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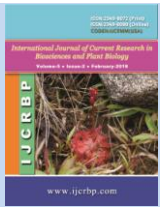

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Therapeutic and Nutritional Delineation of *Dioscorea bulbifera* L.

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ABSTRACT

Dioscorea bulbifera is an important medicinal plant for novel pharmaceuticals since most of the bacterial pathogens are developing resistance against many of the currently available antimicrobial drugs. The antimicrobial activity of all the extracts of *D. bulbifera* against *Staphylococcus aureus, Escherichia coli, Aspergillus, Pseudomons, Candida, Streptococcus pyogenes, Streptobacillus, Klebsiella pneumoniae, Bacillus megaterium, Pseudomonas fluorescens, Bacillus subtilis* and *Xanthomonas sp.;* the agar well diffusion method (E Test) to determine MIC, MBC and Potentiation effect on Gentamicin were investigated. Using Soxhlet extraction, methanolic extracts were prepared from dried bulbs, leaves and stems of *D. bulbifera* which were used to evaluate their Antimicrobial Susceptibility, Synergistic effect, Phytochemical analysis, FTIR, Nutritional analysis and Antioxidant activity. Data interprets that the pattern of inhibition depends upon the plant part used, the solvent chosen, the extraction procedure, temperature, the assaying method and the organisms tested. *S. aureus* was found to be most suscebtible, while *Xathomonas sp.* was resistant to all the organic extracts. The MIC values obtained by agar well diffusion method and broth dilution method had shown little discrepancies. The combinations of extract for synergistic activity against the tested pathogens were found to be additive for bacterial pathogens and antagonistic for fungal pathogens. Combinations of stem and bulb were found to give better synergistic activity. Alkaloids, terpenoids, phenols and tannins were present in all the extracts. Stem extract was found to be better followed by bulbs and leaves. FTIR analysis showed the presence of various functional groups. Stem extract showed the maximum antioxidant activity. Nutritional analysis showed significant levels of nutrients present in bulbs. There are very scanty research papers available on the work done on leaves and stems of *D. bulbifera.*

Introduction

Dioscorea bulbifera (commonly known as the air potato, air yam, bitter yam, cheeky yam, potato yam) is a species of true yam in the yam family Dioscoreaceae (Shajeela et al., 2011). It is native to Africa, Asia, Australia (Schultz, 1993; Duxbury et al., 2003), India, Maldives, China, Japan, Philippines, and Indonesia. It is widely cultivated and has escaped to become naturalized in many regions (Latin America, West Indies, Southeastern United States, and various oceanic islands).

D. bulbifera is a perennial vine with broad leaves and two types of storage organs. The plant forms bulbils in the leaf axils of the twining stems and tubers beneath the ground. These tubers are like small, oblong potatoes. Some varieties are edible and cultivated as a food crop, especially in West Africa. The tubers of edible varieties often have a bitter taste, which can be removed by boiling. They can then be prepared in the same way as other yams, potatoes, and sweet potatoes.

The air potato is one of the most widely consumed yam species. It can grow up to 150 ft tall. Air potato can grow extremely quickly, roughly 8 inch per day, and eventually reach over 60 ft long. It typically climbs to the top of trees and has a tendency to take over native plants. New plants develop from bulbils that form on the plant, and these bulbils serve as a means of dispersal. The aerial stems of air potato die back in winter, but resprouting occurs from bulbils and underground tubers.

D. bulbifera is used in Bangladesh for the treatment of leprosy and tumours (Murray et al., 1984) and by the native people of the Western highlands of Cameroon for the treatment of pig Cysticercosis, though the tubers after collection during farming period are totally destroyed and burned because of their high bitterness (Chandra Subhash et al., 2012). In India the cooked wild tubers are known to be consumed by Palliyar and Kanikkar tribes (Shanthakumari et al., 2008).

Air potato has been used as a folk remedy to treat conjunctivitis, diarrhea, dysentery and among other ailments. Uncultivated forms, such as those found growing wild in Florida, can be poisonous. These varieties contain the steroid diosgenin, which is a principal material used in the manufacture of a number of synthetic steroidal hormones, such as those used in hormonal contraception (Duke et al., 1993). There have been claims that even the wild forms are rendered edible after drying and boiling, leading to confusion over actual toxicity.

