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Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts

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ABSTRACT

Thin layer chromatography (TLC) profile of *Dioscorea pentaphylla* L. tuber extracts was performed by using eight different solvent systems. Chloroform: methanol :: 9:1 was found appropriate for fractionation through column chromatography showing maximum number of spot(s) / band(s). Total six fractions were collected and antibacterial activity was examined using disc diffusion assay and agar well diffusion assay. Results indicated that fraction F6 might have an active antibacterial agent (Rf: 0.82). TLC profile and FT-IR analysis of *D. pentaphylla* tuber extracts can be useful in characterization of different secondary metabolites found in this species.

1. Introduction

Dioscorea species is the major wild tuber crop in the Similipal Biosphere Reserve (SBR) of Odisha as per availability and consumption palatability. It is locally known as "Bân Aâlu" or "Sânga" (Kumar *et al.,* 2013). Genus *Dioscorea* belongs to the family Dioscoreaceae, representing more than 600 species worldwide (Coursey, 1967). About 13 species of *Dioscorea* are recorded in SBR (Kumar *et al.,* 2012; Misra *et al.,* 2013). *D. oppositifolia*, *D. bulbifera, D. wallichi*, *D. hamiltonii*, and *D. spinosa* are usually found in foot-hills whereas *D. puber, D. pentaphylla, D.hispida, D. bellophylla, D. Glabra*, *D. belophylla* and *D. tomentosa* are found in moderate and high altitude. *D. alata* is a cultivated one and mostly found in rural and tribal gardens of SBR. Among all these species, *D. pentaphylla* (Plate 1) is the most common among tribal communities because of its easy storage and effective disease curing potential. Literature indicate the sound ethno-pharmacological values of *D. pentaphylla*. Tubers of this vine are used to cure joint swelling (Edison *et al.,* 2006), to improve body immunity (Kamble *et al.,* 2010), for stomach pain (Choudhary *et al.,* 2008), to cure rheumatic swellings and abdominal pain after delivery (Swarnkar and Katewa, 2008), in fever (Padal *et al.,* 2012), digestive tracts problems (Choudhary *et al.,* 2008), for poor health (Rani *et al.,* 2011) and skin infections (Kumar *et al.,* 2013; Misra *et al.,* 2013).

However, very less reports are available on the bioactive compounds present in *D. pentaphylla*. But researchers have reported the active constituents present in *Dioscorea* species in general such as diosgenin (Ghosh *et al.,* 2014), allantoin (Berthemy *et al.,* 1999; Yoon *et al.,* 2008), cyaniding-3-glucoside (Ozo *et al.,* 1984**)**, dioscorins (Lu *et al.,* 2012), steroidal saponin (Sautour *et al.,* 2004), daucosterol (Ma *et al.,* 2005), bafoudiosbulbins (Teponno *et al.,* 2006; 2008), diterpenoids (Teponno *et al.,* 2007), â-sitosterol (Aderiye *et al.,* 1996), flavonoids and alkaloids (Poornima and Ravishankar, 2009; Sakthidevi and Mohan, 2013), furostanol (Kim *et al.,* 2011), 2-hydroxy-4-

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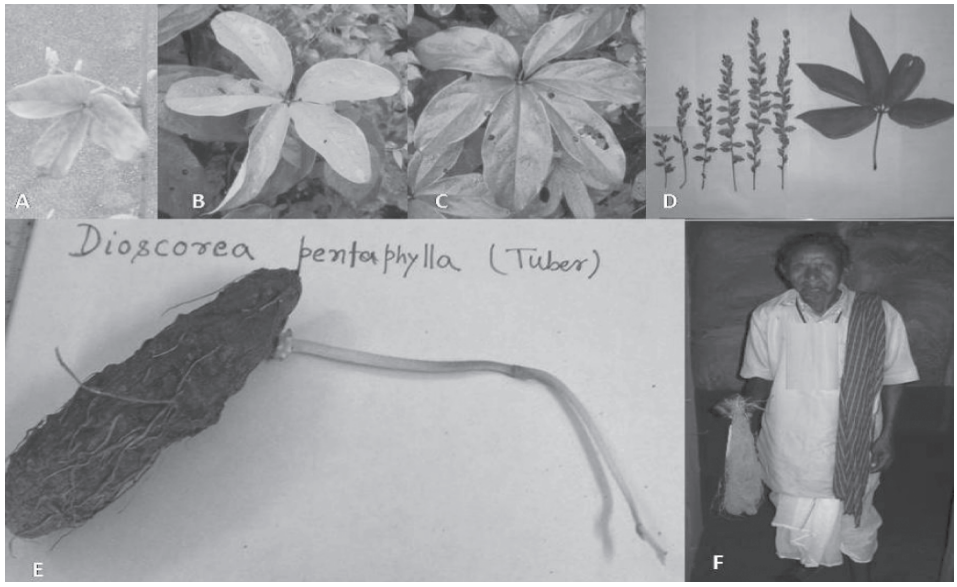

