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Research Article

PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL PROPERTIES OF *DIOSCOREA BULBIFERA* TUBER

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ABSTRACT

Objective: The inhibitory properties of successive extracts from *Dioscorea bulbifera* (Dioscoreaceae) tubers have been evaluated for the presence of phytochemical constituents and antimicrobial efficacy against multidrug-resistant (MDR) clinical isolates was evaluated.

Methods: The tuber of *D. bulbifera* was oven dried and extracted successively with n-hexane, chloroform, methanol, ethanol, and water. The antimicrobial potential of successive extracts against MDR isolates was studied by agar well-diffusion method. Qualitative phytochemical analysis was performed.

Results: Qualitative phytochemical analysis demonstrated the presence of steroids, flavonoids, cardiac glycosides, saponins, and reducing sugars in almost all the extracts tested. Anthraquinones, phlobatanins, and tannins were not reported in any extracts tested. The *in vitro* antimicrobial activity of various solvents and water extracts of *D. bulbifera* was further investigated against ten MDR bacteria and three fungi, respectively. Aqueous and chloroform extracts were found to be more potent being capable of exerting significant inhibitory activities against the majority of the isolates such as *Escherichia coli, Acinetobacter* sp., *Salmonella paratyphi, Klebsiella pneumoniae,* and *Candida albicans.* The highest inhibitory activity was observed for *K. pneumoniae* with wide inhibition zone diameters (17 ± 0.15 mm), followed by *E. coli* 1(13 ± 0.11) mm, and *Acinetobacter* sp. (11 ± 0.12).

Conclusion: Based on the present study, the extracts of *D. bulbifera* tubers have shown excellent activity against MDR microbial cultures tested. Further study is recommended for clinical evaluation, of the efficacy of crude extract in herbal medicine that can serve as a base for the development of novel potent drugs and phytomedicines.

Keywords: Antimicrobial activity, *Dioscorea bulbifera,* Multidrug-resistant, Phytochemical analysis.

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INTRODUCTION

Prevalence of antibiotic-resistant strains of bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases. The continuous emergence of Gram-positive and Gram-negative MDR bacteria drastically reduces the efficacy of our antibiotic armory, and consequently, increases the frequency of therapeutic failure [1]. Approximately 60% of world's population still relies on medicinal plants for their primary healthcare. Medicinal plants have been used as a source of remedies since ancient times all around the globe. Most medicinal plants are known to produce certain bioactive molecules which are responsible for their antimicrobial properties [2]. As a result, numerous studies have been carried out in different parts of the world to extract plant products for screening antimicrobial activity. *Dioscorea bulbifera*, the Air potato, is a true yam species in the dioscoreaceae, or true yam family. It is also known as Varahi in Sanskrit, Kaachil in Malayalam, and Dukkar Kand in Marathi. The Air potato plant is native to Africa and Asia. It is an invasive species in many tropical areas, including Florida. The various extracts of bulbs of the plant have been reported to be antihyperlipidemic, antitumor, antioxidant, anorexiant, analgesic and anti-inflammatory, and antihyperglycemic [3]. Air potato has been used as a folk remedy to treat conjunctivitis, diarrhea, and dysentery, among other ailments [4]. *D. bulbifera* has been traditionally used to lower glycemic index, thus providing a more sustained form of energy and better protection against diabetes and obesity. It also possesses anticancer properties [5]. Isolation and structural elucidation of seven new clerodane diterpenoids, namely, Bafoudiosbulbins A-G from *D. bulbifera* were reported earlier [6,7]. Antimicrobial activity of *D. bulbifera* was reported against mycobacteria and Gram-negative bacteria involving multidrug-resistant (MDR) bacteria [8]. Okwu *et al.* [9] reported greater antibacterial activity of methanol extract of the wild-type *D. bulbifera* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Streptococcus pyogenes.*

In the present investigation, *D. bulbifera* was selected, as one of the medicinally important plants. There are few scientific reports on the phytochemical analysis and antimicrobial properties of this plant. The present study aimed at evaluating the phytochemical screening and *in vitro* antimicrobial activity of various extracts of *D. bulbifera* tuber.

