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Bio-activity guided fractionation and antimicrobial screening of active fractions from *Dioscorea alata*

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ABSTRACT

Dioscorea alata L. Commonly known as Water yam and Betel yam and purple yam it contains about 600 species distributed throughout in the world, but mostly in tropical region. Many species of *Dioscorea* genus are economically important crops of worldwide and many of them have been used in the pharmaceutical industry. *In vitro* propagation of *Dioscorea* species pave the way to meet the demand of this economically important plant. This review summarizes some of the important reports on phytochemical tests and Evaluation of cold methanol extract of *Dioscorea alata* for anti-microbial screening. The medicinal plants used in the treatment of antimicrobial activity are possibly rich sources of potential lead compounds. Chromatographic separation of cold methanol tuber extract of *Dioscorea alata* are inhibiting the growth of *S. aureus, S. pyogens, T. rubrum, T. mentagrophytes, F. flocussum, Candida albicans* at> 250 µg/ml whereas against *P. acne,* it is inhibiting the growth at 62.5 µg/ml. A semi- preparative work needs to be carried out by using ZIC-HILIC column to isolate active constituent from *D. alata* tubers, which shown promising activity against *P. acne.*

Key words: *Dioscorea alata,* Cold methanol tuber extract, Anti-microbial screening.

INTRODUCTION

Dioscorea alata Linn. (Fig.1) (Family: *Diascoreaceae*), known in cultivation. The tuber is used in leprosy (Kartikar and Basu, 2000), piles and gonorrhoea, antioxidant and hypolipidaemic (Lin *et al.*, 2004). Alkaloids, (Chopra *et al.*, 1956), anthocyanins (Yoshida *et al.*, 1991), glycosides (Ozo *et al.*, 1984), carbohydrates (Kovassi *et al.*, 1990) are reported from the plant.

Dioscorea alata L. Commonly known as : Kath Alu (As.), Kham Alu (Beng, TGC), Thaphukhlong (DI), anra (HR), Bahra (HM), Ruichin (Karbi). in English as Water yam (Chopra *et al.*, 1999), Winged yam', 'Betel yam. Sanskrit-Pindalu (Chopra *et al.*, 1999), Hindi-Chupri alu, Telugu Pendalamu. Habitat: Climber. Brief description: Leaf cordate, dark green; stem angular. Biological status: frequent. Part used: Tuber. Ethno botanical and ethno-medicinal uses: Tubers are boiled with arums, mushrooms, cooked with vegetables and mixed with rice. Tuber paste is applied on cancerous wounds, leprosy, gonorrhoea, blood pressure and in skin diseases. 2-3 gm of paste of the tuber is tied on the infected part of the body (Abhyankar RK, Upadhyay R 2011; Choudhury K et al., 2008; Poornima GN, Rai RV., 2009, Khumbongmayum AD et al., 2005).

Dioscorea alata L. is an important tuber crop and is a staple food for millions of peoples in tropical and subtropical countries (Edison S *et al.*, 2006). Root and Tuber crops are the most important food crops after cereals. Tuber crops find an important place in the dietary habits of small and marginal farmers especially in the food security of tribal population. India hold a rich genetic diversity of tuber crop especially yam Dioscorea (Hann SK *et al.*, 1995).

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Diosgenin an aglycone is a chemical substance found in *Dioscorea* and are used commercially in pharmaceutical industry. Apart from diosgenin, dioscorin, dioscin and other alkaloids are also found. Root contains phytosterols, alkaloids, tannin and rich source of starch. Other substance found are aluminium, ascorbic acid, ash, beta-carotene, calcium, chromium, cobalt, iron, magnesium, manganese, niacin, otassium, phosphorus, protein, riboflavin, selenium, silicon, sodium, thiamine, tin, zinc. Barnali Dutta (2015).

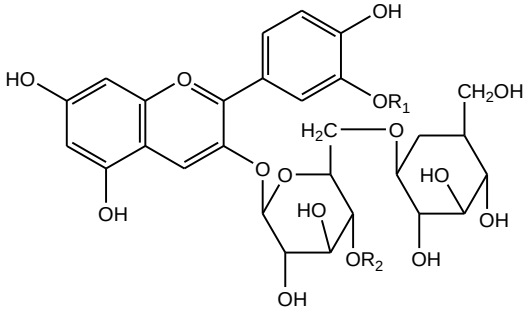
Medicinal uses reported from Ayurvedic literature:

Tuber:

Tuber is anthelmintic; useful in leprosy, piles and gonorrhoea (Kirtikar and Basu, 2000; Behl *et al.*, 1993). Tubers are given orally in haemorrhoids and syphilis (Kumar Sharma *et al.*, 2001), food ingredient (Mali *et al.*, 2003), antioxidant and hypolipidaemic (Lin *et al.*, 2004), anti-fungal (Aderiye *et al.*, 1996).