Phytochemicals have complex and unique structure, and their production is often enhanced by both biotic and abiotic stresses (Okwu et al., 2016). There are very scanty research papers available on the work done on leaves and stems of *D. bulbifera*.

Materials and methods

Collection of plant material

Disease free fresh plant materials of *Discorea bulbifera* were collected from Anjeneri Dist. Nashik, Maharashtra, India. The plant material was made dust free by washing it in running tap water and then in sterilised water. The bulbs, leaves, stems were shade dried in a dark room until complete dryness was observed. The bulbs, leaves and stems were then homogenised into fine powder and stored in air tight glass container at 4ºC until future use.

Preparation of plant extracts

Twenty five gram of dried powder of *D. bulbifera* bulbs, leaves and stem was taken into a cotton thimbles separately and extracted with 250ml of solvent Methanol using Soxhlet apparatus for 6-8 hrs (Chandra Subhash et al., 2012). The temperature parameter is of utmost importance for the activity of extracts as the higher temperature will adversely affect the activity of extracts. The solvent was then evaporated to dryness using rotatory vacuum evaporator to obtain only the dried extract (Bholay et al., 2015). The final quantity was measured and it was diluted appropriately before further procedures.

Pathogenic organisms

Authenticated pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans*, *Streptobacillus sp.*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Bacillus megaterium* and *Pseudomonas fluorescens* for antimicrobial activity were procured from Dr. Vasantrao Pawar Memorial Medical College Adgaon, Dist. Nashik, Maharashtra, India. *Xanthomonas spp* were procurred from Mahatma Phule Krishi Vidhyapeeth, Rahuri, Ahmednagar, Maharashtra, India.

The procurred pathogens were subcultured on their respective culture media like Nutrient agar, MacConkeys agar, Sabouraud dextrose agar, Cetrimide agar, YDCA agar and stored in refrigerator at 4^{o}C.

Antimicrobial activity of *D. bulbifera*

Agar well diffusion technique described by James et al. (2009) was used for evaluating antimicrobial activity. The suspension of pathogens were prepared using $BaSO_4$ turbidity standard equivalent to 0.5 McFarland which contained 2 x 10^8 CFU/ml were seeded into MH agar plates. Then using a sterile borer wells were digged in Muller Hinton agar plates and respective extracts of bulbs, leaves and stem (140 mg/ml, 210 mg/ml and 140 mg/ml respectively) were inoculated (25µl/well). The solvent was used as a negative control. The whole procedure was performed in duplicate and under sterile conditions in Laminar Air Flow. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 hr. The plates were then incubated at 37/28ºC for bacteria/ fungi respectively for 18-24 hrs after which diameters of zones of inhibition (mm) were measured (Moses et al., 2013).

Minimum Inhibitory Concentration (MIC)

Agar well diffusion method – E-test: The agar well diffusion test for determination of MIC values was performed as given by Bholay et al. (2015).

Broth dilution method: Under sterile conditions the additions were done as per the required dilutions and the tubes were incubated at appropriate temperature for 24 hrs in rotatory shaking incubator at 150 rpm. MIC values for respective extracts against respective pathogens were recorded in terms of turbidity. Turbidity was measured in terms of O.D. by keeping negative control as a blank (Gaur Apurv et al., 2016).

Minimum Bactericidal Concentration (MBC)

From each respective concentrations of MIC tubes loopful of inoculum was taken and spread on Sterile Mueller Hinton agar plates in each respective quadrant. The plates were then incubated at appropriate temperature for 24 hrs in incubator. The tube containing the lowest concentration of extract that fails to yield growth in sub-cultured plates was taken to be MBC of the extract for the test culture (Gaur Apurv et al., 2016).

Synergistic effect of *D. bubifera* extracts

The plant extracts in combination of two were used to evaluate the synergistic effect against the different microbial pathogens. Well diffusion technique by Kirby-Bauer was performed to check synergistic effect of extract on pathogens. The extracts were mixed in equal proportions (1:1) aseptically.

