



## Validation of tribal claims on *Dioscorea pentaphylla* L. through phytochemical screening and evaluation of antibacterial activity

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### ABSTRACT

The present study elucidates the ethnobotanical uses of various parts of *Dioscorea pentaphylla* L. by the rural and tribal communities of Similipal Biosphere Reserve, Odisha and its adjoining areas. Further, it validates the tribal claims using phytochemical screening and antibacterial activity. Acetone, Methanol and aqueous extracts showed relevant zone of inhibition against two Gram positive bacteria *Streptococcus mutans* (MTCC\*497) and *Streptococcus pyogenes* (MTCC1926); three Gram-negative bacteria *Vibrio cholera* (MTCC3906), *Shigella flexneri* (MTCC1457) and *Salmonella entericatypfi* (MTCC1252).

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### 1. Introduction

Research evidences of the recent years have shown the urgent need for new antimicrobial agents to replenish, the arsenal of anti-infective agents or drugs on the light of antibiotic resistance reported in pathogenic microbes. It is reported that, on an average, two or three antibiotics are launched each year (Osborn, 1996). After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that, the effective life span of any antibiotics is limited (Eisenberg *et al.*, 1993; Alper, 1998; Wise, 2011). Therefore, it is necessary to know more about antimicrobial resistance (AMR), the mechanisms of action and screening of secondary metabolites from bio-wealth.

Plant materials are of wide use in traditional systems of medicine, and in several communities of the developing world (Fischbash and Walsh, 2009). They are the only resources available for the treatment of different microbial infections among many rural and tribal communities. Most of the common plants have been reported to have antimicrobial activity, still a number of unexplored wild plants are available in the forests having good ethnic values as traditional medicine (Dianella, 2012). There is need for the screening of bioactive compounds present in such plants

and the antimicrobial activities of their extracts (Ginsburg and Deharo, 2011). Among those unexplored plants wealth, genus *Dioscorea* is very common, found abundant in wild forests of Odisha (Kumar and Satpathy, 2011; Kumar *et al.*, 2012; Misra *et al.*, 2013). There are 11 wild species of this genus found in Odisha (Kumar *et al.*, 2012). Among them, *Dioscorea pentaphylla* L. (Plate-1: A, B and C) is easily available (Sinha and Lakra, 2005; Kumar *et al.*, 2012).

*D. pentaphylla* L. (Dioscoreaceae; local name- Panja Sanga) is a tuberous monocot vine bearing aerial bulbils (Fig. 1). It is left twining much more slender climber, more or less prickly below, 3-5 foliate leaves. Leaves are glabrous/pubescent beneath. Leaflets of lower leaves rarely larger, centre ones obovate or elliptic suddenly cuspidate or acuminate, cuneate towards base. Axillary racemes and sometimes terminaly paniced, or the axillary racemes sometime branched. Spike of female flower is long and solitary or 2-nate rarely paniced. Capsule large oblong and deflexed. Elongate obpyriform bulbils. Tubers are oblong or clavate, proceeding direct from the base of the aerial stem and thickening downwards (Saxena and Brahmam, 1995).

Tribal communities use its part as food and against microbial infections. The Hill-Kharia tribe, Mankirdia tribe, Santhal tribe, Ho tribe, Kolha tribe, Munda tribe and Bhumij tribe of Odisha use rhizome of *D. pentaphylla* against skin