METHODS

Plant material and extracts preparation

The tuber of *D. bulbifera* was oven dried at 50°C, ground and extracted successively with n-hexane, chloroform, methanol, ethanol, and water. The collected plant material was identified and authenticated by Prof. P. D. Sharma, Retd. Botanist, Delhi University, New Delhi, India. The extracts were concentrated at reduced pressure to dryness using a Soxhlet evaporator for 48 hrs [10]. After complete solvent evaporation, extracts were dissolved in 10% DMSO to a final concentration of 20 mg/ml and stored at 4ºC till further analysis.

Microbial cultures and growth conditions

The microbial cultures included MDR isolates of *Enterobacter* sp., *Salmonella paratyphi*, *Salmonella typhi*, *S. aureus, E. coli*, *Klebsiella pneumoniae, P. aeruginosa,* and *Acinetobacter* sp. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and sub-cultured on to nutrient broth for 24 hrs before testing. Three fungal isolates studied include *Candida albicans,*

Aspergillus niger, and *Rhizopus nigricans.* The cultures were maintained on potato dextrose agar at 4°C. These microbial isolates served as test pathogens for antimicrobial activity assay.

Phytochemical analysis

The extracts were subjected to phytochemical screening for the presence of saponins, tannins, steroids, phlobatanins, anthraquinones, cardiac glycosides, alkaloids, reducing sugars, and flavonoids using wet reactions [11,12].

Antimicrobial activity assay

The agar well-diffusion method was employed with slight modifications [13] to determine the antibacterial activities for various solvent and aqueous extracts of *D. bulbifera*. About 25 ml of nutrient agar and potato dextrose agar was poured into each Petri plate. Once the agar solidified, the cultures were inoculated on the surface of the plates (1×10^8 cfu/ml). Subsequently, the surface of the agar was punched with a 6 mm diameter wells. Each well was filled with 50 µl of each plant extract. The concentration of the extracts employed was 20 mg/ml. Control wells containing the same volume of hexane, chloroform, methanol, ethanol, and distilled water and DMSO were made. After 24 hrs incubation at 37°C, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in mm. All tests were performed in triplicate and the antimicrobial activity was expressed as the mean of inhibition.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the various extracts of *D. bulbifera* revealed that flavonoids, steroids, reducing sugars, saponins, cardiac glycosides, and reducing sugars are generally present in almost all the extracts. However, some phytoconstituents were absent in some extracts as reported (Table 1). This variation in the results could be due to the difference in the polarity of solvents used for extraction [9]. Anthraquinones and phlobatanins were absent in all the extracts tested. In a similar study, phenols and saponins were invariably found to be present in all the solvent extracts from tubers of *Dioscorea pentaphylla* [14]. Preliminary phytochemical screening of the ethanolic, methanolic, and aqueous extracts of tuber of *D. bulbifera* revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, tannins, and saponins [15]. Chandra *et al.* [5] reported the presence of alkaloids, steroids, flavonoids, and tannins in the tuber extracts of *D. bulbifera.* The bioactive agents present have health-promoting effects which may be responsible for the antibacterial and antifungal activities.