Potentiation effect of *D. bubifera* extracts

The plant extracts in combination with the antibiotic Gentamicin and Fluconazole was used to evaluate the potentiation effect against the different microbial pathogens. The concentration of antibiotic used was 25µg/µl. All the extracts were mixed with antibiotic in equal proportions (1:1) aseptically. The suspension of pathogens were prepared using $BaSO_4$ turbidity standard equivalent to a 0.5 McFarland which contained 2×10^8 CFU/mL. The direct colony suspension method recommended by CLSI is the most convenient

method for inoculum preparation. Well diffusion technique by Kirby-Bauer method was used for evaluating potentiation activity of extracts. Gentamicin and Fluconazole were used as control. The whole procedure was performed under sterile conditions in Laminar Air Flow. The plates were kept for prediffusion at 4°C in refrigerator. The procedure was performed in duplicates. The plates were then incubated and observations were recorded, the inhibition zones were measured using a antibiotic zone scale to the nearest mm.

Phytochemical analysis of *D. bubifera* extracts

D. bubifera extracts were screened for alkaloids, saponines, tannins, phenols, flavonoids, terpenoids and steroids as per standard protocols by Sodium hydroxide test, Mayer's test, Foam test, Salkowiskis test and Ferric chloride test (Kratika Kumari et al., 2013; Chandra Subhash et al., 2012).

FTIR analysis of *D. bubifera* extracts

For FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1filter paper by using high pressure vacuum pump. The samples were diluted to 1:10 with the same solvent. FTIR analysis was performed using Shimadzu IR Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. Each and every analysis was repeated twice for the spectrum confirmation (Antony et al., 2013).

Antioxidant activity of *D. bubifera* extracts

DPPH assay (Goveas and Abraham, 2013)

The free radical scavenging activity of the extracts was measured using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). Briefly, to 1ml of different concentrations of methano0lic stem and leaf extract, 1ml of DPPH 0.1Mm was added. The mixture was mixed and left to stand for 30 min in the dark and the absorbance was recorded at 517nm. An equal amount of DPPH and Methanol served as control. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the following formula,

DPPH Scavenging activity (%) =

[(A control-A sample / A control) ×100]

Nutritional analysis of *D. bubifera* bulbs

Nitrogen content was estimated by the micro-Kjeldhal method and crude proteins were calculated (N×6.25). The contents of crude fiber and ash were estimated by AOAC methods. Nitrogen free extract was obtained by difference method by substracting the sum of the proteins, fats, ash, and fibers from the total dry matter. From the triple acid digested sample, sodium, potassium, calcium and magnesium were analysed using an atomic absorption spectrophotometer. Phosphorous was estimated colorimetrically (Shajeela et al., 2011).

Results and discussion

Antimicrobial activity of *D. bubifera* extracts

The maximum zone of inhibitions of methanolic extract of bulbs extract were observed against *B. megaterium* followed by *E. coli, S. aureus, Strepto. pyogenes, K. pneumoniae, C. albicans, Streptobacillus, P. fluorescence, Xantho. sp. 1, B. subtilis, A. niger, Pseudomonas sp.* and *Xantho. sp.2.*

The maximum zone of inhibitions of methanolic extract of leaves extract were observed against *Staph. aureus* followed by *E. coli, Strepto. pyogenes, K. pneumoniae, B. megaterium, Xantho. sp. 2, A. niger, C. albicans, Streptobacillus, Pseudomonas spp, Xantho. sp. 1, B. subtilis* and *P. fluroscence.* The maximum zone of inhibitions of methanolic extract of stem extract were observed against *A. niger, Staph. aureus* followed by *E. coli, Strepto. pyogens, K. pneumoniae, Pseudomonas sp., C. albicans, Streptobacillus, B. megaterium, Xantho. sp. 2, P. fluroscence, B. subtilis, Xantho. sp. 1.* The results are summarized in Table 1.

MIC of *D. bubifera* extracts

The MIC values of methanolic extract of bulbs for *B. megaterium* and stem for *A. niger* were 17.50 mg/ml by both agar diffusion E-test and broth diffusion method. While MIC value of methanolic extract of leaves for *S. aureus* by agar diffusion E-test was 13.12 mg/ml and by broth dilution method was 26.50 mg/ml as shown in Table 2.

MBC of *D. bulbifera* extracts

The MBC values of methanolic extracts of bulbs against *B. megaterium*, leaves against *S. aureus*, stem against *A. niger* as given in Table 2 were found to be 25.50, 19.12 and 29.50 mg/ml respectively. The results indicate that the leaf extracts of *D. bulbifera* is more potent than bulbs and stem.