Plate 1: A-C Morphological variations (leaves) of *D. pentaphylla*; D- 5-leaflets with fruits of *D. pentaphylla*; E- Tuber of *D. pentaphylla*; F- Ho tribe with tuber of *D. pentaphylla.*

methoxyacetophenone (Jeong *et al.,* 2011), paeonols (Miyazawa *et al.,* 1996) and different types of polyphenols (Martin and Ruberte, 1976). Keeping the above ethno-pharmacological values and bioactive compounds in *Dioscorea* species in view the present paper deals with the bioactive compounds present in crude tuber extracts of *D. pentaphylla* specially using TLC profiling and FTIR analysis along with fraction of active extract and their antibacterial activity.

2. Materials and methods

2.1 Collection of experimental plant species and preparation of plant extracts

The samples were collected and kept in polybags tagged with the botanical name. The collected experimental plant was propagated and grown in the garden of Dept. of Botany, Ravenshaw University for phytochemical and antibacterial activities of the plant extracts. The tuber of experimental plant was collected and dried at room temperature under shade and was powdered. The powdered material was kept in thimble and extraction was carried out using the Soxhlet apparatus (Tiwari *et al.,* 2011). The residues was collected and left for air drying and dried crude extracts were stored in refrigerator for further experimental work.

2.2 Chromatographic studies and FT-IR analysis

Chromatographic analysis was done using standard methods to evaluate the secondary metabolites (Kumar *et al.,* 2009; Bhatnagar *et al.,* 2012; Seelinger *et al.,* 2012; Baragi *et al.,* 2014**).** The mobile phases were taken as per polarity index in single, double and triple combining solvent systems such as n-hexane, Chloroform : Methanol, Chloroform : Ethyl acetate : Formic acid (CEF) and Ethyl acetate : Methanol : Water (EMW). The FT-IR analysis was carried out using potassium bromide (KBr) pellet method (Sawant *et al.,* 2007).

2.3 Antibacterial activity

The tuber extracts of experimental plant part were screened for antibacterial activity against two Gram-positive bacteria, viz., *Streptococcus mutans* (MTCC *497) and *Streptococcus pyogenes* (MTCC 1926), and three Gram-negative bacteria *Vibrio cholera* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella enteric typhi* (MTCC 1252). All 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 (AWD) assay (Allen *et al.,* 1991) and Disc Diffusion (DD) assay (Scorzoni *et al.,* 2007; Zare *et al.,* 2012; Thompson *et al.,* 2013).

2.4 Fractionation of methanol extract

A combination of preparative TLC and column chromatography were used for the initial fractionation of the crude tuber extracts and isolation of the active compounds. The dry powder extract was dissolved in respective solvent. Normal column chromatography was performed with the use of silica gel powder (60-120 mesh, Merck- 0.040-0.063 mm) on a 45 cm glass column with 1.4 cm diameter (Patra *et al.,* 2012).

3. Results and discussion

The field collection survey has revealed that the tuber of this vine is frequently used as food and against different microbial infections. Among them, skin infections are very common infections cured by the vegetative parts of *D. pentaphylla*. The *Santhal, Bathudi* and *Ho* tribes of the SBR use its paste against the skin infections. The above results provided the proof that the vegetative parts of the *D. pentaphylla* might have contained bioactive compounds responsible for such activity. Such reports are also available from many other previous works (Misra *et al.,* 2012; Kumar *et al.,* 2013).

The methanol, acetone and aqueous extracts of *D. pentaphylla* tuber were taken with eight different mobile phases. It was observed that the methanol extract showed highest numbers of visible bands with all used mobile phases followed by acetone and aqueous extracts showing highest number of spots (5) (Fig. 1) with mobile phase C:M (9:1) and C:M (8:2) followed by 3 spots in C:E:F and C:M (6:4) and one spot with C:M (4:12), C:M (7:3) and E:M:W while the acetone extract showed highest visible spots (3) with C:E:F and C:M (9:1) followed by 2 spots with E:M:W, C:M (8:2), C:M (6:4) and C:M (4:12) (Table 1).

Table 1

TLC (thin layer chromatography) profiling of tuber extracts of *D. pentaphylla* L.