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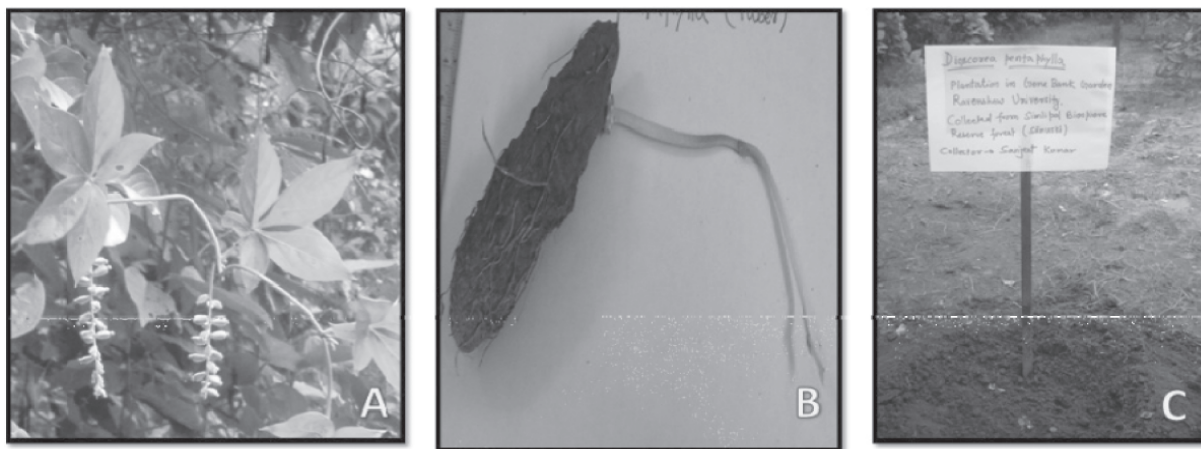


Plate-1: A- Leaves and fruits of *Dioscorea pentaphylla*; B- Tuber of *Dioscorea pentaphylla*; C- Plantation of *Dioscorea pentaphylla* at Dept. of Botany, Ravenshaw University, Cuttack

infections (Misra *et al.*, 2013). All these evidences make a sound platform for the study on antibacterial activity of plant extracts. In this study, an attempt has been made to validate the tribal claims (ethnobotanical values) to evaluate the bioactive compounds present in *Dioscorea pentaphylla* L.

## 2. Materials and Methods

### 2.1 Assessment of ethnobotanical data

The field work was conducted with the rural and tribal communities of adjoining areas (Jashipur, Karanjia, Bisoi, Kendumundi and Padampur) of Similipal Biosphere Reserve (SBR) during 2010-2013. The methodological framework was followed as per standard technique of ethno-biological approaches of Christian and Brigitte (2004). The information on plant used as traditional medicine against different pathogens and disorders were collected through questioners with different rural and tribal communities (Lohar, Mankirdia, Ho, Santhal, Kolha. Hill-Kharia and Munda). The pharmacological and medicinal properties of *Dioscorea pentaphylla* L. were confirmed by cross check with informants. Plant species was confirmed using standard flora (Saxena and Brahmam, 1995).

### 2.2 Collection of plant material

Tubers of *D. pentaphylla* L. were collected from the village Padampur of district Mayurbhanj. Collected tubers were planted in the experimental garden of Botany Department, Ravenshaw University, Cuttack. Plant parts were collected from mature plant in the garden. They were washed properly and were cut into small pieces and left for air drying. The dried materials were crushed to powder with mechanical device and were kept it in air tight container for phytochemical screening and antibacterial activity.

### 2.3 Extraction and detection of bioactive compounds

As per polarity index three solvents (acetone, methanol and aqueous) were selected for extraction. Extraction was done using soxhlet apparatus following the protocol of Tiwari *et al.* (2011). The powder of tuber was poured in the thimble at the ratio of 20 g per 250 ml of solvents at the temperature of 60° C (Methanol), 50 ° C (Acetone) and 100 ° C (Aqueous). Collected residue was then dried at room temperature. Qualitative detection of the bioactive compounds was done on n-Hexane, acetone, methanol, and aqueous extracts of different parts standard procedure (Sofowora, 1993).