Table 1. Antimicrobial susceptibility testing of *D. bulbifera* extracts, diameter in mm.

Pathogens	Methanolic extract of bulbs	Methanolic extract of leaves	Methanolic extract of stems	Gentamicin	Fluconazole
E.coli	18.20 ± 0.5	18.10 ± 0.8	19.20 ± 0.3	26.00 ± 0.5	-
Staph. Aureus	18.15 ± 0.7	20.20 ± 0.8	24.15 ± 1.3	27.00 ± 0.6	-
B. subtilis	13.20 ± 0.7	12.04 ± 0.7	12.16 ± 0.4	27.06 ± 0.4	-
Pseudomonas spp.	13.15 ± 0.8	13.16 ± 0.5	17.20 ± 0.5	21.07 ± 0.9	-
P. fluroscence	14.03 ± 0.7	11.20 ± 0.8	13.10 ± 0.6	17.09 ± 0.7	-
K. pneumoniae	16.20 ± 0.5	15.15 ± 0.6	18.15 ± 0.5	23.02 ± 0.5	-
Strepto. Pyogens	17.10 ± 0.4	17.10 ± 0.5	19.20 ± 0.3	22.03 ± 0.6	-
Streptobacillus	14.15 ± 0.4	13.20 ± 0.9	15.16 ± 0.5	18.03 ± 0.4	-
B. megaterium	23.10 ± 0.5	15.15 ± 0.5	14.20 ± 0.7	27.04 ± 0.5	-
Xantho. spp.1	13.20 ± 0.8	12.10 ± 0.5	12.15 ± 0.8	15.07 ± 0.5	-
Xantho. spp.2	09.40 ± 0.5	15.10 ± 0.4	14.10 ± 0.3	18.09 ± 0.8	-
A. niger	13.16 ± 0.5	14.15 ± 0.6	24.16 ± 0.4	-	27.08 ± 0.5
C. albicans	15.16 ± 0.9	14.10 ± 0.8	17.10 ± 0.6	-	20.04 ± 0.3

Table 2. MIC and MBC values of bulbs, leaves and stem extracts of *D. bubifera*.

Extracts	MIC- Agar diffusion		MIC - Broth dilution		MBC - Agar well diffusion	
	E-test Conc. of extract (mg/ml)	Inhibition zone (mm)	Conc. of extract (mg/ml)	Turbidity	Conc. of extract (mg/ml)	Inhibition zone (mm)
Bulbs (*B. megaterium*)	140.00	24 ± 0.5	140.00	-	35.00	-
	070.00	20 ± 0.4	070.00	-	29.50	-
	035.00	17 ± 0.4	035.00	-	25.50	-
	017.50	13 ± 0.7	017.50	-	21.50	+
	008.75	11 ± 0.3	008.75	+	17.50	+
Leaves (*S. aureus*)	210.00	26± 0.4	210.00	-	35.00	-
	105.00	22 ± 0.2	105.00	-	26.50	-
	052.50	19 ± 0.5	052.50	-	22.12	-
	026.50	17 ± 0.3	026.50	-	19.12	+
	013.12	14 ± 0.5	013.12	+	16.12	+
	006.56	10 ± 0.3	006.56	+	13.12	-
Stems (*A.niger*)	140.00	22 ± 0.4	140.00	-	35.00	-
	070.00	18 ± 0.7	070.00	-	29.50	-
	035.00	15 ± 0.5	035.00	-	25.50	+
	017.50	12 ± 0.3	017.50	-	21.50	+
	008.75	11 ± 0.4	008.75	+	17.00	+

Synergistic effect of *D. bulbifera* extract

As compared to antimicrobial activity of stem extract alone, the combinations of stem extract of *D. bulbifera* have shown considerable additive effect against all the pathogens. Bulb and stem combination of extracts had shown maximum synergistic effect against *A. niger* followed*by B. megaterium, S. aureus, S. pyogenes* and *E. coli* (Table 3).

Table 3. Synergistic effect of *D. bulbifera* extracts on different pathogens.