Chemical constituents isolated from *Disocorea alata:*

Acylated anthocyanins: Cyanidin and peonidin-3-gentiobiosides (Shoyama and Nishioka, 1990).



1. R_1 = H, R_2 = Sinapoyl — Cyanidin
2. R_1 = H, R_2 = H — Cyanidin-3-*O*-gentiobioside
3. R_1 = Me, R_2 = Sinapoyl — Peonidin-3-*O*-(4"-Sinapoyl gentiobioside) (Alatanin 2)

Uses:

Anti-oxidant (Dabeer *et al.*, 2005), Anti-mutagenicity (Galvand *et al.*, 2004), Gastric protective effects (Sessiano *et al.*, 2003), prevention of inflammation (Galvano *et al.*, 2004).

Compounds reported from *D. alata*

Dihydropinosylin (1), Demethylbatatasin (2), Batatasin (3) and Batatasin (4) found in the *Dioscorea alata* (Clines *et al.*, 1989). Batatasins were reported as growth inhibitors (Hashimoto *et al.*, 1972).

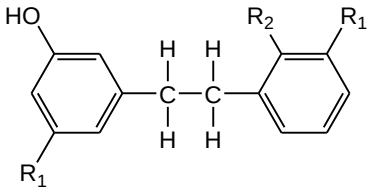
Three new anthocyanins Alatanin A, B and C were isolated from the tubers of *Dioscorea alata* (Yoshida *et al.*, 1991).

Cyanidin-3-glucoside, (+)-catechin and the procyanidin dimers “B-1” and “B-3” as phenolic constituents of *Dioscorea alata* tubers were reported by (Ozo *et al.*, 1984).

Soluble sugars such as monosaccharides, disaccharides and polyols from the leaves, stem and tubers of yam were identified. D-fructose, D-glucose and polyols (2-deoxyribitol, 6-deoxysorbitol, and glycerol) were found in the leaves (Kovassi *et al.*, 1990).

In an effort for the selection of plants with anti-microbial potential based on bioactivity-guided selection, *Dioscorea alata* showed anaerobic antibacterial activity. But no systematic approach has been made to identify the active fractions from the tubers.

The tubers of *D. alata* extract exhibited anti-fungal activity. In view of this, tubers of *Dioscorea alata* is collected and subjected for extraction, fractionation in search of active constituents in crude extract. The crude extract is tested against different organisms and then the active extracts were subjected for isolation of active constituent by using different chromatographic techniques.



1. R_1 = OH, R_2 = R_3 = H — Dihydropinosylin
2. R_1 = OH, R_2 = OH, R_3 = H — Demethyl batatasin IV
3. R_1 = OMe, R_2 = H, R_3 = H — Batatasin III
4. R_1 = OMe, R_2 = OH, R_3 = H — Batatasin VI

MATERIAL AND METHODS

Collection, Extraction and Phytochemical Tests Of *Dioscorea Alata*

Collection and drying of plant:

Tubers of *Dioscorea alata* collected from Tirumala hills, Andhra Pradesh. The tubers of plant were collected in August 2006 at its mature stage, washed with running water to remove mud. The tubers were dried and chopped into pieces (approximately 1-2 cm in length). Immediately sprayed with alcohol to cease the enzymatic degradation of secondary metabolites. The chopped tubers were then shade dried.

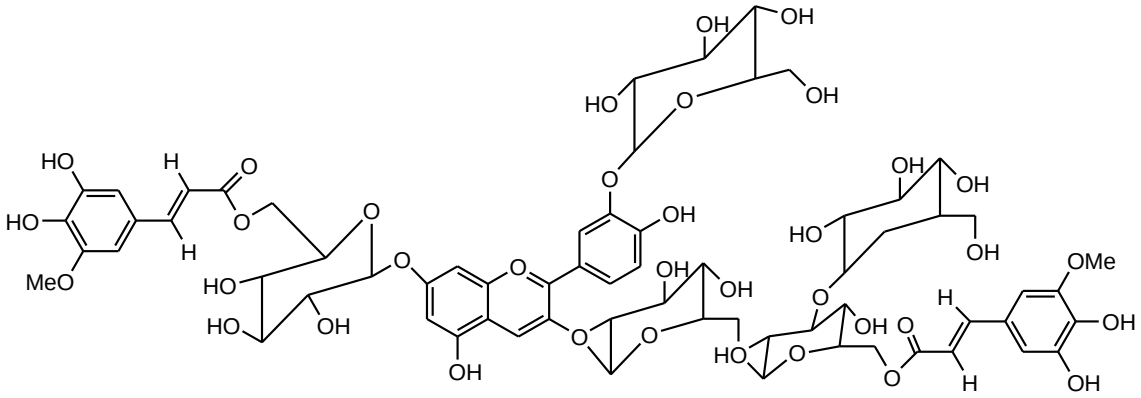
Extraction of plant:

Cold extraction: The chopped material was (500 gms) then immersed in methanol (1000 ml) with occasional shaking for two days. The extract was then filtered and concentrated using Buchii evaporator under reduced temperature. The extract so obtained was air-dried, weighed (6.64 gms) packed and stored in a refrigerator.

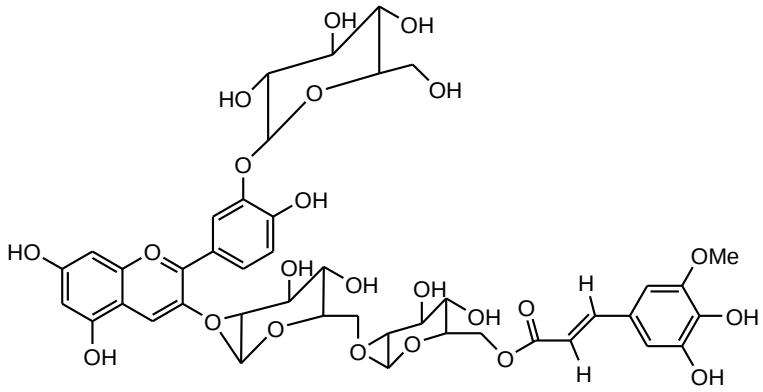
RESULTS AND DISCUSSION:

Phytochemical Tests Of *Dioscorea alata:*

The phytochemical test of extract is studied as described by Harborne (vide Part-I, 4.3.3). The results

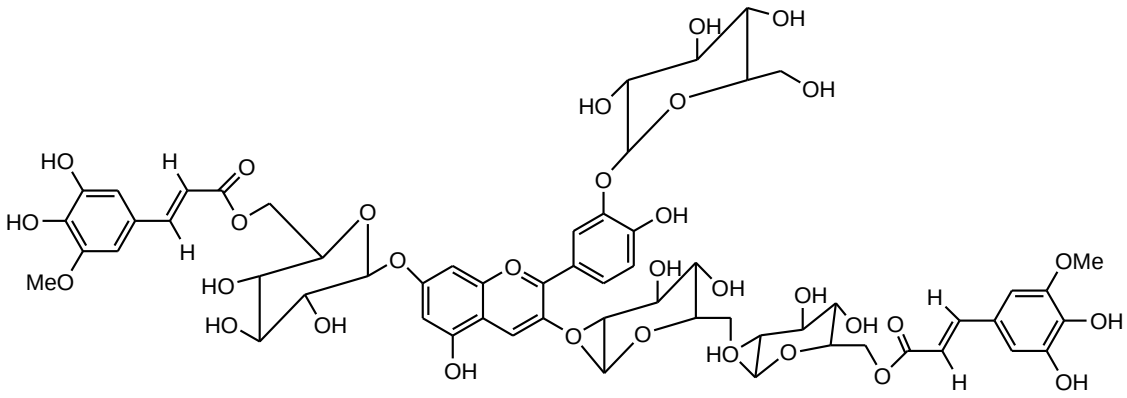


Alatanin A



Alatanin B

Uses: Colouring agent (Bridle and Timberlake, 1997)



Alatanin C

SOURCE: Yoshida *et al.*, 1991

are shown in Table-1.

The cold methanol extract of *D. alata* tubers found to have least comparable MIC values compared with standard drugs. The strength of this crude extract of tubers in inhibition of growth of *S. aureus, S. pyogens, Candida albicans, T. rubrum, T. mentagrophytes, F. floccusum, P. acne* may be due to the presence of diosgenin, a steroidal sapogenin present in Dioscoreace plants.

The cold methanol extract of tubers of *D. alata* are inhibiting the growth of *S. aureus, S. pyogens, T. rubrum, T. mentagrophytes, F. flocussum, Candida albicans* at> 250 µg/ml whereas against *P. acne,* it is inhibiting the growth at 62.5 µg/ml. (Table.2).