### 2.4 Antibacterial activity

The extracts of *D. pentaphylla* were screened for antibacterial activity against two Gram positive bacteria *Streptococcus mutans* (MTCC \*497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella enterica typhi* (MTCC 1252) collected from Institute of Microbial Technology (IMTECH), Chandigarh. Nutrient broth was used to maintain broth cultures. Antimicrobial activity was done using Agar Well Diffusion assay (Allen *et al.*, 1991) with slight modification. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (dimethyl sulfoxide; Sigma) and twofold serial dilutions were made in amount of 100 µl per well ranged from 0.5, 1.0 and 2.0 mg / ml. Samples (100 µl) were added by sterile syringes into the wells and allowed to diffuse at room temperature for 2 h. Only the solvent (DMSO) was poured into the wells in another set of plates as part of negative control (Amanda *et al.*, 2012). The positive control set consisted of standard antibiotics Kanamycin with disc potency 10µg in place of samples. Plates were

incubated at  $35 \pm 2^\circ\text{C}$  for 18-24 h. after which the zones of inhibition were measured to determine the antibacterial activity.

### 2.5 Data analysis

Triplicates were maintained and the experiment was repeated thrice. Mean and SD (standard deviation) was performed to evaluate triplicate values of zone of inhibition (cm) of samples.

## 3. Results and discussion

Field survey revealed that *Dioscorea pentaphylla* L. is very popular as food and medicine among the tribal communities of SBR and its adjoining areas (Table 1). Tubers are bitter in taste but after traditional preparation such as continuous washing overnight and boiling, these are consumed as vegetables. Tuber paste is also used against skin infections and other diseases.

The qualitative analysis of phytochemical screening has shown the medicinal potential of the plant. Terpenoids,

Tannin, Saponin, Glycosides, Reducing sugar, Flavonoids and Phenolic compounds are major bioactive compounds present in the tuber, leaf and bulbils of *D. pentaphylla* (Table 2). Presence of these bioactive compounds is thought to be effective against different bacterial and fungal infections. The antibacterial activity of acetone, methanol and aqueous extracts of tuber showed significant zone of inhibition (cm) against *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457), *Salmonella enteric typhi* (MTCC 1252), *Streptococcus pyogenes* (MTCC 1926) and *Streptococcus mutans* (MTCC \*497). Aqueous extract of tuber showed excellent activity against MTCC 1926 (Table 3; Fig. 2). It has been observed that methanol and acetone extract were found to be active against Gram-negative bacteria *Vibrio cholera* (MTCC 3906). Therefore it is presumed that these compounds can be used for formulation of new drugs which may be effective against cholera and can also fight against antimicrobial resistance. The extracts also showed good growth inhibition against Gram-positive bacteria *Streptococcus mutans* (MTCC \*497).

Table 1

Ethnobotanical values of *Dioscorea pentaphylla* L. among the tribal communities (adjoining areas of SBR)

Plant Part(s)	Collection site(s)	Races	Medicinal Use(s)	Mode of use(s)	Other use(s)
Tubers	Padampur	Santhal	Skin Infections	Macerated tuber paste is applied externally on lesions	Edible
	Jashipur haat	Mankardia	Cold	Approx 250 gm tuber is boiled with about 1 lit of water and juice is prepared. One cup of juice with salt is taken thrice a day to remove cough.	Edible
	Bisoi	Ho	Constipation	One year old tubers are left overnight in running water and this tubers are used as chips to cure stomach pain and constipation problems.	Edible
	Karanja	Santhal	Poor appetite	After successive boiling, the tubers are eaten as vegetables and to reduce poor appetite twice a week.	Edible
	Haatibaadi	Santhal	Against cut	Approx 200 gm of fresh tuber is crushed with water and made into paste, which is used externally on cut and other similar wounds thrice a day till cure.	Edible
Leaves	Padampur	Munda	Against joint pain	Leaves paste made with Karanja oil ( <i>Pongamia pinnata</i> ) and is rubbed on joint	

Table 2  
Qualitative analysis of bioactive compounds in plant parts of *Dioscorea pentaphylla* L.