Combinations → Pathogens ↓	**Bulbs + Stem**	**Bulbs + Leaves**	**Leaves + Stem**
E. coli	22± 0.5	18 ± 0.5	20 ± 0.6
S. aureus	25 ± 0.6	21 ± 0.5	23 ± 0.5
K. pneumoniae	19 ± 0.5	14 ± 0.6	16 ± 0.5
Pseudomonas sp.	19 ± 0.5	15 ± 1.0	17 ± 0.5
S. pyogenes	22 ± 0.6	18 ± 0.6	20 ± 0.6
Streptobacillus	16 ± 1.0	14 ± 0.5	15 ± 0.5
B. megaterium	25 ± 1.0	16 ± 1.0	21 ± 0.6
B. subtilis	15 ± 0.5	13 ± 0.8	14 ± 0.5
A. niger	26 ± 0.8	15 ± 1.0	21 ± 1.0
C. albicans	19 ± 1.0	16 ± 0.5	17 ± 0.6
P. fluroscence	18 ± 0.5	13 ± 1.0	15 ± 1.0
Xantho. sp. 1	18 ± 0.5	13 ± 1.0	15 ± 1.0
Xantho. sp. 2	17 ± 0.5	15 ± 0.5	16 ± 0.8

Potentiation effect of *D. bubifera* extracts

The combination of gentamicin and leaves extracts of *D. bulbifera* showed maximum potentiation activity than any other combinations against both bacteria and fungi (Table 4). Among all the bacteria *S. aureus* and *B. megaterium* were most sensitive while *Xantho. sp.1* was resistant. Among the fungi *A. niger* was most sensitive while *C. albicans* was resistant towards the combination of fluconazole and leaves. These results indicate that other combinations of extracts with gentamicin could not potentiate its effect against the pathogens used.

Table 4. Potentiation effect of *D. bulbifera*extracts on standard antibiotics.

Extract→ Pathogens ↓	**GEN + bulbs**	**GEN + leaves**	**GEN + stem**	**FLC+ bulbs**	**FLC + leaves**	**FLC + stem**
E. coli	20± 0.8	21± 0.8	18± 0.8	-	-	-
S. aureus	17± 0.7	22± 0.8	20± 0.7	-	-	-
B. subtilis	13± 0.4	18± 0.5	11± 0.8	-	-	-
K. pnemoniae	18± 0.5	18± 0.4	13± 0.7	-	-	-
Pseudomonas sp.	14± 0.8	19± 0.7	12± 0.8	-	-	-
Xantho. sp.1	10± 0.7	14± 0.4	11± 0.4	-	-	-
Xantho. sp.2	10± 0.2	17± 0.5	13± 0.8	-	-	-
Strepto. pyogenes	15± 0.5	17± 0.5	14± 0.4	-	-	-
Streptobacillus	17± 0.7	20± 0.8	16± 0.8	-	-	-
B. megaterium	18± 0.8	22± 0.7	16± 0.5	-	-	-
P. fluroscence	15± 0.8	19± 0.5	14± 0.8	-	-	-
A. niger	-	-	-	14± 0.8	22± 0.7	18± 0.8
C. albicans	-	-	-	14± 0.8	20 ±0.7	15± 0.8

(GEN = Gentamicin, FLC = Fluconazole)

Phytochemical analysis of *D. bulbifera* extracts

These results as presented in table- 5 indicated that the phytochemical constituents of extracts varied significantly depending upon the plant parts used i.e. bulbs, leaves and stem. Methanolic extract of bulbs were rich in alkaloids, saponins, steroids, terpenoids, phenols and tannins.

Methanolic extract of leaves and stem showed the presence of flavonoids, alkaloids, steroids, terpenoids, phenols and tannins. These results illucidate that the presence of different bioactive principles in different extracts could be due to the solubility of the phytochemical compounds in different solvents, as a simple rule is followed "like dissolves like".

Table 5. Phytochemical analysis of *D. bulbifera* extracts.

Extracts→ Phytochemicals ↓	**Methanolic extract of bulbs**	**Methanolic extract of leaves**	**Methanolic extract of stems**
Flavanoids	-	+	+
Alkaloids	++	+	+
Saponins	+++	-	-
Steroids	+++	+	+
Terpenoids	++	+	+
Phenols	++	+	+
Tannins	++	+	+

FTIR analysis of *D. bulbifera*extracts

The FTIR spectrum was used to identify the functional group of active components based on the peak value in the region of infrared radiation. The bulbs, leaves and stem extracts of *D. bulbifera* was passed into the FTIR and the functional groups of components were separated based on its peak ratio. The results of *D. bulbifera* bulbs, leaves and stems FTIR analysis, confirmed the presence of alcoholic phenols group in chemical compounds which shows major peak at 3371.57 cm, while the peak at 524.64cm shows minor peak which confirmed the presence of alkyl halide groups in all the three extracts.