The *Dioscorea alata* has shown a positive test for steroids, saponins. Steroidal sapogenins containing preparations are physiologically active constituents that have been used to treat human diseases. Interestingly, this class of natural products is becoming the subject of anti-infective research.

i) Activity of crude extract of Tubers of *D. alata* against *C. albicans*

The cold methanol extract of *D. alata* is found to be inhibiting the growth of *C. albicans* at MIC of 1000 μg/ml.

ii) Activity of tubers crude extract against *S. aureus*

The cold methanol extract of tubers of *D. alata* found to inhibit the growth of *S. aureus* at MIC of 500 μg/ml.

iii) Activity of tubers of crude extracts against *S. pyogens*

The cold methanol extract of tubular *D. alata* is found to be inhibiting the growth of *S. pyogens* at MIC of 250 μg/ml.

iv) Activity of tubers crude extracts against *T. rubrum*

The cold methanol extract of tubers of *D. alata* is found to be inhibiting the growth of *T. rubrum* at MIC of 500 μg/ml.

v) Activity of tubers crude extracts against *T. mentagroplzytes*

The cold methanol extract of *D. alata* is found to be inhibiting the growth of *T. mentagrophytes* at MIC of 1000 μg/ml.

vi) Activity of *D. alata* tubers cold methanol extract against *E.floccusum*

The cold methanol tubers extract of *D. alata* is found to be inhibiting the growth of *E. floccusum* at MIC of 500 μg/ml.

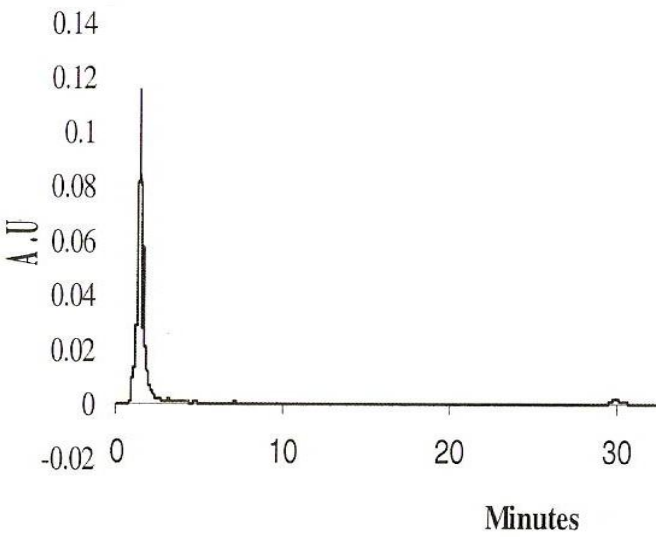
vii) Activity of *D.alata* tubers extract against *P. acne*

The cold methanol extract of *D.alata* tubers inhibit the growth of *P. acne* at 62.5 μg/ml.

Chemical examination/HPLC separation of active fraction from cold methanol extract of *D. Alata*

The cold methanol extract of tubers of *D. alata* extract has shown a promising activity against *P. acne*. Chromatographic separation of cold methanol tuber extract of *Dioscorea alata* was attempted for separation using isocratic elution by Ace 5C18 column. With Ace 5C 18 column all the constituents are eluting with the solvent front. (Fig.2).

Figure-2. Analytical work on cold methanol extract of *D. alata* tubers using ACE 5C 18 Column.



Analytical profile of *D. alata* tubers cold methanol extract on ACE 5C18 column:

Conditions and Equipment:

Concentration of sample 1 mg/ml prepared in 60:40 v/v methanol : water

Mobile phase	:	65:35 v/v methanol: water
Column	:	Ace 5C 18 (250 x 4. 6 mm ID)
Flow rate	:	1.5 ml/min
Injection volume:		20 μl

Table-1. Plant part, method of extraction, phytochemical test results

Plant Part	Method of extraction	Solvent of extraction	Secondary metabolites					
			S	A	F	P	G	Sa
Dioscorea alata Tubers	Cold	Methanol	+	+	+	+	+	+

S — Steroids; A — Alkaloids; F — Flavonoids; P - Phenols; G — Glyosides; Sa — Saponins; + — Present.