Plant part(s)	Solvent used	Bioactive compound(s) detected
Tubers	n-Hexane	Terpenoids
	Acetone	Tannin, Flavonoids, Glycosides and Reducing sugar
	Methanol	Tannin, Phenolic compounds and Steroids
	Water	Saponin, Reducing sugar and Glycosides
Leaves	n-Hexane	No bioactive compounds detected
	Acetone	Tannin, Saponin, Flavonoids, Terpenoids, Glycosides and Reducing sugar
	Methanol	Tannin, Saponin, Phenolic compounds and Glycosides
	Water	Tannin, Saponin, Flavonoids, Reducing sugar and Phenolic compounds
Bulbils	n-Hexane	Terpenoids
	Acetone	Tannin, Flavonoids, Glycosides and Reducing sugar
	Methanol	Tannin, Phenolic compounds and Steroids
	Water	Saponin, Reducing sugar and Glycosides

The compounds present in this extract might be playing active role against primary etiologic agents of coronal caries and root caries for oral diseases such as dental and periodontal caused by *Streptococcus mutans* (Hay *et al.*, 1994), *Streptococcus sobrinus* (Ellen *et al.*, 1985), *Streptococcus oralis* (Sansone *et al.*, 1993) etc. The extracts also showed relevant results against Gram-negative bacteria *Salmonella enterica typhi* (MTCC 1252).

*Salmonella enterica typhi* is considered as deadly bacteria causing typhoid fever and being responsible for the dead of more than 6 lakh people annually all over the world (Falkaw *et al.*, 2004). Strains were tested for their vulnerability using chloramphenicol, trimethoprim and amoxicillin and found all strains are resistance (Philippa *et al.*, 1998) to all these formulation. The tuber extracts being

effective against this fatal strain, can be used in new drug formulation against *Salmonella enterica typhi* (MTCC 1252).

The present study validates the tribal claims as reported for medicinal uses of the plant parts. The santhal tribe of Padampur reported that the tuber of *Dioscorea pentaphylla* L. used against different types of skin infections, which is correct as tannin presence in methanol extract and saponin presence in aqueous extract (Okwe and Okwe, 2004; Aderotimi and Samuel, 2006; Mohan and Kalidas, 2010) created zone of inhibition against *Streptococcus poyogenes* (Table 3 & 4; Fig 2). At concentration 500 µg/ml, aqueous extract showed higher zone of inhibition (1.20 cm) than acetone (0.92 cm) and methanol extract (1.00 cm) against *Streptococcus polygenes* (MTCC 1926).

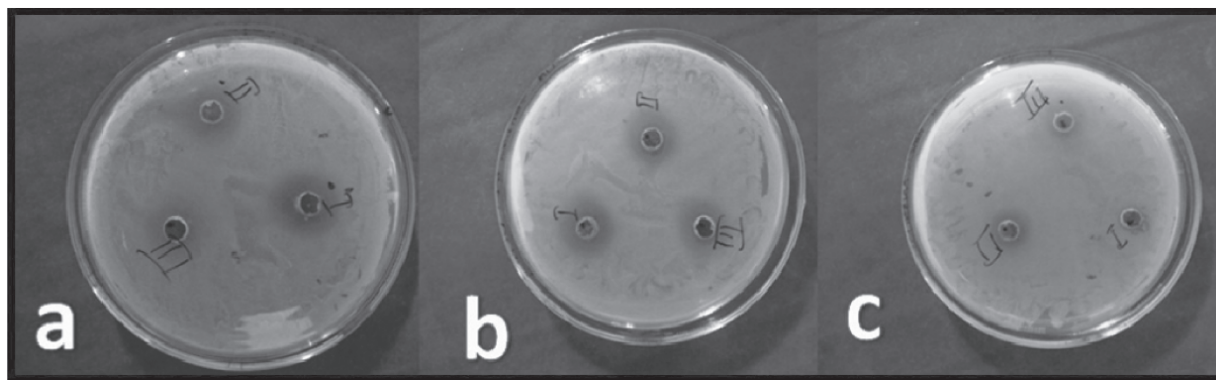


Fig. 2. Zone of inhibition of aqueous extract against MTCC 1926 (a), MTCC 1457 (b), and MTCC 3906 (c). I: 2000 µg/ml; II: 1000 µg/ml; I: 500 µg/ml.

Table 3

Antibacterial activity of *Dioscorea pentaphylla* tuber extracts with standard.