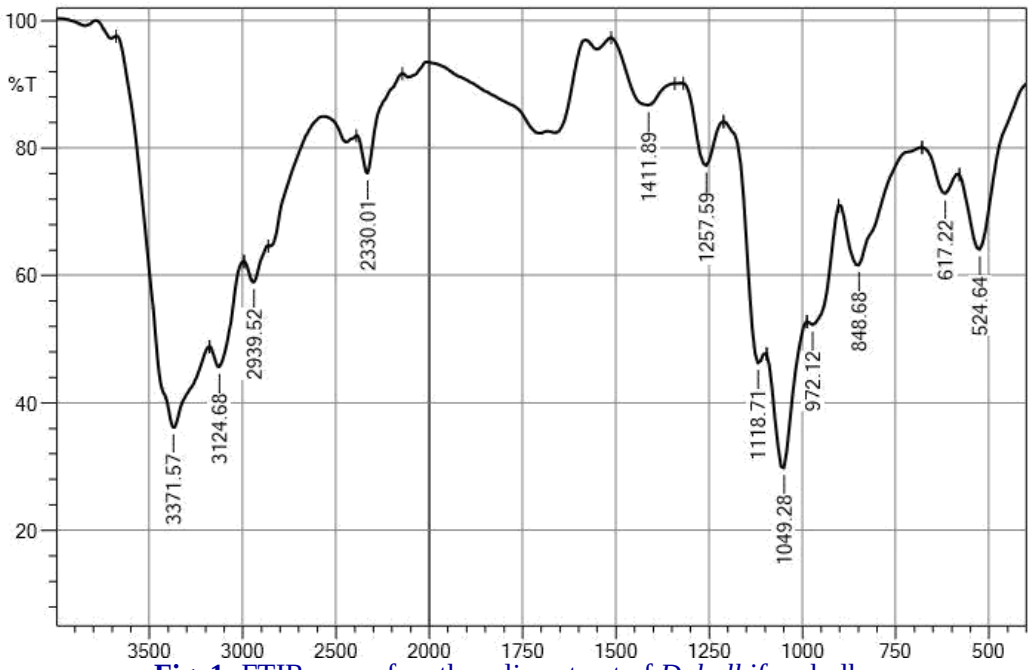

Fig. 1: FTIR scan of methanolic extract of *D. bulbifera* bulbs.

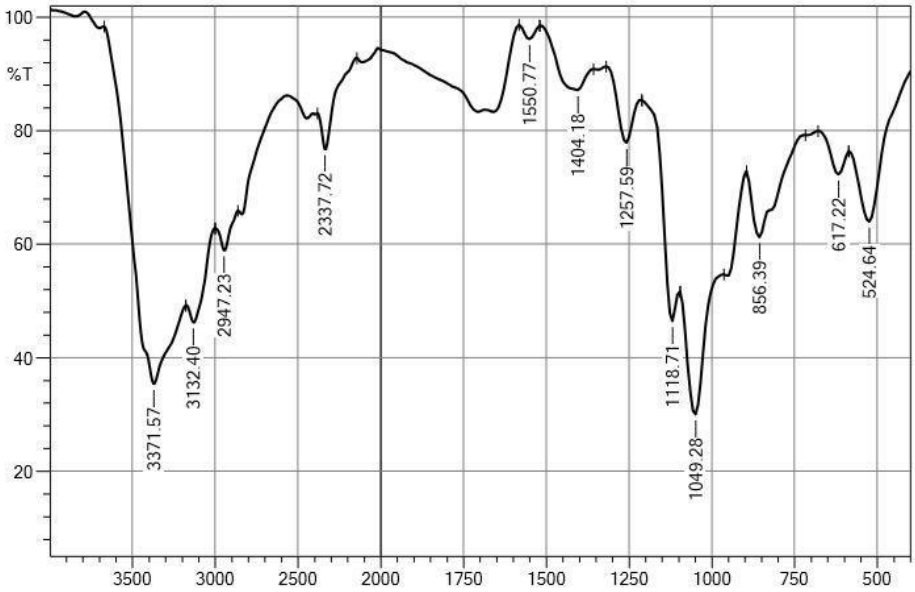

Fig. 2: FTIR scan of methanolic extract of *D. bulbifera* leaves.