Table 2: Evaluation of cold methanol extract of *D. alata* for anti-microbial screening. MIC values for crude extracts by agar dilution method

Plant part	Solvent of extraction	MIC values in μg/ml against microorganisms						
		C.a	*S.a*	*S.p*	*T.r*	*T.m*	*E.f*	*P.a*
Tubers	Cold methanol	1000	500	250	500	1000	500	62.5

C.a - Candida albicans, S.a- Staphylococcus aureus, S.p- Streptococcus pyogens, T.r- Trichophyton rubrum, T.m.- Trichophyton mentagrophytes,

Wavelength	:	215 nm
Temperature	:	21°C
Pump	:	Shimadzu LC-6A liquid chromatopae.
Detector	:	Shimadzu SPD — 6AV UV-VIS Spectrophotometric detector.
Integrator	:	Shimadzu C- R5A chrornatopac.

D. alata cold methanol extract solutions were made up with approximately 1 mg/ml of extract.

1. Methanol: Water (90:10 v/v)
2. Methanol: Water (80:20 v/v)
3. Methanol: Water (70:30 v/v)
4. Methanol: Water (60:40 v/v)

The solutions were chromatographed by RP-HPLC using an isocratic mobile phase of methanol and water in the proportions equal to that of the sample diluent i.e., sample solution no. 4 was chromatographed using a mobile phase methanol: water (65:35) by maintaining above mentioned chromatographic conditions.

The isocratic elution of the sample no. 4 was not best resolved by all the above mobile phases, however the maximum number of eluents were eluting with the solvent front and the resolution was poor as shown in Fig.5.11. Hence, the decision was taken to change the column from Ace 5C18 to ZIC-HILIC so that the sample resolution and retention can be improved.

Isocratic HPLC separation by using ZIC — HILIC column:

Instruction for Use-ZIC —HILIC

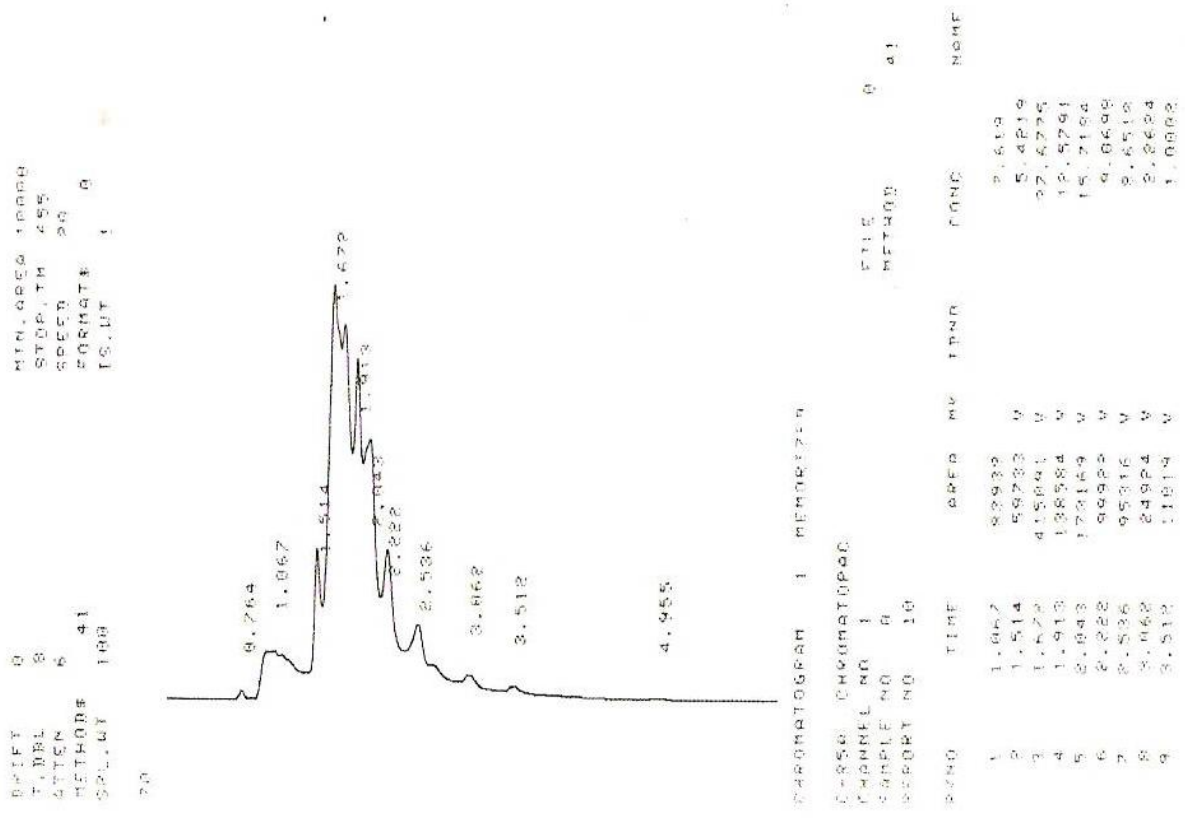
The ZIC-HILIC column has a zwitterionic stationary phase covalently attached to porous silica. The permanent and hydrophilic zwitterion functionality makes the column suitable for application in hydrophilic interaction liquid chromatography (HILIC). Weak electrostatic interactions between charged analyses and the neutral zwitterionic stationary phase results in a unique selectivity and especially for analytes that are poorly retained on reverse phase columns.

