

In-vitro Screening of Antibacterial Activities of Selected Wild Tuberos Plants of Odisha, India

Jugajyoti Swain¹, Sanjeet Kumar², Sagarika Parida^{3*}, Padan Kumar Jena¹

¹Department of Botany, Ravenshaw University, Cuttack, INDIA.

²Biodiversity and Conservation Lab., Ambika Prasad Research Foundation, Odisha, INDIA.

³School of Applied Sciences, Centurion University of Technology and Management, Odisha, INDIA.

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ABSTRACT

Tuberous plants are the resources of many useful bioactive compounds. It suggests that they may probably protect the humans against microbial pathogens. Keeping this in view, an attempt has been made to analyze the microbial potentials. Eight species of tuberous plants (*Dioscorea belophylla*, *D. esculenta*, *D. glabra*, *D. oppositifolia*, *D. tomentosa*, *D. wallichii*, *D. hamiltonii* and *D. hispida* var. *daemonia*) were collected from peripheral areas of Similipal Biosphere Reserve, Mayurbhanj, Khordha, Cuttack and Puri districts of Odisha. Methanol, acetone and aqueous extracts were extracted and bioactive compounds were detected. In all the extracts of the above selected plants, phenolic compounds, tannin and saponin were detected as the chief bioactive compounds. Antibacterial activities were carried out using agar well diffusion and disc diffusion assay against selected bacterial pathogens. *Streptococcus mutans* (MTCC 497), *Streptococcus pyogenes* (MTCC 1926), *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252). The minimum inhibitory concentration (MIC) values were determined using broth dilution assay. Results revealed that all the extracts showed significant antibacterial activity using the said methods. Methanol extract of all experimental plants showed highest activity against MTCC 1926 and MTCC 497. Present investigation highlights the antibacterial potential of tuberous plants available in Odisha state.

Key words: Agar well diffusion, Antibacterial activities, Bioactive compounds, Disc diffusion assay, Tuberous plants.

Correspondence:

Dr. Sagarika Parida,

Department of Botany,
School of Applied
Sciences, Centurion
University of
Technology and
Management, Odisha,
INDIA.

Phone no: 7008298535

Email: sagarika.parida@cutm.ac.in

INTRODUCTION

Tuberous plants are the oldest known plants having food and medicinal values. These plants are widely distributed altitudinally from sea. In India they are distributed throughout the country. They are used in pharmaceutical products, horticulture, household purposes and are also ecologically important as good indicators of environmental conditions. They are traditionally used in China, Europe, North American and India to cure illness of cardiovascular system, tonsillitis, bronchitis, tympanitis, in skin diseases and burns.^[1-3]

They also possess anticancer and antimicrobial activity due to their unique chemical constituents. Antimicrobial resistance (AMR) is now a global burning problem and as a result these microorganisms are no longer responding to the drugs designed to kill them. Evolvement of new strains of micro-organisms is the main reason for AMR. The above cited medicinal and pharmacological values indicated that the tuberous plants provide a base line because of presence of new antimicrobial compounds against the new strains of micro-organisms to fight.^[4] AMR creating a permanent loss of antibiotics which indicates the need of reverse pharmacological research. Keeping all this in view, an attempt has been made to detect and the secondary metabolites present in selected tuberous plants available in Odisha and their antibacterial activity. Present paper highlights the medicinal and pharmacological importance of the tuberous plants.

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MATERIALS AND METHODS

Collection of Wild Tuberos Plants for Experimental Work

The samples (tubers) and their medicinal values were collected from different parts of Odisha (Figure 1a; Figure 1c) and kept in poly bags tagged with the botanical name and sorted out as per standard sampling procedure and passport description.^[5]

Preparation of Extracts

Soxhlet method and percolation were adopted to obtain different extracts.^[4,6] The collected experimental plant materials (Figure 1d) were dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored in refrigerator for further phytochemical analysis and antibacterial activities.

Phytochemical Analysis

Phyto-chemical analyses were carried out using standard procedure to identify the bioactive compounds.^[7-9]

Antibacterial Activity

The extracts of experimental plant parts were screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252). All used MTCC (Microbial Type Culture Collection) bacterial strains (Figure 1e) were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using slight modification of standard methods of agar well diffusion assay^[10] disc diffusion method^[11] and broth dilution assay.^[12]

Agar Well Diffusion Assay

Agar well diffusion method^[10] was followed to test the antibacterial activity of extracts of experimental plant parts against the five bacterial strains. Nutrient agar plates were prepared as per manufacturer's instructions. 100 µl of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100 % DMSO (Sigma) and twofold serial dilutions were made in amount of 100 µl per well at concentration of 0.25 and 0.5 mg/ml.

