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Article

Antioxidant activity, antibacterial potential and characterization of active fraction of *Dioscorea pentaphylla* L. tuber extract collected from Similipal Biosphere Reserve, Odisha, India

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Dioscorea pentaphylla L., a wild tuber is used both as food and medicines among different ethnic groups of Similipal Biosphere Reserve, India. Tubers are used against skin infections. In order to establish and confirm tribal claims, methanol extract was subjected to fractionation. The active fraction (DP1) was subsequently used for further purification and NMR (Nuclear magnetic resonance) characterization. The phytochemical analysis revealed the presence of saponin groups. The antibacterial activity of DP1 was done against selected bacterial strains (*Salmonella typhi*, *Shigella flexneri*, *Streptococcus pyogenes*, *Streptococcus mutans* and *Vibrio cholerae*) using DD (disc diffusion), AWD (agar well diffusion) and broth dilution assay. The activity was compared with antibiotics Penicillin and Kanamycin. It was observed that DP1 showed significant inhibitory activity against the tested bacteria. The characterization of DP1 through NMR analysis and presence of proton in carbon position at C-3, C-19, C-18, C-21 and C-27 was same as the known compound "Diosgenin". Therefore, isolated compound was confirmed to be Diosgenin. The study for the first time showed that, diosgenin present in *D. pentaphylla* tuber was responsible for antibacterial and antioxidant potential. Present study highlights the importance of *Dioscorea* species as sources of diverse secondary metabolites for the isolation of active compound(s).

Keywords: *Dioscorea pentaphylla*/extract/active fraction. *Dioscorea pentaphylla*/ antibacterial activity/ antioxidant. NMR characterization.

INTRODUCTION

The use of natural products with therapeutic properties by common man is as ancient as human civilization and, for long, plant products were the main sources of traditional medicines (Ji, Li, Zhang, 2009). The industrial revolution and the development of organic chemistry resulted in preference for synthetic products for pharmacological treatment (Rats, 2001; Kwik, 2015). Even if we consider the impact of the discovery of the penicillin, obtained from micro-organisms, as on anti-infection therapy, the importance of natural products can never be ignored. About 25% of the drugs prescribed worldwide come from plants, and many such active compounds are in current use (Shu, 1998; Ciddi, 2012). Most of important drugs obtained from plants are digoxin from *Digitalis* species, quinine from *Cinchona* species, vincristrine and vinblastine from *Catharanthus roseus* (L.) G. Don., atropine from *Atropa belladonna* L., morphine codeine from *Papaver somniferum* L., and diosgenin from *Dioscorea* species etc. Natural compounds from plant sources are being used for designing and formulating new drugs (Rats, 2001; Dittbrenner *et al.,* 2009; Tostmann *et al.,* 2010; Cragg, Newman, 2005; Archana, Paul, Tiwari, 2011; Heena, Lele, 2012; Chavez *et al.,* 2013; Lahlou, 2013).

Most of the wild plants have been reported to have antimicrobial activity (Mikayel, Margarit, Armen, 2017). Still number of wild unexplored / neglected plants are available in the forest having both food as well as