Antimicrobial activity assay

The antimicrobial activity of *D. bulbifera* tuber extracts against 10 MDR bacteria and three fungi was assessed (Table 2). The results from the agar well-diffusion method revealed that the various solvent and aqueous extracts showed significant to moderate antibacterial activity toward all tested strains except *P. aeruginosa, Salmonella typhi,* and *Acinetobacter* sp. Aqueous and chloroform extracts were found to be more potent being capable of exerting significant inhibitory activities against the majority of the isolates such as *E. coli, Acinetobacter* sp., *S. paratyphi, K. pneumoniae,* and *C. albicans.* The highest inhibitory activity was observed for *K. pneumoniae* with wide inhibition zone diameters (17 ± 0.15 mm), followed by *E. coli* (13 ± 0.11) mm, and *Acinetobacter* sp. (11 ± 0.12). All the extracts showed poor antifungal activity and inhibited the growth of only *C. albicans*. Similar studies are reported by Prakash and Hosetti [14], where all the extracts from *Dioscorea pentaphylla* exhibited predominant antibacterial activity against *S. aureus* (ATCC-20852), *P. aeruginosa,* and *K. pneumoniae,* respectively, and five clinically isolated pathogenic fungi, *Trichophyton rubrum, Microsporum gypseum, Trichophyton tonsurans, Microsporum audouini,* and *C. albicans.* On contrary, Dahiya and Purkaysatha [16] reported potential antibacterial activity of ethanolic and methanolic extracts against MDR bacteria as compared to hexane and chloroform extracts.

Table 1: Phytochemical analysis of various extracts of *D. bulbifera* tubers

Phytoconstituents	*D. bulbifera*				
	H	C	M	E	Aq
Reducing sugar	-	+	+	-	+
Flavonoids	++	++	+	+	+
Steroids	+	++	-	+	++
Tannins	-	-	-	-	-
Phlobatanins	-	-	-	-	-
Saponin	+	+	-	-	+
Cardiac glycosides	++	+	-	+	+
Anthraquinones	-	-	-	-	-

H: Hexane extract, C: Chloroform extract, M: Methanol extract, E: Ethanol extract, Aq: Aqueous extract, +: Present (in lower amount), ++: Present (in higher amount), -: Not present, *D. bulbifera: Dioscorea bulbifera*

Table 2: Antimicrobial activity of *D. bulbifera* by agar well-diffusion assay

Test microorganism	Zone of inhibition (in mm)				
	H	C	M	E	Aq
Acinetobacter sp.	-	-	-	-	11±0.12
Escherichia coli 1	-	9.2±0.22	-	-	13±0.11
Escherichia coli 2	-	-	-	-	-
Enterobacter aerogenes	-	-	-	-	10.3±0.23
Klebsiella pneumoniae	-	10±0.14	-	-	17±0.15
Salmonella typhi	-	-	-	-	-
Salmonella paratyphi	9.6±0.11	-	-	-	9.0±0.22
Staphylococcus aureus 1	-	-	-	-	-
Staphylococcus aureus 2	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-
Candida albicans	-	8.9±0.15	-	-	10.8±0.11
Aspergillus niger	-	-	-	-	8.6±0.12
Rhizopus nigricans	-	-	-	-	-

Zone of inhibition is the mean of three readings, H: Hexane extract, C: Chloroform extract, M: Methanol extract, E: Ethanol extract, Aq: Aqueous extract, -: No inhibition, *D. bulbifera: Dioscorea bulbifera*

Out of three extracts, ethanol extracts possessed better minimum inhibition concentration against all the bacterial strains. Kuete *et al.* [8] reported antimicrobial activities against mycobacteria and Gram-negative bacteria involving MDR bacteria using the methanol extracts and six compounds isolated from the bulbils of *D. bulbifera.* Seetharam *et al.* [17] reported *in vitro* antimicrobial activity of successive extracts of *D. bulbifera* (bulbils) which showed significant activity against *Aspergillus fumigates, R. nigricans, K. pneumoniae,* and *S. aureus.*

CONCLUSION

The present study emphasizes the phytochemical analysis and antimicrobial potential of successive series of *D. bulbifera* against MDR microbial cultures. The extracts of *D. bulbifera* tubers have shown excellent activity against *K. pneumoniae, E. coli, Acinetobacter* sp., and *C. albicans* and are the potential candidates to be developed into the next generation of antimicrobials to combat pathogenic microorganisms. The results are promising and supported the traditional use of *D. bulbifera* for the treatment of bacterial and fungal infections.