100 µl of samples were added by sterile syringes into the wells in three above mentioned concentration and allowed to diffuse at room temperature for 2 h. Plates were incubated at $35 \pm 2^\circ\text{C}$ for 18-24 h. Kanamycin served as standard antibiotics control. Triplicates were maintained and the experiment was repeated thrice. For each replicates the readings (diameter of zone of inhibition in cm) were taken and the mean \pm SD values (diameter of zone of inhibition) were recorded

Disc Diffusion Assay

Antibacterial activity using disc diffusion assay was done using the 6 mm of disc prepared from whattman filter paper.^[11] Each extracts were dissolved in dimethyl sulfoxide. The sets of dilutions (10 µg/ disc and 50 µg/ disc) of crude extracts and standard drugs were prepared. 6 mm of discs were kept in the drugs for 12 h before placing to the agar plates. The zones of growth inhibition around the disks were measured after 18 to 24 h of in incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks and values less than 8 mm were considered as not active against microorganisms.

MIC Using Broth Dilution Assay

All the extracts of experimental plant parts were screened for their antibacterial activity.^[12,13] Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of trypticase soya broth. The broth was incubated for 8 ± 1 h at $35 \pm 2^\circ\text{C}$ until there was visible growth. Mc Farland No.5 standard and PBS (Phosphate Buffer Saline) were used to adjust the turbidity to get 105 cfu/mL.

Data Interpretation

After the incubation, the tubes showing no visible growth after 8 h till 12 h were considered to be inhibition of bacteria which represent MIC (minimum inhibitory concentration) values of a respective concentration. Inoculums control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each replicates. The readings were taken as foresaid.



Figure 1: Field and lab work on experimental plants, a) Collection of medicinal values, b) *Dioscorea oppositifolia*, c) Collection of tubers, d) Processing of tubers of *D. esculenta*, e) Microbial strains.

RESULTS

Methanol, acetone and aqueous extracts of the said plants were analysed and noticed that the saponin, flavonoids and phytosterols were present in all the three extracts (Table 1). Saponin are amphipathic glycosides and traditionally it has been used as detergents as it act as foaming and surface active agents.^[14]

Based on the above reported activities of present secondary metabolites and researchers of recent years have emphasised that there is urgent need to screen new anti-bacterial agents in the light of antibiotics resistance offered by pathogenic microbes. The antibacterial activities were carried out and it was observed that the antibacterial activity of studied plant showed significant zone of inhibition using AWD (agar well diffusion) and DD (disc diffusion) assays (Table 2; Table 3). It was also examined that MIC (Minimum Inhibitory Concentration) of the experimental showed significant values as compared to standard Kanamycin (Table 4). It was observed that acetone extract of DB (*Dioscorea belophylla*) showed highest zone of inhibition ($21.6 \pm 0.05\text{mm}$) against MTCC 497 at concentration of 0.25 mg/ml, while highest zone of inhibition ($22.6 \pm 0.02\text{ mm}$) was recorded in methanol extract at concentration of 0.5 mg/ml against MTCC 3906. It was observed that acetone extract of DE (*Dioscorea esculenta*) showed highest zone of inhibition ($20.3 \pm 0.02\text{ mm}$) against MTCC 497 at concentration of 0.5 mg/ml, while maximum zone of inhibition ($11.5 \pm 0.05\text{ mm}$) was recorded in methanol extract followed by aqueous extract (10.0 ± 0.00) at same concentration against MTCC 1457 (Table 2).

Table 1: Bioactive compounds present in the selected plant extracts.

Plant name	Methanol extract							
	Saponin	Flavonoids	Phenolic compounds	Tannin	Phytosterols	Alkaloids	Phlobotannins	Glycosides
DB	++	++	+	+	+	+	++	+
DE	-	++	+	+	+	+	++	+
DG	+++	++	+	+	+	+	+	+
DO	++	+++	+	+	+	+	+	++
DT	++	+++	+	+	+	+	+	++
DW	+++	+++	+	+	+	+	++	+++
DH	+++	+++	+	+	+	+	+++	+++
BHD	+++	+++	+	+	+	+	+	+++
Acetone extract								
DB	+++	++	+	+	+	-	+	+
DE	+++	++	+	+	+	-	+	+
DG	+++	++	+	+	+	-	+	+
DO	+++	++	+	+	+	-	+	+
DT	+++	++	+	+	+	-	-	+
DW	+++	++	+	+	+	-	-	+
DH	+++	++	+	+	+	-	-	+
DHD	+++	++	+	+	+	-	+	+
Aqueous extract								
DB	+++	++	+	+	+	-	+	+
DE	+++	++	+	+	+	-	+	+
DG	+++	++	+	+	+	-	+	+
DO	+++	++	+	+	+	-	+	+
DT	+++	++	+	+	+	-	-	+
DW	+++	++	+	+	+	-	-	+
DH	+++	++	+	+	+	-	-	+
DHD	+++	++	+	+	+	-	+	+

(-: Not Detected; +: Present; ++: Moderate; +++: Rich; DB: *Dioscorea belophylla*; DE: *D. Esculenta*; DG: *D. glabra*, DO: *D. Oppositifolia*; DT: *D. Tomentosa*; DW: *D. Wallichii*; DH: *D. hamiltonii* and DHD: *D. hispida* var. *daemona*)

DG (*Dioscorea glabra*) showed highest zone of inhibition (15.6 ± 0.02) against MTCC 1926 at concentration 0.5 mg/ml in methanol extract as compared to acetone and aqueous extract at same concentration. Methanol extract of DO (*Dioscorea oppositifolia*, Figure 1b) showed highest zone of inhibition (12.6 ± 0.02) against MTCC 1457 and MTCC 1926 as compared to acetone extract with less inhibition zone (10.6 ± 0.02) against MTCC 3906 and MTCC 497 at concentration 0.5 mg/ml, as compared aqueous extract at same concentration (Table 3). Methanol extract of DT (*Dioscorea tomentosa*) showed highest zone of inhibition (15.1 ± 0.0) against

Table 2: Antibacterial activity of experimental plant parts using agar well diffusion assay.