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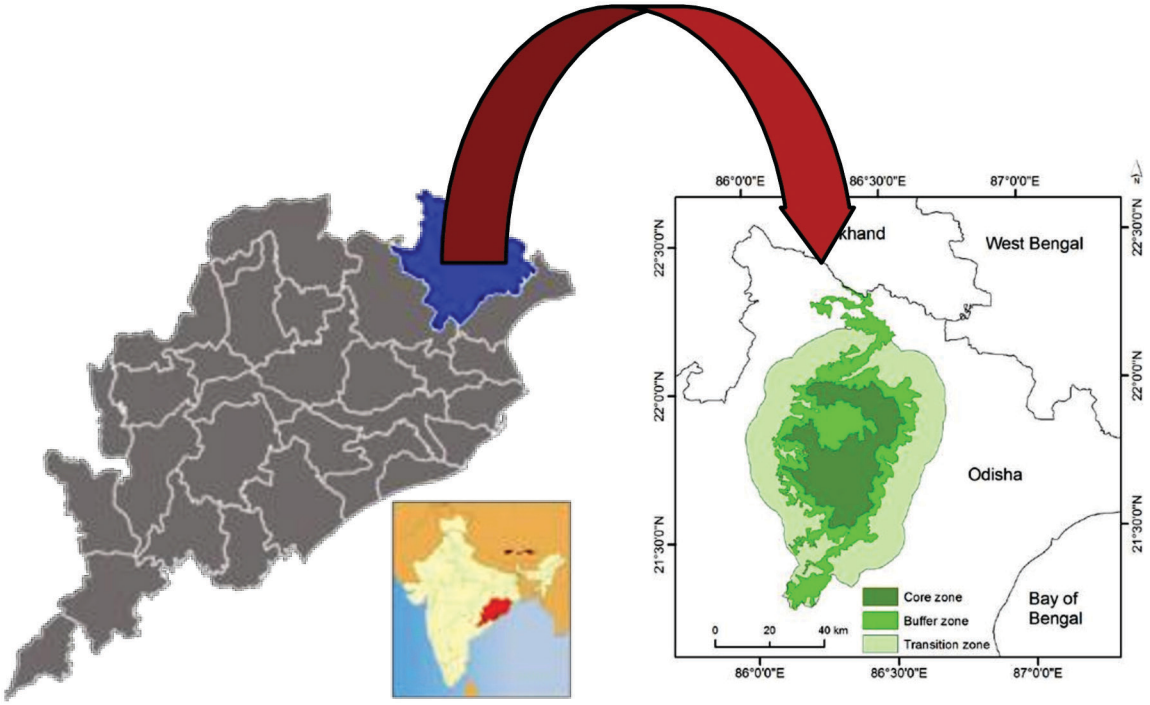

FIGURE 1 - Collection site of Experimental plant

medicinal values. Among the indigenous forest food plants, the tubers and roots are the most important wild medicinal foods after grains (Birgitta, Gullick, 1999). Many of these tubers are used for preparation of medicines against diseases by the rural and tribal people (Tabassum, Hamdani, 2014). Therefore, there is an urgent need for screening primary and secondary metabolites in many such wild plants species. Among such wild tubers, India harbours a rich genetic diversity of tropical root and tuberous plants such as "Yams" (Bān Aālu), aroids and several others like ginger, arrowroot, zedoary, ginger lily, wild turmeric and some orchids. In Similipal Biosphere Reserve (Figure 1), Odisha, India, the tuberous edible medicinal plant *D. pentaphylla* is one such tuber that acclaims unique importance. It is locally known as "Panja Aalu" or "Panja Sānga" (Kumar *et al.,* 2017). Tribal communities use different preparations out of this tuber as medicine, particularly against inflammation, anti-aging and diseases caused by microbial pathogens. They attribute antioxidant activity and antimicrobial activities due to the expression of browning properties and presence of secondary metabolites in them.

Keeping this in mind, in the present study an attempt has been made to study the browning properties and antioxidant of tuber extracts and to characterize the constituents present in active fraction of the tuber extract of *D. pentaphylla* (Figure 2) against tested

FIGURE 2 - Vegetative parts of *D. pentaphylla* L.

bacterial strains. The antioxidant and antimicrobial assay of *D. pentaphylla* extracts were carried out. TLC (Thin Layer Chromatography) profiling of active extract and fractionation was done. NMR (Nuclear Magnetic Resonance) analysis of the spot of active fraction was done to identify the bioactive compound(s) present in the tubers of *D. pentaphylla*.

MATERIAL AND METHODS

Selection and collection of experimental plant (tuber)

The experimental plant was collected from the Padampur village, in the peripheral area of Similipal Biosphere Reserve, Odisha, India and was kept in poly bags tagged with the botanical name as per standard sampling procedure and passport description (Hawkes, 1980; Christian, Brigitte, 2004). The collected germplasm of experimental plant was propagated and grown in the gene bank of Department of Botany, Ravenshaw University, Cuttack, India for further experimental work.