The FTIR values of *D. bulbifera* bulbs and leaves were524.64, 617.22, 848.68, 972.12, 1049.28, 1118.71, 1257.59, 1411.89, 2330.01, 2939.52, 3124.68, 3371.57 whose functional groups are Alkyl halides, Alkyl halides, Aromatic, Ethers, Aliphatic amines, Aliphatic amines, Secondary alcohol, Nitrogen, Nitriles, Alkane, Alkene and Alcoholic phenols respectively (Fig. 1 and Fig. 2).

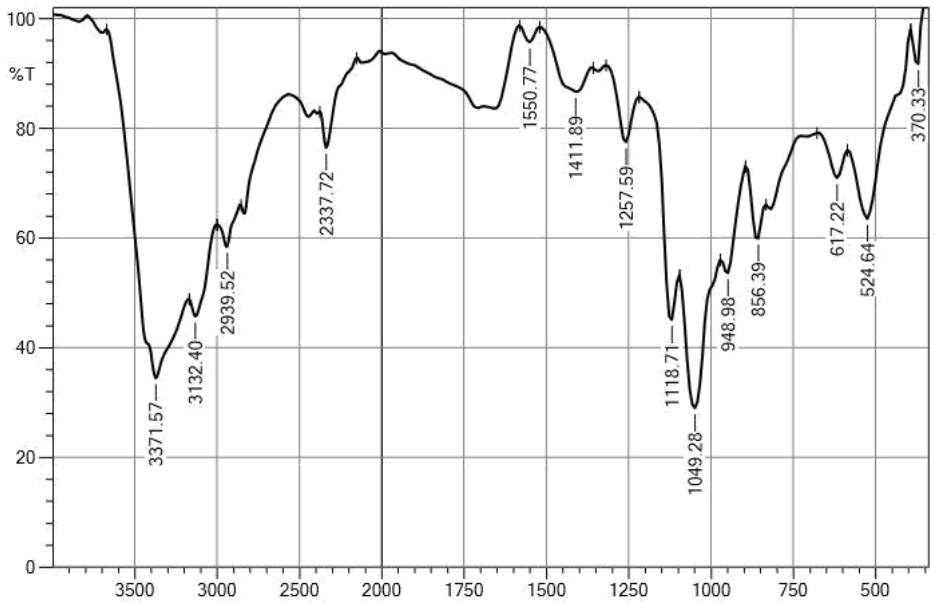

Fig. 3: FTIR scan of methanolic extract of *D. bulbifera* stem.

The FTIR values of *D. bulbifera* stems were 370.33, 524.64, 617.22, 856.39, 948.98, 1049.28, 1118.71, 1257.59, 1411.89, 1550.77, 2337.72, 2939.52, 3132.40, 3371.57 whose functional group were Alkyl halides, Alkyl halides, Alkyl halides, Aromatic, Ethers, Aliphatic amines, Aliphatic amines, Secondary alcohol, Nitrogen, Primary amines, Carboxylic acid, Alkane, Alkene and Alcohol including phenols respectively (Fig. 3).

Anti-oxidant activity of *D. bulbifera* extracts

The free radical scavenging activity of *D. bulbifera* was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of bulbs, leaves, and stem are shown in Table 6 and the DPPH inhibition of ascorbic acid is shown in fig 9. The stem extract showed maximum scavenging activity at a concentration of 200 mg/ml and the lowest was found at the concentration of 50 mg/ml of bulbs extract. The reduction in the number of DPPH molecule can be correlated with the available no of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in the extracts. The wide range of antioxidant activity may be attributed to the wide variety of bioactive compound as compared to the standard ascorbic acid.

Nutritional analysis of *D. bulbifera* bulbs

The level of nutrients in *D. bulbifera* bulbs such as ash, crude fat, crude fibre, total nitrogen, total proteins, carbohydrates, organic matter, insoluble ash and soluble ash were (3.15, 1.29, 8.21, 0.67, 3.63, 26.54, 96.87, 2.28 and 2.03%) and also minerals such as nitrogen, sodium, calcium, magnesium, potassium and phosphorous were (0.49, 0.86, 0.94, 0.92, 0.62 and 0.41 mg/ 100gm) respectively. The results indicate the high nutritional value of bulbs as shown in Table 7.