Zone of Inhibition of DB (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	7.4 ± 0.05	8.3 ± 0.02	8.5 ± 0.02	0.25 mg/ml
MTCC 1457	8.1 ± 0.05	8.3 ± 0.02	8.4 ± 0.02	
MTCC 3906	8.1 ± 0.02	8.1 ± 0.02	8.6 ± 0.02	
MTCC 1926	21.2 ± 0.02	21.2 ± 0.12	8.8 ± 0.02	
MTCC 497	21.6 ± 0.05	20.1 ± 0.02	9.1 ± 0.02	
MTCC 1252	21.1 ± 0.02	22.4 ± 0.02	9.1 ± 0.02	0.5 mg/ ml
MTCC 1457	21.3 ± 0.05	21.3 ± 0.02	9.5 ± 0.02	
MTCC 3906	21.5 ± 0.02	22.6 ± 0.02	9.5 ± 0.02	
MTCC 1926	21.2 ± 0.02	21.2 ± 0.02	9.8 ± 0.05	
MTCC 497	21.4 ± 0.02	22.4 ± 0.02	20.2 ± 0.02	
Zone of Inhibition of DE (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.1 ± 0.02	9.3 ± 0.02	8.0 ± 0.00	0.25 mg/ml
MTCC 1457	8.2 ± 0.02	9.0 ± 0.00	8.0 ± 0.00	
MTCC 3906	8.1 ± 0.02	9.0 ± 0.00	8.0 ± 0.00	
MTCC 1926	8.0 ± 0.02	9.0 ± 0.00	8.0 ± 0.00	
MTCC 497	8.0 ± 0.02	9.4 ± 0.02	8.0 ± 0.00	
MTCC 1252	8.4 ± 0.02	9.4 ± 0.02	9.0 ± 0.00	0.5 mg/ ml
MTCC 1457	8.3 ± 0.02	11.5 ± 0.05	10.0 ± 0.00	
MTCC 3906	8.6 ± 0.02	8.0 ± 0.00	8.0 ± 0.00	
MTCC 1926	20.1 ± 0.07	9.8 ± 0.02	9.0 ± 0.00	
MTCC 497	20.3 ± 0.02	9.6 ± 0.02	9.0 ± 0.00	
Zone of Inhibition of DG (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.5 ± 0.05	9.1 ± 0.02	7.8 ± 0.02	0.25 mg/ml
MTCC 1457	8.2 ± 0.05	9.1 ± 0.02	7.3 ± 0.02	
MTCC 3906	8.3 ± 0.02	9.3 ± 0.02	7.8 ± 0.02	
MTCC 1926	8.6 ± 0.02	8.6 ± 0.12	7.8 ± 0.02	
MTCC 497	8.0 ± 0.05	9.3 ± 0.02	7.3 ± 0.02	
MTCC 1252	8.3 ± 0.02	10.6 ± 0.02	7.3 ± 0.02	0.5 mg/ ml
MTCC 1457	8.0 ± 0.05	10.6 ± 0.02	7.3 ± 0.02	
MTCC 3906	8.6 ± 0.02	15.1 ± 0.02	7.3 ± 0.02	
MTCC 1926	8.0 ± 0.02	15.6 ± 0.02	7.5 ± 0.05	
MTCC 497	8.6 ± 0.02	15.1 ± 0.02	7.3 ± 0.02	
Zone of Inhibition of DO (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.5 ± 0.05	9.1 ± 0.02	7.0 ± 0.00	0.25 mg/ml
MTCC 1457	9.2 ± 0.05	9.1 ± 0.02	8.0 ± 0.00	
MTCC 3906	9.3 ± 0.02	9.3 ± 0.02	8.0 ± 0.00	
MTCC 1926	10.6 ± 0.02	10.6 ± 0.12	8.0 ± 0.00	
MTCC 497	10.0 ± 0.05	9.3 ± 0.02	8.0 ± 0.00	