Browning values and tuber extract preparation

Browning values were qualitatively estimated with the rate of colour changing. Soxhlet method was adopted (Tiwari *et al.,* 2011) to obtain the methanol extract of *D. pentaphylla* tuber. The tubers were collected and 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 experimental work.

Estimation of antioxidant activity

In order to study the antioxidant activity of experimental plant extracts, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and metal chelating activity were evaluated. The standard methods were adopted for the said scavenging activity. DPPH was carried out followed by Cao, Sofic and Prior (1997) and metal chelating activity was done using Gouda *et al.* (2014). The DPPH activity was expressed as EC_{50} values (effective concentration showing 50% of inhibition activity). DPPH was carried out using 5.0 mL of dilutions (100 µg/mL) of the experimental compounds and standard were mixed with 1 mL of a 0.001 % ethanolic solution of DPPH. DPPH solution was freshly prepared in each experiments and was stored in dark at 4± 2 °C. The compounds were incubated for 20-30 minutes in the dark at 30±2 °C. After incubation, Spectrophotometer readings were taken at 517 nm. All determination was performed in triplicate for better documentation. The Metal Chelating Activity of the plant extracts was determined using Gouda *et al.* (2014). About 1ml of plant extract added to a solution of 0.5 mL ferrous chloride (0.2mM), and then about 0.2 mL. of Ferozin (5 mM) was added to it and incubated at room temperature for 10 minutes. The absorbance of the solution was then measured at 562 nm.

Antibacterial activity of *D. pentaphylla* tuber extracts

Antibacterial activity using Disc diffusion assay was done with the 6 mm of disc prepared from Whatman filter paper (Amanda *et al.,* 2012). Each extract was dissolved in dimethyl sulfoxide. The sets of three dilutions (0.5, 1.0 and 2.0 mg/mL) of crude extracts and standard drugs were prepared. 6 mm of discs were kept in the drugs for 12 h before placing on the agar plates. The zones of growth inhibition around the disks were measured after 18 to 24 hrs of 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.

Fractionation of methanolic extract of *D. pentaphylla* tuber

First author already reported that the methanol extract showed highest antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); and three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252) (Kumar, Behera, Jena, 2013; Kumar, Jena, 2014). Therefore, a combination of preparative TLC and column chromatography (Raman 2006) were used for the initial fractionation of the crude extracts and isolation of the active compounds. The dry powder extract was dissolved in respective solvent (acetone, methanol and aqueous). 10 µL of the extract was applied as a drop at the origin of the preparative TLC plate with pre determined mobile phase (Bhatanagar *et al.,* 2012). The active well visualized bands were confirmed.

The bands that are to be analyzed were marked and the silica containing the compounds was scraped off repeatedly and collected in closed container. The collected samples were centrifuged with respective solvent. The supernatant was collected and kept for further experiments. Normal column chromatography was performed with the use of silica gel powder (60-120 mesh, Merck- 0.040-0.063 mm, and code-61806205001730) on a 45 cm glass column of 1.4 cm diameter. The previously collected

supernatant was loaded on the packed column. A gradient mobile phase, composed of different ratios of chloroform and methanol, was used in order to elute the compounds over the range of polarities. The used mobile phase for active visualized bands on TLC was also used in column. Fractions were collected and monitored on TLC again with respective mobile phase and confirmed the spot at same Rf (retention factor) (Puspa, Weeraddana, 2011; Kuete *et al.,* 2012; Sathiavelu, Arunachalam, 2012). The antibacterial activity of the fraction is reported (Kumar, Jena, 2014) and the confirmed fraction was concentrated. The concentrated fractions were dissolved in a small volume of respective solvent and subjected to phytochemical analysis, NMR analysis and antibacterial activity (Present study).

Antibacterial activity of active spot (DP-1)

The methanol fraction of *D. pentaphylla* tuber was screened for antibacterial activity against two Gram-positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); and three Gram-negative bacteria *Vibrio cholerae* (MTCC 3906), *Shigellaflexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 1252). All of the used MTCC (Microbial Type Culture Collection) bacterial strains were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using slight modification of standard methods of Agar Well Diffusion assay (Allen, Molan, Reid*,* 1991), Disc Diffusion method (Scorzoni *et al.,* 2007; Amanda *et al.,* 2012; Zare *et al.,* 2012; Thompson *et al.,* 2013**)** and Broth dilution assay (Rai *et al.,* 2010).