Strain(s)	Zone of inhibition (cm)			Extract / Standard
	500 µg/ml	1000 µg/ml	2000 µg/ml	
MTCC 3906	0.55 ± 0.07	1.05 ± 0.07	1.00 ± 0.14	Methanol Extract
MTCC 1252	0.85 ± 0.07	1.10 ± 0.00	1.45 ± 0.07	
MTCC 1457	1.20 ± 0.14	1.35 ± 0.21	1.50 ± 0.14	
MTCC 1926	1.00 ± 0.14	1.40 ± 0.21	1.50 ± 0.00	
MTCC *497	0.85 ± 0.21	1.20 ± 0.41	1.40 ± 0.14	
MTCC 3906	1.05 ± 0.07	1.27 ± 0.03	1.41 ± 0.01	Acetone Extract
MTCC 1252	0.95 ± 0.07	1.37 ± 0.03	1.72 ± 0.10	
MTCC 1457	0.87 ± 0.03	1.19 ± 0.01	1.44 ± 0.07	
MTCC 1926	0.92 ± 0.10	1.29 ± 0.01	1.77 ± 0.03	
MTCC *497	0.92 ± 0.10	1.2 ± 0.14	1.49 ± 0.01	
MTCC 3906	1.10 ± 0.14	1.10 ± 0.14	1.30 ± 0.14	Aqueous Extract
MTCC 1252	0.70 ± 0.14	0.85 ± 0.07	1.05 ± 0.07	
MTCC 1457	1.10 ± 0.14	1.15 ± 0.21	1.45 ± 0.07	
MTCC 1926	1.20 ± 0.14	1.50 ± 0.14	1.60 ± 0.14	
MTCC *497	0.85 ± 0.21	1.20 ± 0.00	1.47 ± 0.03	
MTCC 3906		1.63 ± 1.53		Kanamycin
MTCC 1252		1.56 ± 0.58		
MTCC 1457		1.73 ± 0.58		
MTCC 1926		1.76 ± 0.58		
MTCC *497		1.73 ± 0.58		

Note: MTCC 3906- *Vibrio cholera*; MTCC 1252- *Salmonella enterica typhi*; MTCC 1457- *Shigella flexneri*; MTCC 1926- *Streptococcus pyogenes*; MTCC \*497- *Streptococcus mutans*.

Table 4

Correlation of Tribal Claims with bioactive compounds & antibacterial activity of *Dioscorea pentaphylla* L. tuber extracts for validation.

Plant Parts	Tribal claims	Correlation with bioactive compounds	Correlation with antibacterial activity	Reference
Tubers	Skin infections	Tannin presence in methanol extract and saponin presence of aqueous extract of <i>Dioscorea pentaphylla</i> L. tuber may be responsible for curing skin infections.	Antibacterial activity of aqueous extract against <i>Streptococcus polygenes</i>	Okwe and Okwe, 2004; Mohan and Kalidas, 2010; Aderotimi and Samuel, 2006
	Cold	Presence of Terpenoids in n-Hexane extract might be responsible		Musa <i>et al.</i> , 2009
	Cut and wounds	Presence of flavonoids in acetone extract might be responsible		Musa <i>et al.</i> , 2009
Leaves	Joint pain	Presence of flavonoids in acetone and methanol extract might be responsible.		Majumadar <i>et al.</i> , 2008
Bulbils	Not bitter in taste	No indication of Saponin in aqueous may be indicated less bitterness properties.		Magdolena, 2009

#### 4. Conclusion

*Dioscorea pentaphylla* L. a wild tuber crop abundant in Similipal Biosphere Reserve forest and its adjoining areas possesses wide ethnobotanical values among the rural and tribal communities of the study areas. Presence of bioactive compounds has shown the potential antibacterial and pharmacological action of this wild tuber. Present study might be useful to supplement scientific information to establish tribal claims for the presence of phytochemicals and medicinal values. Further research is needed to document the detail bioactive compounds present in such wild plants predominant in Odisha and their probable use against known pathogens and antibiotic resistance as well.

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