MTCC 1252	10.3 ± 0.02	11.6 ± 0.02	9.3 ± 0.02	0.5 mg/ ml
MTCC 1457	10.0 ± 0.05	12.6 ± 0.02	9.3 ± 0.02	
MTCC 3906	10.6 ± 0.02	12.1 ± 0.02	9.3 ± 0.02	
MTCC 1926	10.0 ± 0.02	12.6 ± 0.02	9.5 ± 0.05	
MTCC 497	10.6 ± 0.02	12.1 ± 0.02	9.3 ± 0.02	
Zone of Inhibition of DT (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	10.0 ± 0.02	11.8 ± 0.07	8.6 ± 0.02	0.25 mg/ml
MTCC 1457	10.1 ± 0.02	11.5 ± 0.05	8.6 ± 0.02	
MTCC 3906	10.6 ± 0.02	11.8 ± 0.02	8.8 ± 0.02	
MTCC 1926	10.3 ± 0.02	12.1 ± 0.02	8.6 ± 0.02	
MTCC 497	11.6 ± 0.02	12.3 ± 0.02	8.6 ± 0.02	
MTCC 1252	10.0 ± 0.05	12.1 ± 0.02	9.8 ± 0.02	0.5 mg/ ml
MTCC 1457	10.1 ± 0.02	12.3 ± 0.02	10.5 ± 0.05	
MTCC 3906	11.6 ± 0.05	12.8 ± 0.02	9.8 ± 0.02	
MTCC 1926	11.8 ± 0.02	13.1 ± 0.02	10.0 ± 0.00	
MTCC 497	12.8 ± 0.07	10.6 ± 0.11	10.0 ± 0.00	
Zone of Inhibition of DW (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.6 ± 0.05	9.3 ± 0.02	7.3 ± 0.02	0.25 mg/ml
MTCC 1457	9.3 ± 0.02	9.6 ± 0.02	7.3 ± 0.02	
MTCC 3906	9.6 ± 0.02	9.8 ± 0.02	8.1 ± 0.02	
MTCC 1926	9.8 ± 0.02	10.1 ± 0.02	8.1 ± 0.02	
MTCC 497	10.3 ± 0.02	9.8 ± 0.02	8.5 ± 0.05	
MTCC 1252	9.8 ± 0.02	9.8 ± 0.02	8.5 ± 0.05	0.5 mg/ ml
MTCC 1457	10.1 ± 0.07	11.0 ± 0.05	9.3 ± 0.02	
MTCC 3906	10.6 ± 0.02	11.6 ± 0.02	9.6 ± 0.02	
MTCC 1926	10.6 ± 0.02	12.1 ± 0.02	10.1 ± 0.05	
MTCC 497	10.3 ± 0.02	10.6 ± 0.02	9.6 ± 0.02	
Zone of Inhibition of DH (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	9.3 ± 0.02	10.3 ± 0.02	7.5 ± 0.05	0.25 mg/ml
MTCC 1457	9.3 ± 0.02	11.3 ± 0.02	7.8 ± 0.02	
MTCC 3906	9.8 ± 0.02	10.6 ± 0.02	7.5 ± 0.05	
MTCC 1926	11.3 ± 0.02	11.6 ± 0.02	8.3 ± 0.02	
MTCC 497	10.6 ± 0.02	11.6 ± 0.02	8.1 ± 0.02	
MTCC 1252	10.3 ± 0.02	11.3 ± 0.02	8.3 ± 0.02	0.5 mg/ ml
MTCC 1457	10.3 ± 0.02	11.8 ± 0.02	8.8 ± 0.02	
MTCC 3906	10.6 ± 0.02	12.1 ± 0.02	9.1 ± 0.02	
MTCC 1926	12.3 ± 0.02	12.8 ± 0.02	9.6 ± 0.02	
MTCC 497	11.3 ± 0.02	12.3 ± 0.02	9.8 ± 0.02	

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Zone of Inhibition of DHD (mean \pm SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	7.8 \pm 0.02	7.6 \pm 0.02	7.1 \pm 0.02	0.25 mg/ml
MTCC 1457	7.5 \pm 0.02	8.1 \pm 0.02	7.0 \pm 0.02	
MTCC 3906	7.6 \pm 0.02	8.1 \pm 0.02	7.3 \pm 0.02	
MTCC 1926	8.1 \pm 0.02	8.5 \pm 0.05	7.8 \pm 0.05	
MTCC 497	8.2 \pm 0.02	9.3 \pm 0.02	8.6 \pm 0.02	
MTCC 1252	8.6 \pm 0.02	8.8 \pm 0.02	7.6 \pm 0.02	0.5 mg/ml
MTCC 1457	9.3 \pm 0.02	9.6 \pm 0.02	8.5 \pm 0.05	
MTCC 3906	9.6 \pm 0.02	9.1 \pm 0.02	8.5 \pm 0.05	
MTCC 1926	9.6 \pm 0.02	11.0 \pm 0.02	9.0 \pm 0.02	
MTCC 497	9.8 \pm 0.02	10.0 \pm 0.02	9.3 \pm 0.02	

(MTCC 3906: *Vibrio cholerae*; MTCC 1252: *Salmonella typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans*; SD: Standard Deviation, DB: *Dioscorea belophylla*, DE: *D. esculenta*, DG: *D. glabra*, DO: *D. oppositifolia*, DT: *D. tomentosa*, DW: *D. wallichii*, DH: *D. hamiltonii* and DHD: *D. hispida* var. *daemonia*).

MTCC 1926 followed by MTCC 3906 (12.8 \pm 0.02) while MTCC 497 was shown comparatively less zone of inhibition (12.8 \pm 0.07) at concentration 0.5 mg/ml and aqueous extract was shown less mm of zone of inhibition at same concentration (Table 2).