Phytochemical assays of DP1

Phyto-chemical analysis of DP1 was carried out using standard procedure to identify the possible bioactive compound(s) (Harborne, 1973; Trease, Evans, 1989; Sofowara, 1993).

*Test of Tannin***:** The powder DP1 was boiled in 10 mL of distilled water and filtered using "whatman filter paper" of filter grade 42. 2 mL of filtrate was taken in a test tube and 3-5 drops of 0.1 % ferric chloride solution was added. The brownish green or blue black colouration indicated the presence of tannins.

*Test for Saponin***:** The powder DP1 was boiled in 15 mL of distilled water and filtered using Whatman filter paper of filter grade 42. 5 mL of filtrate was mixed with 2 mL of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

*Test of Flavonoids***:** 6 mL of dilute ammonium solution was added to a portion of the aqueous filtrate of DP1 followed by addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

*Test of Terpenoids***:** DP1 was mixed with 1 mL of methanol and 2.5 mL of chloroform and 3 mL of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

*Test of Glycosides***:** DP1 powder was treated with 1 % ferric chloride solution and was put into water bath for 5 minutes at 100 °C. The mixture was cooled, and equal volume of benzene was added. The benzene layer was separated, and 5 mL of ammonia solution was added. Formation of rose pink colour indicated the presence of glycosides.

*Test of Phenolic compounds***:** DP1 powder was treated with 3-5 drops of 1% ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

Test for Reducing Sugar: DP1 powder was dissolved with distilled water and filtered. The filtrate was boiled with 2 drops of Fehling's solution A and B for 5 minutes. An orange-red precipitate was obtained, which indicated the presence of reducing sugar.

*Test for Steroids***:** DP1 was dissolved in 2 mL of methanol and again dissolved in 5 mL chloroform and then 5 mL of concentrated sulphuric acid was added. Formation of 2 phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

*Test for Alkaloids***:** DP1 powder was mixed with 5 mL of 1% aqueous HCl on water bath and then filtered. 2-5 drops of Dragendorff's reagent were added in the filtrate. The occurrence of orange-red precipitate indicated the presence of alkaloids in the sample extract.

^{1}H NMR analysis for estimation of active spot / bands

^{1}H NMR analysis of spot DP1 found in the active fraction of methanol extract of *D. pentaphylla* tuber was recorded on a NMR-400 MHz and also the chemicals shifts were recorded (Singh *et al.,* 2014). The results were compared with reference graph and number of protons present in C position (Ghosh *et al.,* 2014).

RESULTS AND DISCUSSION

Nutraceutical foods are very important for an increasing global population. Biodiversity is the hub of uncountable such foods, yet we only make use of a few. Among them, *Dioscorea* species play a vital role in

supplementing the requirement of food and medicines to rural and tribal people. The results of present study revealed that the tuber of *D. pentaphylla* showed high rate of browning. The browning properties (Figure 3) are directly proportional to the antioxidant activities. The estimation of antioxidant revealed that the organic extracts of tubers showed excellent activity with standards. It was observed that acetone extract showed highest (89 µg/mL EC_{50} values for DPPH and 86 µg/mL EC_{50} values for Metal chelating) antioxidant activity (Table I) while Bhandari *et al.* (2003) documented the browning values of *D. bulbifera, D. Deltoids, D. triphylla* and *D. versicolor* along with the total phenol content (mg/100g) in *D. bulbifera*, *D. deltoida*, *D. triphylla* and *D. versicolor* and Lubag *et al.* (2008) reported the antioxidant activity of *D. alata* (61 %) using the relative lipid peroxidation. In the year of 2011, Roy *et al.* (2011) documented the free radical scavenging activity using DPPH assay of ethanolic extract of *D. villosa* (87.72%) while Lincy and Mohan (2013) studied the antioxidant activity of *D. spicta* using DPPH assay (57.51% at 1000 µg/mL), hydroxyl radical scavenging activity (49.33 % at 1000 µg/mL).