Mobile phase	Ratio	Rf Values
	Methanol extract	
E:M:W	(40:5.4:4)	0.81
C:E:F	(5:4:1)	0.51, 0.77, 0.84
C: M	(9 :1)	0.65, 0.81, 0.82, 0.83, 0.93
C: M	(8:2)	0.30, 0.46, 0.90, 0.92
C: M	(7:3)	0.80
C: M	(6:4)	0.40, 0.42, 0.68
C: M	(4:12)	0.81
n-hexane	-	-
	Acetone extract	
E:M:W	(40:5.4:4)	0.82, 0.87
C:E:F	(5:4:1)	0.48, 0.75, 0.81
C: M	(9 :1)	0.36, 0.52, 0.70
C: M	(8:2)	0.40, 0.80
C: M	(7:3)	-
C: M	(6:4)	0.57, 0.65
C: M	(4:12)	0.82, 0.87
n-hexane	-	-
	Aqueous extract	
E:M:W	(40:5.4:4)	0.81
C:E:F	(5:4:1)	0.85
C: M	(9 :1)	0.58, 0.88
C: M	(8:2)	0.76, 0.86
C: M	(7:3)	0.70
C: M	(6:4)	0.85
C: M	(4:12)	0.72
C: M	-	-

Abbreviations - E:M:W-Ethyl acetate: Methanol: Water; C:E:F- Chloroform: Ethyl acetate: Formic acid; C:M- Chloroform: Methanol

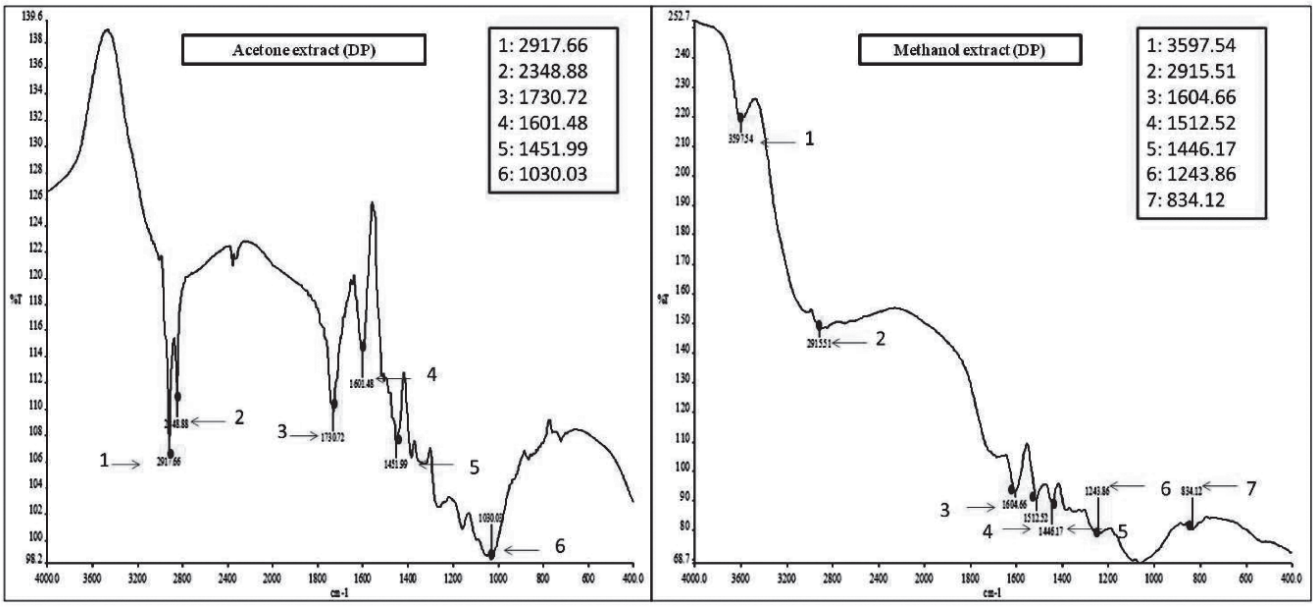

Fig. 1 FT-IR analysis of organic tuber extracts of *D. pentaphylla* L.

Though the organic extract showed highest visible bands aqueous extract also showed good bands. The aqueous extract showed two spots with C:M (9:2) and C:M (8:2) followed by one spot with all rest used mobile phases. The TLC profiling revealed that the used extract showed no visible bands with n-hexane.