Methanol extract of DW (*Dioscorea wallichii*) showed highest zone of inhibition (12.1 \pm 0.02) against MTCC 1926 followed by MTCC 3906 (11.6 \pm 0.02) and MTCC 1457 (11.6 \pm 0.05) while both the strains *i. e.*, MTCC 3906 and MTCC 1926 had shown less zone of inhibition (10.6 \pm 0.02) in acetone extract at concentration 0.5 mg/ml. DH (*Dioscorea hamiltonii*) showed highest zone of inhibition (12.8 \pm 0.02) and 12.3 \pm 0.02 mm against MTCC 1926 in methanol and acetone extract at concentration 0.5 mg/ml respectively. DHD (*Dioscorea hispida* var. *daemonia*) showed maximum zone of inhibition against MTCC 1926 (11.0 \pm 0.02) and MTCC 497 (10.0 \pm 0.02) in methanol extract at concentration 0.5 mg/ml. This was observed that DHD had shown less zone of inhibition in comparison to other seven *Dioscorea* species in agar well diffusion method (Table 2). The antibacterial activity of studied plants were analysed using AWD assay and revealed that all species showed significant zone of inhibition against tested microbial strains. It was observed that acetone and methanol extract showed highest inhibitory activity against MTCC 3906 and MTCC 497. The methanol extract of DT showed highest activity against MTCC 1926 (Table 2).

The antibacterial activity of experimental plants using DD assay also showed the relevant zone of inhibition against used microbial strains. The activity also showed highest against MTCC 3906 and MTCC 497. MTCC

Table 3: Antibacterial activity of experimental plant parts using disc diffusion assay.				
Zone of Inhibition of DB (mean \pm SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.00 \pm 0.57	9.00 \pm 0.00	ZI \leq 7.00	10 μ g/disc
MTCC 1457	ZI \leq 7.00	9.30 \pm 0.50	ZI \leq 7.00	
MTCC 3906	8.00 \pm 0.00	10.00 \pm 0.00	ZI \leq 7.00	
MTCC 1926	9.30 \pm 0.57	10.60 \pm 0.50	ZI \leq 7.00	
MTCC 497	ZI \leq 7.00	8.30 \pm 0.57	ZI \leq 7.00	
MTCC 1252	14.00 \pm 1.00	14.60 \pm 0.57	10.60 \pm 1.50	50 μ g/disc
MTCC 1457	14.30 \pm 0.57	14.00 \pm 1.70	10.30 \pm 0.50	
MTCC 3906	14.30 \pm 0.57	14.00 \pm 0.00	10.60 \pm 0.50	
MTCC 1926	14.00 \pm 0.00	14.00 \pm 0.00	10.00 \pm 0.00	
MTCC 497	14.60 \pm 0.57	14.00 \pm 1.00	10.00 \pm 0.00	
Zone of Inhibition of DE (mean \pm SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.00 \pm 0.00	9.00 \pm 0.00	8.00 \pm 0.00	10 μ g/disc
MTCC 1457	9.30 \pm 0.57	9.00 \pm 0.00	7.30 \pm 0.57	
MTCC 3906	9.60 \pm 0.57	9.00 \pm 0.00	7.00 \pm 0.00	
MTCC 1926	9.00 \pm 0.00	9.30 \pm 0.57	7.60 \pm 0.57	
MTCC 497	9.00 \pm 0.00	9.00 \pm 0.00	9.30 \pm 0.57	
MTCC 1252	12.00 \pm 0.00	13.00 \pm 0.00	9.00 \pm 0.00	50 μ g/disc
MTCC 1457	12.00 \pm 1.00	14.60 \pm 0.57	10.30 \pm 0.57	
MTCC 3906	12.30 \pm 0.57	14.00 \pm 1.73	10.60 \pm 0.57	
MTCC 1926	12.30 \pm 0.57	14.00 \pm 0.00	10.00 \pm 0.00	
MTCC 497	12.00 \pm 0.00	14.00 \pm 0.00	10.00 \pm 0.00	
Zone of Inhibition of DG (mean \pm SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.00 \pm 0.57	8.00 \pm 0.00	ZI \leq 7.00	10 μ g/disc
MTCC 1457	ZI \leq 7.00	8.30 \pm 0.57	ZI \leq 7.00	
MTCC 3906	8.00 \pm 0.00	9.00 \pm 0.00	ZI \leq 7.00	
MTCC 1926	9.30 \pm 0.57	10.60 \pm 0.57	ZI \leq 7.00	
MTCC 497	ZI \leq 7.00	8.30 \pm 0.57	ZI \leq 7.00	
MTCC 1252	11.30 \pm 0.57	10.00 \pm 0.00	8.30 \pm 0.57	50 μ g/disc
MTCC 1457	10.30 \pm 0.57	12.60 \pm 0.57	9.00 \pm 0.00	
MTCC 3906	11.30 \pm 0.57	10.00 \pm 0.00	8.30 \pm 0.57	
MTCC 1926	11.30 \pm 0.57	10.00 \pm 0.00	8.30 \pm 0.00	
MTCC 497	10.30 \pm 0.57	11.00 \pm 0.00	8.30 \pm 0.00	