Researches of the recent years have emphasized that there is an urgent need of research for new antimicrobial agents or drugs in the light of antibiotic resistance offered by pathogenic microbes. Keeping these in view, the extracts of the *D. pentaphylla* were investigated for their anti-microbial values using disc diffusion assay. The results revealed that methanol extract showed highest zone of inhibition followed by acetone and aqueous extracts at all taken concentrations against all tested bacterial strains. It was also noted that the highest inhibition was exhibited by methanol extract of *D. pentaphylla* tuber against *S. pyogenes* at all the used concentrations (Table II). The results were in conformity with the traditional uses among the aboriginals of SBR as reported by the tribal of Padampur, Hatibadi, Durdura. They use the tuber against skin infections, against cut, wounds and microbial infections (Kumar, Behera, Jena, 2013). All the three extracts were having excellent inhibitory effects, so the tuber extracts might be quite effective in controlling the diseases caused by *V. cholerae, S. typhi, S. flexnerii, S. mutans* and *S. pyogenes*.

As the methanol extract of *D. pentaphylla* (tuber) showed highest zone of inhibition, therefore it was taken with eight different mobile phases for TLC and column chromatography analysis. TLC analysis showed that the methanol extract showed significant number of visible bands (Kumar, Jena, 2014). The column chromatography of *D. pentaphylla* revealed that there are six fractions named F1, F2, F3, F4, F5 and F6 which were collected. The antibacterial activity of fractions showed that only F6 exhibited the zone of inhibition against all tested bacterial strains (Kumar, Jena, 2014). Kuete *et al.* (2012) also documented the antibacterial activity of methanol extract and fractions from the bulbils of *D. bulbifera* to be active against *E. coli*, *M. tuberculosis*, *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa*. The experiment was

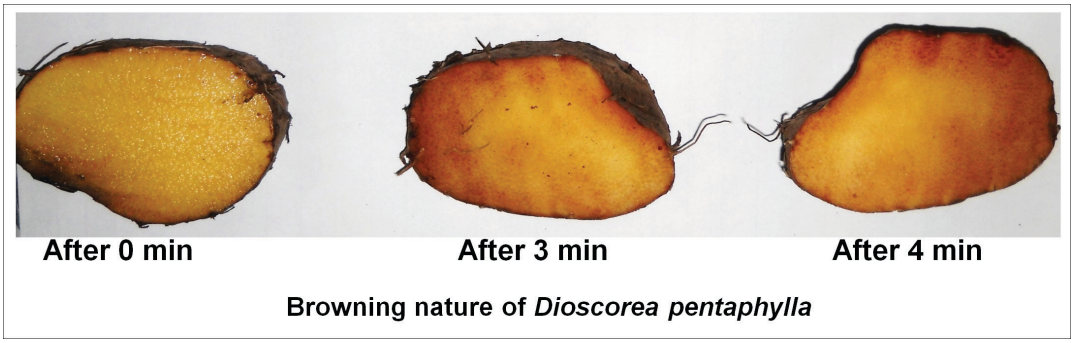

FIGURE 3 - Browning properties of *D. pentaphylla*

TABLE I - Antioxidant activities of *Dioscorea pentaphylla* tuber extracts (100 µg/mL)

Plant extract	DPPH scavenging activity (EC_{50} value)*		Metal chelating activity (EC_{50} value)*	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract
D. pentaphylla	82.07± 0.08	89.41 ± 0.39	81.47 ± 0.36	86.52 ± 0.55
BHT	132.86 ±0.20		97.92	0.62

