscorea deltoidea* of Uttarakhand are the rich sources of diosgenin. Diosgenin play an important role in the cholesterol metabolism. Diosgenin, a steroid saponin, is the product of hydrolysis by acids, strong bases, or enzymes of saponins extracted from the tubers of *Dioscorea deltoidea*. The sugar-free (aglycone), diosgenin is used for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products. The plant tubers are also believed to possess activities such as antimicrobial, antioxidant, stomachic and hypoglycemic activities<sup>[2,3]</sup>.

### 2. Materials and Methods

#### 2.1 Plant Material

The fresh parts of tubers of *Dioscorea deltoidea* were collected from adjoining area of Dhunglwali village (Dist- Chamoli Uttarakhand) in the month of September – November 2012. The plant was authenticated by botanist Prof. R. D. Guar, Department of Botany and the voucher specimen number is GUH 7569. H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

#### 2.2 Preparation of plant Extract

The plant material was separated into its selected part tuber air dried ground to moderately fine powder and soxhlet extracted with increasing polarity solvent (petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water)<sup>[4]</sup>.

Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of tuber were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be

air dried (weight of crude extract 500 gm). The various concentrated extracts were stored in air tight container for further studies.



Fig 1: *Dioscorea deltoidea* tuber & bail

### 2.3 Media

Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc. all product of Himedia Laboratories Mumbai (India) were used in this study.

### 2.4 Bacterial Strains

Ten bacterial strains were used namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter gergoviae*, *salmonella enterica typhi*, *shigella flexneri*, *staphylococcus epidermidis*, *streptococcus pyogenes*, and *Bacillus cereus*, The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, Customer no, 3921.

### 2.5 Fungal Strains

Three fungal strains were used namely *Candida albicans*, *Aspergillus flavus* and *Aspergillus parasiticus*, The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

### 2.6 Antibacterial assay

The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts [5,6]. Diluted bacterial culture (100 µl) was spread over nutrient agar plates with a sterile glass L-rod. 10 mg/ml and 50 mg/ml of the each extracts were applied to each filter paper disc (Whatman No. 1, 5 mm diam.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37 °C for 24 hr. After incubation, the diameter of inhibition zones was measured with a caliper.

### 2.7 Antifungal assay

The antifungal activity was tested by disc diffusion method [7,8].

The sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hr. both culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45 °C respectively, and fungal plates were incubated at 25-28 °C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

### 2.8 Phytochemical analysis

The qualitative phytochemical properties of the dried powdered sample were determined using standard methods [9].

## 3. Result and discussion

Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity. The results of antibacterial, antifungal and phytochemical screening activity table 1, 2 and 3 reveals that antibacterial, antifungal and phytochemical screening activity of tubers of *Dioscorea deltoidea* was evaluated against ten bacterial and three fungal human pathogenic strains.

### 3.1 Antibacterial and antifungal activity

The ethanolic tubers extract of *Dioscorea deltoidea* showed significant activity 19±1 mm, 17±1 mm and 15±1 mm against *S. aureus*, *P. aeruginosa* and *E. coli* against food poisoning bacteria and the order of the species based on total antibacterial activity is as follows: *Staphylococcus aureus* > *P. aeruginosa* > *Escherichia coli* (MTCC 729).

### 3.2 Phytochemical screening

The phytochemical screening of plant for the presence of glycosides, flavonoids, phenols, resins and tannins, however alkaloids were minor or absent.

**Table 1:** Antibacterial activity of ten bacterial strains against *Dioscorea deltoidea* plant tubers extract, Disc size, 5 mm, Inhibitory zone size  $\pm 1$  mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