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Zone of Inhibition of DO (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	9.00 ± 0.00	10.00 ± 0.00	ZI ≤ 7.00	10 µg/disc
MTCC 1457	10.00 ± 0.00	10.00 ± 0.00	ZI ≤ 7.00	
MTCC 3906	10.50 ± 0.50	9.00 ± 0.00	ZI ≤ 7.00	
MTCC 1926	11.00 ± 0.00	11.00 ± 0.00	ZI ≤ 7.00	
MTCC 497	10.00 ± 0.00	10.00 ± 0.00	ZI ≤ 7.00	
MTCC 1252	11.00 ± 0.00	13.00 ± 0.00	8.50 ± 0.50	50 µg/disc
MTCC 1457	11.50 ± 0.00	12.00 ± 0.00	8.00 ± 0.00	
MTCC 3906	11.00 ± 0.00	13.00 ± 0.00	8.30 ± 0.57	
MTCC 1926	12.50 ± 0.50	13.00 ± 0.00	8.30 ± 0.57	
MTCC 497	13.00 ± 0.00	13.50 ± 0.00	9.00 ± 0.00	
Zone of Inhibition of DT (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	11.00 ± 0.00	11.50 ± 0.50	9.00 ± 0.00	10 µg/disc
MTCC 1457	13.00 ± 0.00	11.00 ± 0.00	9.16 ± 0.28	
MTCC 3906	10.00 ± 0.00	12.00 ± 0.00	9.83 ± 0.28	
MTCC 1926	12.00 ± 0.00	12.00 ± 0.00	9.00 ± 0.50	
MTCC 497	12.00 ± 0.00	12.00 ± 0.00	9.16 ± 0.57	
MTCC 1252	14.00 ± 0.00	14.00 ± 0.00	10.33 ± 0.28	50 µg/disc
MTCC 1457	15.00 ± 0.50	14.00 ± 0.00	10.16 ± 0.00	
MTCC 3906	13.00 ± 0.50	14.00 ± 0.00	10.00 ± 0.00	
MTCC 1926	14.00 ± 0.00	14.50 ± 0.00	10.00 ± 0.00	
MTCC 497	14.00 ± 0.00	14.00 ± 0.00	10.00 ± 0.00	
Zone of Inhibition of DW (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	10.66 ± 0.28	10.50 ± 0.50	9.00 ± 0.00	10 µg/disc
MTCC 1457	9.83 ± 0.57	10.00 ± 0.00	8.00 ± 0.50	
MTCC 3906	10.16 ± 0.57	10.33 ± 0.28	8.30 ± 0.50	
MTCC 1926	9.83 ± 0.57	10.16 ± 0.28	8.83 ± 0.00	
MTCC 497	11.33 ± 0.28	10.00 ± 0.00	8.30 ± 0.00	
MTCC 1252	11.33 ± 0.28	12.00 ± 0.50	9.00 ± 0.00	50 µg/disc
MTCC 1457	11.16 ± 0.50	12.00 ± 0.00	9.33 ± 0.28	
MTCC 3906	11.16 ± 0.57	12.83 ± 0.57	9.00 ± 0.00	
MTCC 1926	11.00 ± 0.00	12.66 ± 0.28	9.66 ± 0.28	
MTCC 497	11.16 ± 0.28	12.16 ± 0.28	9.00 ± 0.00	

Continued...

Zone of Inhibition of DH (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	8.30 ± 0.28	9.16 ± 0.28	9.30 ± 0.28	10 µg/disc
MTCC 1457	8.50 ± 0.00	9.66 ± 0.28	9.50 ± 0.00	
MTCC 3906	8.83 ± 0.28	10.30 ± 0.28	9.83 ± 0.28	
MTCC 1926	9.33 ± 0.28	10.16 ± 0.57	9.30 ± 0.28	
MTCC 497	9.50 ± 0.00	10.16 ± 0.28	9.50 ± 0.00	
MTCC 1252	10.00 ± 0.50	11.30 ± 0.28	8.50 ± 0.00	50 µg/disc
MTCC 1457	10.50 ± 0.00	11.00 ± 0.86	8.00 ± 0.00	
MTCC 3906	10.16 ± 0.57	11.33 ± 0.28	8.16 ± 0.28	
MTCC 1926	10.16 ± 0.57	12.66 ± 0.28	8.50 ± 0.50	
MTCC 497	10.30 ± 0.28	10.16 ± 0.28	8.50 ± 0.50	
Zone of Inhibition of DHD (mean ± SD, mm)				
Strain	Acetone extract	Methanol extract	Aqueous extract	Concentration
MTCC 1252	9.00 ± 0.00	9.00 ± 0.00	7.33 ± 0.28	10 µg/disc
MTCC 1457	9.00 ± 0.00	9.10 ± 0.28	7.83 ± 0.57	
MTCC 3906	9.00 ± 0.00	9.16 ± 0.57	7.50 ± 0.00	
MTCC 1926	9.50 ± 0.50	9.66 ± 0.28	9.33 ± 0.57	
MTCC 497	10.00 ± 0.00	11.66 ± 0.28	9.83 ± 0.57	
MTCC 1252	9.50 ± 0.00	9.00 ± 0.00	8.30 ± 0.28	50 µg/disc
MTCC 1457	9.30 ± 0.28	9.16 ± 0.28	8.83 ± 0.28	
MTCC 3906	9.00 ± 0.50	9.50 ± 0.50	8.16 ± 0.28	
MTCC 1926	12.50 ± 0.00	12.00 ± 0.86	9.66 ± 0.28	
MTCC 497	12.50 ± 0.50	12.33 ± 0.28	9.16 ± 0.28	