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REFERENCES

1. Rice LB. Unmet medical needs in antibacterial therapy. Biochem Pharmacol 2006;71(7):991-5.
2. Sonibare MA, Soladoye MO, Esan OO, Sonibare OO. Phytochemical and antimicrobial studies of four species of Cola Schott & Endl. (Sterculiaceae). Afr J Tradit Complement Altern Med 2009;6(4):518-25.
3. Tapan S, Kausik C. Antioxidant activities of some wild edible plants of Meghalaya state in India and effect on solvent extraction system. Int J Pharm Pharm Sci 2015;7 Suppl 9:446-50.
4. Duke J*A,* DuCellier JL. CRC Handbook of Alternative Cash Crops. Boca Raton, Florida: CRC Press; *1993.*
5. Chandra S, Sarla S, Abhay MP, Annop B. Nutritional profile and phytochemical screening of Garhwal Himalaya medicinal plant *Dioscorea bulbifera*. Int Res J Pharm 2012;3 Suppl 5:289-4.
6. Teponno RB, Tapondjou AL, Jung HJ, Nam JH, Tane P, Park HJ. Three new clerodane diterpenoids from the bubils of *Dioscorea bulbifera* var. *sativa*. Helv Chim Acta 2007*;*90:1599-5.
7. Teponno RB, Tapondjou AL, Abou-Mansour E, Stoeckli-Evans H, Tane P, Barboni L. Bafoudiosbulbins F and G, further clerodane diterpenoids from *Dioscorea bulbifera* L. var *sativa* and revised structure of Bafoudiosbulbin B. Phytochemistry 2008;69:2374-9.
8. Kuete V, Betrandteponno R, Mbaveng AT, Tapondjou LA, Meyer JJ, Barboni L, *et al.* Antibacterial activities of the extracts, fractions and compounds from Dioscorea bulbifera. BMC Complement Altern Med 2012;12:228.
9. Okwu GI, Enweani IB, Amengialue OO, Obiazi HA. Comparative study of the phytochemical and antibacterial properties of two different varieties of *Dioscorea bulbifera* tubers. Am J Res Commun 2016;4 Suppl 7:46-9.
10. Varaprasad BV, Katikala PK, Naidu KC, Penumajji S. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. Indian J Sci Technol 2009;2 Suppl 4:6839-46.
11. Yun CL, Feng-Xiang S, Xia G. Preliminary phytochemical analysis of *Acanthopanan trifoliatus* (L.) Merr. J Med Plants Res 2011;5 Suppl 17:4059-64.
12. Ghamba PE, Agbo EB, Umar AF, Bukbuk DN, Goje, LJ. *In vitro* antibacterial activity of crude ethanol, acetone and aqueous *Garcinia kola* seed extracts on selected clinical isolates. Afr J Biotechnol 2012;11 Suppl 6:1478-83.
13. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial potentials of *Alangium salvifolium* and *Piper longum* against multi-drug resistant bacteria from clinical isolates. Int J Pharm Pharm Sci 2011;3 Suppl 5:462-5.
14. Prakash G, Hosetti BB. Investigation of antimicrobial properties of *Dioscorea pentaphylla* from mid-western ghats, India. Sci World 2010;8 Suppl 8:91-6.
15. Marandi RR, Britto SJ, George M, Minj E. Pharmacognostic, fluorescent, antibacterial andphytochemical analysis of tuber of *Dioscorea bulbifera* L. from Jharkhand. J Pharmacogn Phytochem 2016;5 Suppl 1:08-4.
16. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian J Pharm Sci 2012;74 Suppl 5:443-50.
17. Seetharam YN, Jyothishwaran G, Sujeeth H, Barad A, Sharanabasappa G, Shivkumar D. Antimicrobial activity of *Dioscorea bulbifera* bulbils. Indian J Pharm Sci 2003;65 Suppl 2:195-6.