*Values in µg/mL

TABLE II - Antibacterial activity of *D. Pentaphylla* tuber extracts

Strains	Zone of inhibition (in mm)					Extract(s)
	10 µg/disc	20 µg/disc	30 µg/disc	40 µg/disc	50 µg/disc	
MTCC 3906	ZI < 7.00	9.00 ±1.00	14.33 ± 0.57	18.00 ± 2.00	24.83 ± 1.32	Methanol
MTCC 1252	ZI < 7.00	7.6 ±1.52	13.33 ±3.05	16.66 ± 0.57	22.33 ± 0.57	
MTCC 1457	ZI < 7.00	7.33± 0.76	11.00 ± 2.64	18.33 ± 1.52	23.00 ± 1.00	
MTCC 1926	ZI < 7.00	13.00 ±2.06	16.66 ± 0.57	22.33 ± 0.57	25.00 ± 1.00	
MTCC 497	ZI < 7.00	8.66 ±1.52	12.33 ± 2.08	17.33 ± 0.57	19.00 ± 0.76	
MTCC 3906	ZI < 7.00	ZI ≤ 7.00	8.66 ± 1.52	11.33 ± 1.52	15.00 ± 1.25	Acetone
MTCC 1252	ZI < 7.00	ZI ≤ 7.00	9.66 ± 1.52	12.00 ± 2.00	14.66 ± 1.25	
MTCC 1457	ZI < 7.00	ZI ≤ 7.00	9.33 ± 1.52	11.33 ± 1.52	13.66 ± 0.76	
MTCC 1926	ZI < 7.00	8.66 ± 1.25	10.33 ± 2.02	12.33 ± 2.08	16.66 ± 1.04	
MTCC 497	ZI < 7.00	ZI ≤ 7.00	8.33 ± 0.76	11.00 ± 1.00	14.00 ± 0.76	
MTCC 3906	ZI < 7.00	ZI ≤ 7.00	8.00 ± 0.50	9.00 ± 1.00	10.66 ± 0.50	Aqueous
MTCC 1252	ZI < 7.00	ZI ≤ 7.00	7.00 ± 0.50	9.00 ± 1.00	8.00 ± 1.00	
MTCC 1457	ZI < 7.00	8.00 ± 0.00	10.33 ± 1.52	11.00 ± 2.64	13.00 ± 1.00	
MTCC 1926	ZI < 7.00	8.00 ± 0.00	10.33 ± 1.04	11.66 ± 3.21	15.33 ± 2.08	
MTCC 497	ZI <7.00	ZI ≤ 7.00	8.33 ± 1.25	9.00 ± 1.00	11.66 ± 1.52	

(ZI < 7.0: zone of inhibition is less than 7.0 mm; ZI ≤ 7. 0: zone of inhibition is less than or equal to 7.0 mm; NI: no inhibition, Disc Diffusion Assay, mean ± SD, n=3) (MTCC 3906: *Vibrio cholerae*, MTCC 1252: *Salmonella typhi*, MTCC 1457*: Shigella flexneri*, MTCC 1926: *Streptococcus pyogenes*, MTCC 497: *Streptococcus mutans*)

further subjected to get the active spot / band using F6 on TLC. It was observed that same spot appeared 23 times out of 25 times of experiments at Rf: 0.82 with respective mobile phase with F6 (Kumar, Jena, 2014). The spot having Rf: 0.82 was named as DP-1 (present study) and results confirmed the spot (DP-1) at the said Rf. The present antibacterial analysis of DP-1 using Agar well Diffusion and Disc Diffusion assay was done and results revealed that it showed significant inhibitory activity against *S. pyogenes* followed by *S. flexneri*, *S. mutans*, *V. cholerae* and *S. typhi* at used concentrations (Table III).

DP-1 was also excellent in inhibiting the growth at concentration of 10 µg/disc against *V. cholerae*, *S. typhi*, *S. flexneri*, *S. pyogenes* and *S. mutans*. Using both assay, DP-1 showed the highest inhibitory activity against MTCC 1926 *S. pyogenes*. The above results indicated that the DP-1, Kanamycin and neomycin are more effective against Gram-positive bacteria *S. pyogenes*. It was also examined that ampicillin is more effective against Gram-negative bacteria. Results confirmed that the compounds might be responsible to formulate drugs against *S. pyogenes* too. (Table III). For further confirmation of the antibacterial activity, broth dilution method was performed for assessment of MIC (Minimum Inhibitory Concentration) values compared to antibiotics. The results revealed that DP-1 showed the lowest MIC values against *S. pyogenes* and *S. mutans* as compared to used antibiotics (Table IV).