(MTCC 3906: *Vibrio cholerae*; MTCC 1252: *Salmonella typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans*; SD: Standard Deviation, DB: *Dioscorea belophylla*, DE: *D. esculenta*, DG: *D. glabra*, DO: *D. oppositifolia*, DT: *D. tomentosa*, DW: *D. wallichii*, DH: *D. hamiltonii* and DHD: *D. hispida* var. *daemona*)

3906 causing skin infections, therefore the studied natural products might be used to formulate new antibacterial drugs to fight against the various types of skin infections. In disc diffusion method, DB showed same zone of inhibition (14.60 ± 0.57) against MTCC 1457 and MTCC 497 in both acetone and methanol extract at a concentration of 50 µg/disc. It was observed that DE showed maximum zone of inhibition (14.06 ± 0.57 mm) against MTCC 497 and MTCC 1252 at concentration of 50 µg/disc in acetone and methanol extract. DG showed highest zone of inhibition (12.60 ± 0.57) against MTCC 1457 at same concentration in methanol

extract as compared to acetone and aqueous extract at same concentration (Table 3).

Methanol extract of DO showed maximum zone of inhibition (13.50 ± 0.00) against MTCC 497 and (13.00 ± 0.00) against both strains of MTCC 3906 and MTCC while acetone extract showed inhibition zone (13.00 ± 0.00) against MTCC 497 at concentration of 50 $\mu\text{g}/\text{disc}$ (Table 4). Acetone extract of DT showed maximum zone of inhibition (15.1 ± 0.05) against MTCC 1457 and MTCC 3906 showed zone of inhibition (12.83 ± 0.57) in methanol extract of DW. DH showed 12.66 ± 0.28 mm zone of inhibition against MTCC 1926 in methanol at concentration 0.5 mg/ml respectively. Acetone extracts of DHD showed maximum zone of inhibition of 12.50 ± 0.00 mm for MTCC 1926 and 12.50 ± 0.50 mm MTCC 497 at concentration 0.5 mg/ml (Table 3). However, the antibacterial activity of the extracts of studied plant against used bacterial strains using AWD and DD were significant (Table 2, 3) but in order to have more assertive conclusion, MIC was estimated. The MIC was carried out using broth dilution assay and the results revealed that among all the three extracts of selected plants, the methanol extract of DT showed the lowest MIC values against all tested pathogenic microbes as compared to standard Kanamycin (Table 4).

DISCUSSION

For the first time antimicrobial activity of these tuberous plants from Odisha state were reported. The active extracts were extracted in different solvents like methanol, acetone and aqueous. Although all the extracts showed varying levels of activity against all the tested bacteria, the methanol extract was found to be more active than other extracts. The antibacterial activity justify the potentials of bioactive compounds present in studied plants such as saponin, tannin etc. The extracts were effective against MTCC 497 and MTCC 1926. MTCC 497 which cause teeth decay, therefore, these extracts will be very effective as primary etiologic agents of coronal caries and root caries for oral diseases as such as dental and periodontal caused by *Streptococcus mutans*, *Streptococcus sobrinus*^[15] and *Streptococcus oralis*^[16] Extracts of said wild tuberous plants can be formulated as new drugs which may be also affective against cholera and against *Salmonella typhi*, which had killed over 600,000 people annually all over the world so it is known as deadly bacteria cause typhoid fever.^[17] MTCC 1926 is responsible for skin infections; therefore, the experimental plant parts will be helpful to formulate new drugs for skin infections. The extracts also showed

Table 4: Estimation of minimum inhibitory concentration (MIC) ($\mu\text{g}/\text{ml}$) of selected experimental plants.