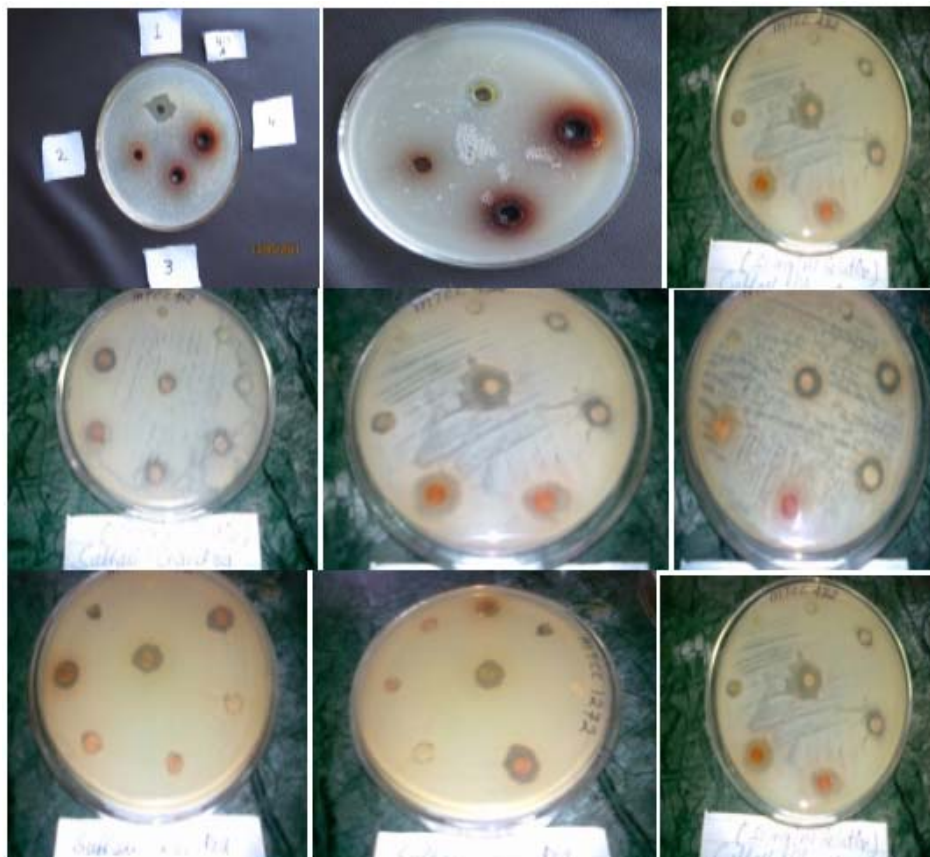
Bacterial Name		Erythro mycin	Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml
<i>Bacillus cereus</i>	1272	12	-	-	-	-	8	10	9	12	12	14	9	10
<i>Escherichia coli</i>	729	14	-	8	-	9	9	12	-	9	13	15	-	9
<i>Pseudomonas aeruginosa</i>	1688	13	-	-	-	-	8	10	8	10	14	17	-	8
<i>Klebsiella pneumonia</i>	432	11	-	-	-	8	-	10	-	11	8	11	7	10
<i>Salmonella enterica typhi</i>	98	10	-	-	-	-	-	9	-	9	7	10	-	12
<i>Shigella flexneri</i>	1457	10	-	8	-	-	8	10	-	10	8	11	-	8
<i>Staphylococcus aureus</i>	902	11	-	9	-	9	9	13	10	13	14	19	8	10
<i>Staphylococcus epidermidis</i>	435	10	-	-	-	-	8	12	-	12	13	14	-	13
<i>Streptococcus pyogenes</i>	1925	12	-	-	-	8	-	9	-	14	9	12	9	9
<i>Escherichia coli</i>	443	13	-	8	-	9	8	10	-	12	10	14	-	10

**Table 2:** Fungal activities of three fungal strains against *Dioscorea deltoidea* plant tubers extract, Disc size, 5 mm, Inhibitory zone size  $\pm 1$  mm, mm means (millimetres) and – indicate (NIZ) No inhibitory zone.

Fungal Name		Ketoconazole	Petroleum ether Extract		Chloroform Extract		Ethyl acetate Extract		Acetone Extract		Ethanol Extract		Water Extract	
Genus /Species /Subspecies	MTCC (Code)	10 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml	10 Mg /ml	50 Mg /ml
<i>Candida albicans</i>	3017	10	-	-	-	-	-	6	-	8	-	8	-	9
<i>Aspergillus flavus</i>	2798	8	-	7	-	-	-	-	-	-	-	9	-	7
<i>Aspergillus parasiticus</i>	2796	9	-	8	-	-	-	-	-	8	-	7	-	9

**Table 3:** Phytochemical screening of *Dioscorea deltoidea* different extract, (+) – Present, (-) – Absent.

Test	Pt. ether Extract	Benzene Extract	Chloroform Extract	Methanolic Extract	Ethanol Extract	Water Extract
<b>Carbohydrates/ glycosides</b>						
(1) Molisch test	(-)	(-)	(-)	(+)	(+)	(+)
(2) Fehling test	(-)	(-)	(-)	(+)	(+)	(+)
(3) Benedict test	(-)	(-)	(-)	(+)	(+)	(+)
<b>Alkaloid</b>						
(1) Mayer's test	(-)	(-)	(-)	(-)	(-)	(-)
(2) Dragendorff test	(-)	(-)	(-)	(-)	(-)	(-)
<b>Flavonoids</b>						
(1) Shinoda/pew	(-)	(-)	(-)	(-)	(-)	(-)
(2) Ammonia	(-)	(-)	(-)	(-)	(-)	(-)
<b>Saponins</b>	(-)	(-)	(-)	(-)	(-)	(-)
<b>Tannins</b>						
(1) Pyrogallol & catechol	(-)	(-)	(-)	(-)	(+)	(+)
(2) Gallic acid	(-)	(-)	(-)	(-)	(+)	(+)
<b>Unsaturated sterol/triterpenes</b>						
1. Liebermann Burchard test	(+)	(+)	(+)	(+)	(+)	(+)
2. Salkowski's test	(+)	(+)	(+)	(+)	(+)	(+)
<b>Resin</b>	(-)	(-)	(-)	(-)	(-)	(-)
<b>Phenolics compound</b>						
(1) Ferric chloride	(-)	(-)	(-)	(+)	(+)	(-)
(2) Nitric acid	(-)	(-)	(-)	(+)	(+)	(+)
<b>Protein and amino acid</b>						
(1) Xanthoprotein	(-)	(-)	(-)	(+)	(+)	(+)

**Fig 2:** Antibacterial and antifungal activity of ten bacterial strains & three fungal strains against *Dioscorea deltoidea* plant tubers extract.

#### 4. Conclusion

The present study results focused on antimicrobial activity and phytochemical screening of *Dioscorea deltoidea* this investigation revealed that antimicrobial and antifungal activity against selected bacterial and fungal strains. Which encourage developing a novel broad spectrum antimicrobial formulation in future. Now our research will be directed to develop a broad spectrum antimicrobial herbal formulation with this plant. Even at low concentrations, these plant species contained potent antimicrobial and antifungal activity nearly equal to that of the commercial fungicide used as a positive control.

#### 5. Acknowledgement

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