The above results encouraged for the qualitative tests of phytochemical screening. The phytochemical screening of DP-1 showed the presence of saponin (Table V). Previous reports have revealed that *Dioscorea* is rich with Diosgenin (Ghosh *et al.,* 2014), a steroid sapogenin. The sugar-free diosgenin is used for the commercial synthesis of cortisone, pregnenolone, progesterone, and other steroid products (Dierassi, 1992). The antibacterial activity of saponin / diosgenin reports are documented in literature against Gram-positive and Gram-negative bacterial strains (Karimi, Jaafar, Ahmad*,* 2011).

All the above experimental results and findings from literature justified that the active component DP-1 might possess some active compound/group of compounds which is/are responsible for antibacterial activities and this might be dosgenin or its analogue. Keeping this in view, NMR analysis for DP-1 was done to analyze the possible functional groups. The results showed Carbon position at C-3, C-19, C-18, C-21 and C-27 were equal to the known compound Diosgenin. The characterization of DP1 is ($C_{27}H_{42}O_3$) (m/z 414 [M]+), mp 201–203 °C, Rf 0.82, silica gel, n-hexane: ethyl acetate (5:2) ^{1}H NMR ($CDCl_3$,300 MHz): 0.8 (C-18 methyl), 0.83 (C-27 methyl),

TABLE III - Antimicrobial activity (zone of inhibition) of fraction (DP1; RF: 0.82) of *D. pentaphylla* tuber

Disc diffusion assay, ZI (mm)						
Drugs	*V. cholerae*	*S. typhi*	*S. flexneri*	*S. pyogenes*	*S. mutans*	Concentration
DP1	12.00 ± 0.00	11.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00	12.00 ± 0.00	10 µg/Disc
	14.00 ± 0.00	14.00 ± 0.00	16.00 ± 0.00	19.00 ± 0.00	15.00 ± 0.00	50 µg/Disc
Kanamycin	16.44 ± 1.53	15.67 ± 0.58	14.00 ± 0.00	18.00± 0.00	17.33 ± 0.57	10 µg/Disc
Neomycin	21.33 ± 0.58	21.33 ± 0.58	21.33 ± 0.58	20.33 ± 0.58	22.67 ± 0.58	10 µg/Disc
Ampicillin	26.00 ± 0.00	24.00± 0.00	28.00 ± 0.00	23.00 ± 0.00	26.00 ± 0.00	10 µg/Disc
Agar Well Diffusion assay, ZI (mm)						
DP1	7.5 ± 0.00	7.5 ± 0.00	7.5 ± 0.00	10.0 ± 0.00	9.0 ± 0.00	100 µg/mL
	15.0 ± 0.00	11.0 ± 0.00	14.0 ± 0.00	16.0 ± 0.00	14.0 ± 0.00	200 µg/mL
Kanamycin	9.0 ± 0.00	9.50 ± 0.00	10.0 ± 0.00	12.0 ± 0.00	11.0 ± 0.00	100 µg/mL
Ampicillin	12.0 ± 0.00	11.0± 0.00	14.0± 0.00	12.0± 0.00	14.0± 0.00	100 µg/mL

(ZI: Zone of Inhibition; mean value ± SD, n=3, MTCC: Microbial Type Culture Collection; mm: millimeter; Rf: retention factor; µg: microgram; DP1: fraction of Methanolic extract of *Dioscorea pentaphylla* tuber at Rf: 0.82; MTCC 3906: *Vibrio cholerae*; MTCC 1252: *Salmonela typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans*)

TABLE IV - Comparatives evaluation of MIC values of *D. Pentaphylla* methanol extract, DP1 along with standards