Table 6. Percent Scavenging activity of *D. bulbifera* extracts.

Extracts	Conc. (mg/ml)	% Scavenging activity
Bulbs	200	36.66
	100	30.00
	50	20.00
Leaves	200	46.66
	100	43.33
	50	33.33
Stems	200	66.66
	100	53.33
	50	46.66

Table 7. Nutritional value of *Dioscorea bulbifera*bulbs.

Nutrients	Values	Nutrients	Values
Ash (%)	3.15±0.61	Carbohydrate (%)	26.54±0.09
Crude fat (%)	1.29±0.14	Organic matter (%)	96.87±0.17
Crude fibre (%)	8.21±0.28	Insoluble ash (%)	2.28±0.13
Total nitrogen (%)	0.67±0.12	Soluble ash (%)	2.03±0.08
Total protein (%)	3.63±0.22	N (mg/100 gm)	0.49±0.65
Na (mg/100 gm)	0.85±0.18	Mg (mg/100 gm)	0.94±0.08
Ca (mg/100 gm)	0.94±0.11	K (mg/100 gm)	0.63±0.14
P (mg/100 gm)	1.41 ± 0.07	-	-

Conclusions

Among all the pathogens tested against methanolic extracts of bulbs, leaves and stem of *D. bulbifera B. megaterium, Staph. Aureus and A. niger* respectively was found to be most sensitive with maximum zones of inhibitions. This result indicates that the antimicrobial activity varied depending upon the type of solvent used. The temperature parameter is of crucial importance when the extracts are being prepared for the antimicrobial activity. So in order to achieve the maximum activity of extracts the temperature should be kept as low as possible. Methanolic extracts exhibited maximum antimicrobial activity as it was more suitable solvent for maximum extraction of bioactive metabolites.

Agar diffusion Method, the E Test was performed successfully and a broad range of MICs was determined for various extracts against the different pathogens. This broad range obtained was used for the determination of MIC by broth dilution method to a narrower range and to check how far the E test and MIC by broth dilution correlate (Rolinson et al., 1972). The MIC values depend upon the

procedure of extraction and may be due to the presence of bioactive principles in the extract. The MIC tubes were used for evaluation of MBC. The MBC value of methanolic extracts of leaves was lower than that of bulbs and stem extracts for *B. megaterium* and *A. niger* respectively.

The combinations of stem extract with every extract had shown highest synergistic effect against *A. niger* followed *by B. megaterium*, *Staph. aureus*, *S. pyogenes and E. coli.* Only leaves combination against *S. aureus*, *B. megaterium* and *A. niger* was able to potentiate the effect of gentamicin and fluconazole. All other combinations in contrast were proved to be showing antagonistic effect.

All the extracts showed the presence of alkaloids, steroids, terpenoids, phenols and tannins which indicates its higher solubility in solvent. The interpretation of results indicated that *D. bulbifera* was highly rich in the diversity of phytochemicals constituents. Plants, including most food and feed plants, produce a broad range of bioactive chemical compounds via their so called secondary metabolism. These compounds may elicit a long range of different effects in man, animals eating the plants dependent on plant species and amount eaten. Plants with potent bioactive compounds are often characterised as both poisonous and medicinal, and a beneficial or an adverse result may depend on the amount eaten and the context of intake. For typical food and feed plants with bioactive compounds with less pronounced effects, the intakes can be regarded as beneficial. Many researchers applied the FTIR spectrum as a tool for distinguishing closely associated plants and other organisms. The results of the present study coincided with the previous observations observed by various plant biologist and taxonomist. The results of the present study developed excellent phytochemical markers to identify the medicinally important plant.

Further advanced spectroscopic studies are required for the structural elucidation and identification of active principles present in the bulbs, leaves and stem of *D. bulbifera.* Highest scavenging activity was observed in stems followed by leaves and bulbs. *D. bulbifera* bulbs contained organic matter, carbohydrates and crude fibers in significant amount. As *D. bulbifera* contains good amount of nutrients and also had shown excellent antimicrobial activity it may be used as combinatorial feed.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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