The column chromatography of *D. pentaphylla* revealed that there were six fractions (F1-F6) among 24 flask having 20 ml of eluants. Each eluant was collected at 30 minutes intervals. The fraction was categorized with physical colour appearance of eluants. The fraction, F1 was collected from 1st to 6th flask, F2 from 7th to 9th flask, F3 from 10th to 13th flask, F4 from 14th to 18th flask, from 19th to 20th flask and F6 from 21st to 24th flask (Table 2; Fig 1a). Each collected fraction was subjected to TLC profiling with used mobile phases.

The F1 showed one spot with CEF having Rf : 0.56, F2 showed 2 spots with C:M (8:2) having Rf : 0.86, 0.88, F3 and F4 did not show any spots with used solvents. F5 showed 3 spots with C:M (9:1) having Rf: 0.65, 0.81 and 0.82 and F6 showed 1 spot with C:M (9:1) having Rf: 0.82. the fraction F5 and F6 were bulked together as they showed

Table 2

Composition of eluants for the fraction of *D. pentaphylla* L. tuber extract in methanol for column chromatography

Eluants	Solvent (ml)	Number of collected conical flask(s)	Name of fraction
100 % n-Hexane	300	1-4	F1
50 % Methanol in n-Hexane	200	5-6	
100 % Methanol	100	7-8	F2
10 % Chloroform in Methanol	100	9	
20 % Chloroform in Methanol	100	10	F3
30 % Chloroform in Methanol	100	11-12	
40 % Chloroform in Methanol	100	13	
50 % Chloroform in Methanol	200	14-17	F4
60 % Chloroform in Methanol	100	18	
70 % Chloroform in Methanol	100	19	F5
80 % Chloroform in Methanol	100	20	
90 % Chloroform in Methanol	200	21-23	F6
100 % Chloroform	100	24	

Table 3

TLC profile for observed spot(s) on respective mobile phase of Fractions (F1-F6) of *Dioscorea pentaphylla* L. (tuber) methanol extract

Fraction	Mobile phase	Ratio	Number of Spot(s)
F1	C:E:F	(5:4:1)	1 (Rf: 0.56)
F2	C:M	(8:2)	2 (Rf: 0.86, 0.88)
F5	C:M	(9 :1)	3 (Rf: 0.65, 0.81, 0.82)
F6	C:M	(9 :1)	1 (Rf: 0.82)

Note: abbreviation of mobile phase are as for Table 1

very similar TLC features i.e. a spot at same Rf as found in the solvent system C:M (9:1) (Table 3).

The antibacterial activity of the extract was measured with zone of inhibition (ZI). Inhibition zones of ZI e" 0.7 cm for AWD and e" 7.0 mm for DD assay were considered sognofocantly toxic nature of the extract to the pathogen and taken positive as per NCCLS (National Committee for Clinical Laboratory) standard. The fraction F1-F4 did not show any activity whereas F5 and F6 showed good activity against the selected strains (Table 4). F5 showed activity against MTCC 1252, MTCC 1457, MTCC 1926, MTCC 497 using DD and activity against MTCC 3906, MTCC 1252, MTCC 1457, MTCC 1926 and MTCC 497 using AWD (Fig. 2b). F6 showed activity against all used microbial strains both with AWD and DD (Fig. 2c) (Table 4). The results encouraged the TLC run for collecting the spots at Rf: 0.82 with mobile phase C: M (9:1). The antibacterial activity and visible spots on TLC ar Rf: 0.82 with F6 might have some correlation with each other. Compound(s) present at said Rf 0.82 might be responsible for the antibacterial activity of *D. pentaphylla* tuber extracts against the strains used. Kuete *et al.* (2012) also documented the antibacterial activity of methanol extract and fractions from the bulbils of *D. bulbifera* against *E. coli*, *M. tuberculosis*, *E. aerogenes*, *K. pneumonia* and *P. aeruginosa*.

It was also observed that methanol and acetone extract only showed the respective visible bands, which give the base-line for the FT-IR analysis. Therefore, the methanol and acetone extract were taken for FT-IR analysis. When the FT-IR analysis carried out, it showed relevant peak with

Table 4

Antibacterial activity of Fraction (F1-F6) against used Microbial strains

	Disc diffusion assay					
	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 1926	MTCC 497	Concentration (µg/disc)
F1	-	-	-	-	-	
F2	-	-	-	-	-	10
F3	-	-	-	-	-	10
F4	-	-	-	-	-	10
F5	-	+	-	+	+	10
F6	+	+	+	+	+	10
			Agar Well Diffusion assay			µg/m^2
F1	-	-	-	-	-	100
F2	-	-	-	-	-	100
F3	-	-	-	-	-	100
F4	-	-	-	-	-	100
F5	-	+	+	+	+	100
F6	+	+	+	+	+	100

(+: inhibition seen; -: inhibition not seen)