Antibacterial agents	*V. cholerae*	*S. typhi*	*S. flexneri*	*S. pyogenes*	*S. mutans*
S1	3.125 µg/mL	3.125 µg/mL	3.125 µg/mL	3.125 µg/mL	3.125 µg/ mL
S2	25 µg/mL	12.55 µg/mL	25 µg/mL	12.5 µg/mL	12.5 µg/ mL
DP-1	100 µg/mL	100 µg/mL	100 µg/mL	50 µg/mL	50 µg/ mL
Inoculums control	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration
Broth control	No Growth	No Growth	No Growth	No Growth	No Growth
DMSO	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration

(DMSO: Dimethyl sulfoxide; S1: Ampicillin; S2: Kanamycin)

TABLE V - Qualitative phytochemical analysis of DP1

Bioactive compounds/ Secondary metabolites	**Compounds detected**
Tannin	Not Detected
Saponin	Detected
Flavonoids	Not Detected
Terpenoids	Not Detected
Glycosides	Not Detected
Cardiac glycosides	Not Detected
Phenolic compounds	Not Detected
Reducing sugar	Not Detected
Alkaloids	Not Detected

0.81 (C-21 methyl), 1.02 (C-19 methyl), 3.38 (C-26), 3.49 (C-3), 4.21 (C-1) and 5.02 (C-6 H) (Figure 4). The number of protons were same at said carbon position, therefore the active compound was proved to be diosgenin.

CONCLUSION

The results of present investigations highlight the antioxidants potentials of *D. pentaphylla* tuber extracts. The antioxidant properties can generate further interest in studying the under-exploited tuber crops for proving the efficacy of these plants as nutraceutical and pharmaceutical foods. The consumption of these crops might play a vital role in preventing human diseases in which free radicals are involved, such as cancer, cardiovascular disease and ageing. The antibacterial

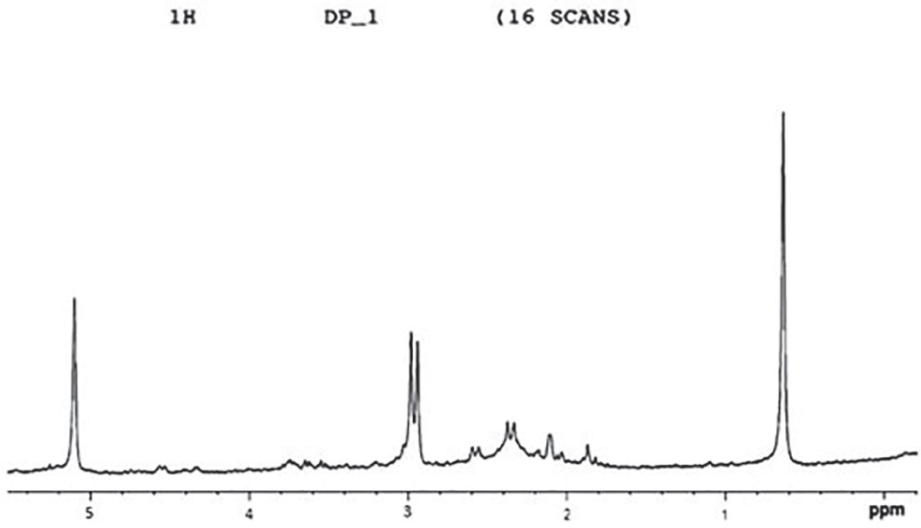

FIGURE 4 - ^{1}H NMR of DP-1 isolated from methanol extract of *D. pentaphylla* tuber.

potential, TLC and Column Chromatography analysis show its importance in the formulation of new antibacterial drugs to fight against Antimicrobial resistance problem. The antibacterial activity of scraped spot (DP-1) showed first report against the tested bacterial strains and tuber of *D. pentaphylla* might be effective to cure the disease caused by the used bacterial strains. The characterization of DP1 proved that the compound present in the scraped spot to be Diosgenin. The present study, for the first time established that diosgenin present in *D. pentaphylla* tuber was more effective against *Streptococcus pyogenes* and *Streptococcus mutans* along with strong antioxidant potential. The study emphasize upon further investigation, to isolate the active compounds present in this tuber for formulation of new drugs against bacterial infections.

CONFLICTS OF INTEREST

Authors declare no conflicts of Interest.

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