The HILIC is a technique suitable for separation of very polar and hydrophilic compounds.

Why HILIC

Despite the fact that reversed phase liquid chromatography (RPLC) is the overall most applied separation technique, and that it can be used for a variety of applications in junction with the most common detection principles, certain solutes, especially polar and hydrophilic compounds, are not retainable. Normal phase liquid chromatography has been the technique of choice for this purpose. Under such condition it is difficult to dissolve polar and hydrophilic compounds. In other words solutes that have little or no retention on

Figure-3. Analytical profile of *D. alata* tubers cold methanol extract on ZIC-HILIC column



RPLC columns generally experience strong retention on HILIC columns.

The ZIC-HILIC column can be used as a tool to change the selectivity or to improve peak resolution for peptides, carbohydrates, protein digests and various polar compounds as amino acids, zwitter ions, acryl amide, cytosine, uracil, urea, glucosylated and glucuronated compounds.

The ZIC-HILIC column can be operated in the pH range 2 to 8, while strongly alkaline solutions and washing with sodium hydroxide should be avoided.

Analytical work on cold methanol extracts of *D. alata* tubers using ZIC-HILIC column (Fig. 3):

Conditions and Equipment

Concentration of sample 1 mg/ml prepared in 70:30 v/v methanol: water

Mobile phase	:	70: 30 v/v methanol: water
Column	:	5µ ZIC HILIC (250 x 4.6 mm i.d.)
Flow rate	:	1.5 ml/min
Volume	:	20 µl
Wavelength	:	254 nm
Temperature	:	21°C
Pump	:	Shimadzu LC-6A liquid Chromatograph.
Detector	:	Shimadzu SPD-6AV UV- VIS Spectrophotometric detector.
Integrator	:	Shimadzu C-R5A chromatopac.

The shortage of new compounds active in dermatological conditions has directed the research to the traditional herbal remedies used in Ayurveda, a traditional Indian form of medicine. The medicinal plants used in the treatment of skin diseases are potentially rich sources of potential lead compounds. The tubers of *Dioscorea alata* cold methanol extract have shown a promising activity against *P. acne* at a concentration of 62.5 µg/ml.

Chromatographic separation of *D. alata* tubers methanol extract was initially carried out on Ace 5C 18 columns. With Ace 5C 18 columns all the components are eluting with the solvent front. The components may be polar. To overcome this ZIC-HILIC column was used instead of the 5C 18 column to separate polar compounds. The analytical work on ZIC-HILIC has been carried out by using the conditions mentioned. A semi-preparative work needs to be carried out by using ZIC-HILIC column to isolate a active constituent from *D. alata* tubers, which shown promising activity against *P. acne*.

CONCLUSION

Dioscorea alata is a climber, used in the treatment of haemorrhoids and syphilis, food ingredient, antioxidant and hypolipidaemic and antifungal diseases. The methanol extract of tubers of the *D. alata* when tested for antimicrobial activity against different microorganisms exhibited inhibition against *Propionobacterium acne* at MIC of 62.5 µg/ml. Phytoconstituents present in the plant crude extract may be synergistically responsible to inhibit the growth of *P. acne*. As all the components present in the methanol extract of tubers were found to be polar, normal phase liquid chromatography has been the choice for the purpose. Instead of RPLC columns, ZIC-HILIC column was used to separate polar compounds. A semi-preparative work needs to be carried out by using ZIC-HILIC column to isolate the active constituent(s) which are responsible for antidermatophytic activity of the methanol extract of *D. alata* tubers against *P. acne*. This may be due to the lack of confidence of young generation in the traditional medicine systems and availability of modern medicines. Proper steps must be taken to protect and conserve these plants as they are used for various medicinal purposes and household food.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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