Name of compounds	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 497	MTCC 1926
DBM	GC	GC	GC	GC	GC
DBA	GC	GC	GC	GC	GC
DBAQ	GC	GC	GC	GC	GC
DE M	500	500	500	500	500
DEA	GC	GC	GC	GC	GC
DEAQ	GC	GC	GC	GC	GC
DG M	500	500	500	500	500
DGA	500	500	500	500	500
DGAQ	GC	GC	GC	GC	GC
DOM	500	500	500	500	500
DOA	500	500	500	500	500
DOAQ	GC	GC	GC	GC	GC
DTM	400	300	300	400	300
DTA	300	300	300	300	300
DTAQ	500	500	500	500	500
DWM	500	500	500	500	500
DWA	500	500	500	500	500
DWAQ	GC	GC	GC	GC	GC
DHM	400	400	500	500	500
DHA	400	400	500	500	500
DHAQ	500	500	500	GC	GC
DHD M	500	500	500	500	500
DHDA	500	500	500	500	500
DHDAQ	GC	GC	GC	400	400
Standard	25	12.5	25	12.5	12.5
Inoculums control	GC	GC	GC	GC	GC
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth

(M: methanol, A: acetone, Aq: aqueous, GC: growth in all concentration, MTCC 3906: *Vibrio cholerae*; MTCC 1252: *Salmonella typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans*, Standard: Kanamycin, DB: *Dioscorea belophylla*, DE: *D. esculenta*, DG: *D. glabra*, DO: *D. oppositifolia*, DT: *D. tomentosa*, DW: *D. wallichii*, DH: *D. hamiltonii* and DHD: *D. hispida* var. *daemona*)

relevant inhibitory effect against *S. typhi* (MTCC 1252). *S. typhi* is considered as deadly bacteria causing typhoid fever.^[17] Some strains of *S. typhi* were also tested by other researchers for their vulnerability using chloramphenicol, trimethoprim and amoxicillin and many of these strains were found to be resistant to all these formulation.^[18]

It was reported that the tuber extract of *Dioscorea alata* against *Vibrio cholera* (MTCC 3909), *Salmonella typhimurium* (MTCC 1252), *Shigella flexneri* (MTCC 1457) *Streptococcus pyogenes* (MTCC 1926) and *S. mutans*

(MTCC 1497) were evaluated and revealed that the tuber extract had highest activity against *Streptococcus pyogenes*.^[19] In this study it was found that as the tuber extracts being effective against these ten fatal strains, among these varieties DHD, DH, DT was found to be effective against MTCC 1926 strain; DO and DE against strain MTCC 497; DG and DE for MTCC 1457 strain and DW showed promising effect against MTCC 3906 by inhibiting the growth. Since these tubers are rich in bioactive phytochemical compounds, therefore the extracts showed prominent properties against human pathogenic micro organisms. As indiscriminate use of antibiotics has led antimicrobial resistance to drugs, therefore, new antibacterial sources may be beneficial towards discovery of new potent antimicrobial drugs.

CONCLUSION

Present study highlights the phytochemistry and the pharmacological properties of the experimental tuberous plants. Results of qualitative and quantitative analysis of bioactive compounds / secondary metabolites justify their medicinal importance. It was observed that in both well diffusion and disc diffusion assay both methanol and acetone extracts showed good response against these strains in comparison to aqueous extracts for these eight tested species. This study revealed that as the tuber extracts being effective against these fatal strains, Among these varieties DHD, DH, DT was found to be effective against MTCC 1926 strain; DO and DE against strain MTCC 497; DG and DE for MTCC 1457 strain and DW showed promising effect against MTCC 3906 by inhibiting the growth. This study revealed that as the tuber extracts being effective against these fatal strains, the bioactive compounds of this tuber might be useful in formulating drugs against the cited strains of *S. typhi*. However antibacterial activities of tuberous plants were found to be active against gram negative bacteria. This makes the advantages of selected tuberous plants as antimicrobial agent. Our study showed that all the selected bacterial strains are more or less sensitive to all the tuberous plants fractions. Further research is being carried out for isolation of bioactive chemical constituents from the active fractions and their mode of action on microbial cells.

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CONFLICT OF INTREST

Authors declare no conflict of interest.

ABBREVIATIONS

MTCC: Microbial Type Culture Collection; **MTCC 497:** *Streptococcus mutans*; **MTCC 1926:** *Streptococcus pyogenes*; **MTCC 3906:** *Vibrio cholera*; **MTCC 1457:** *Shigella flexneri*; **MTCC 1252:** *Salmonella typhi*; **AMR:** Antimicrobial resistance; **AWD:** Agar well diffusion; **DD:** Disc diffusion assays; **MIC:** Minimum inhibitory concentration; **DB:** *Dioscorea belophylla*; **DE:** *Dioscorea esculenta*; **DG:** *Dioscorea glabra*; **DO:** *Dioscorea oppositifolia*; **DT:** *Dioscorea tomentosa*; **DW:** *Dioscorea wallichii*; **DH:** *Dioscorea hamiltonii*; **DHD:** *Dioscorea hispida* var. *daemona*.

SUMMARY

This study highlights the medicinal and pharmacological importance of the eight species of *Dioscorea* collected from peripheral areas of Similipal Biosphere Reserve, Mayurbhanj, Khordha, Cuttack and Puri districts of Odisha. In-vitro antibacterial screening of acetone, aqueous and methanolic extract of these *Dioscorea* species was evaluated against four bacterial species by agar well diffusion and disc diffusion assay methods. The minimum inhibitory concentration (MIC) values were also determined using broth dilution assay. The results showed significant antibacterial activity in methanol extract of all experimental tuberous species with highest activity against MTCC 1926 and MTCC 497. This results indicate the presence of phytochemicals in the selected tuberous plant species against specific bacterial strains.

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