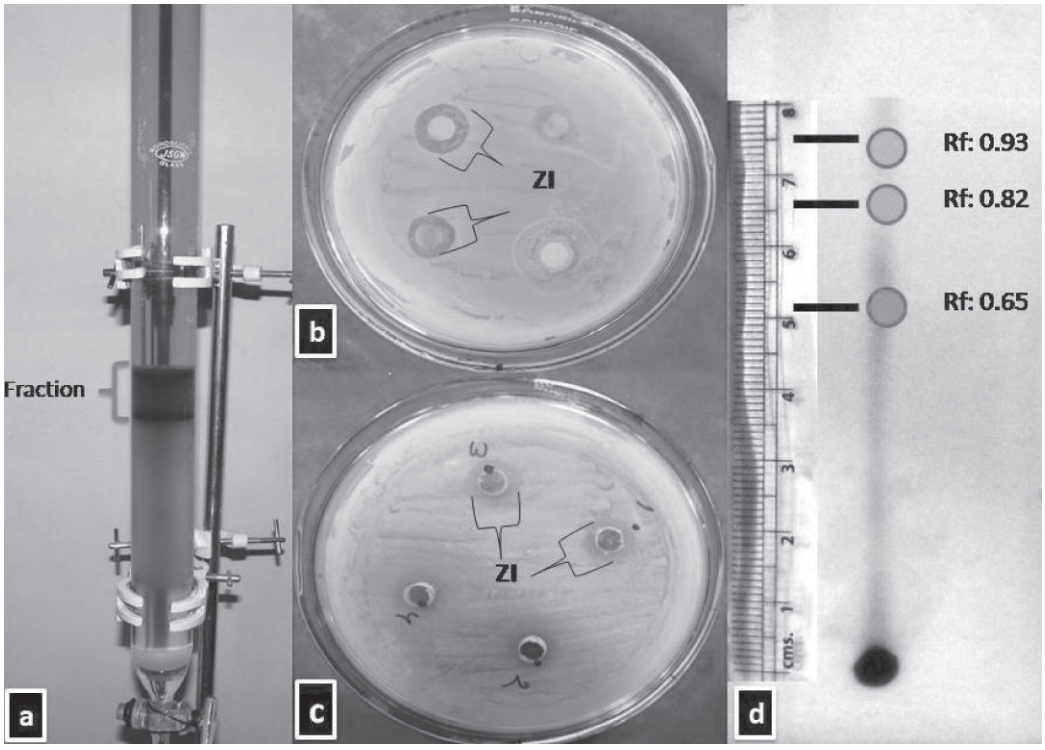

Fig. 2. Chromatographic profiling and antibacterial activity of *D. pentaphylla* tuber extract, (a). Fractionation of methanol extract (tuber), (b). Antibacterial activity of F6 using disc diffusion against MTCC 1926, (c). Antibacterial activity of F6 using agar well diffusion assay against MTCC 1926, (d). Active visible band(s) / spot(s) of methanol extract (tuber); (ZI: zone of inhibition)

acetone and methanol extract (Fig. 2). The acetone extract of *D. pentaphylla* (tuber) showed five peaks value representing five different possible functional groups. The peak values are 2848.88 which lies between 2700-2900 indicating C-H stretching, 1730.72 (1730-1750) indicating aliphatic esters C = O stretching, 1601.48 (1600-1690) representing amide –I, C = O stretching, 1451.99 (1400-1500) first overturn N-H and O-H stretching and 1030. 03 (1020-1060) representing S = O stretching (Kong and Shooning, 2007; Stuart, 2004). The methanol extract of this vine showed six peaks values representing six possible functional groups. The peak values 1604.66 (1600-1690) represents amide-I, C = O stretching, 1446.17 (1400-1500) representing N-H and O-H stretching functional groups.

As per the ethnobotanical report collected from ethnic tribes of SBR, *D. pentaphylla* is used frequently against different microbial infections (Kumar *et al.,* 2013). Therefore, the present study was more designed to analyse the antibacterial activity of fractions (methanol extract of tuber) of *D. pentaphylla*. Fraction F5 and F6 showed excellent activity (Zone of inhibition) against all the used bacterial strains. The inhibitory activity exhibited by all the extracts against MTCC 1926, that cause skin infections, justifying the claims to use the tuber extract of this vine against skin infections. The activities of fractions of the *D. pentaphylla* (methanol tuber extract) as tested found the extracts suitable natural anti-microbial agents. The present study warrants more specific research to identify the active compounds of fractions F5 and F6, which can be utilized for formulation of new antimicrobial drugs against some common microbial diseases prevalent in rural and tribal Odisha.

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