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Research Article
GCMS Analysis and Hypolipidemic Activity of *Dioscorea bulbifera* (L) in High Fat Diet Induced Hypercholesterolemic Rats

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Abstract

Background and Objective: Hypercholesterolemia is the leading cause of the development of various diseases that made pharmaceutical companies turn towards herbal products with fewer side effects. Hyperlipidemic and reactive oxygen species in the body are important factors for the development of cardiovascular disease such as hypercholesterolemia. The present study investigated the phytochemical analysis using GCMS and hypolipidemic activity of ethanolic extract of *D. bulbifera* tubers. **Materials and Methods:** In the present study, the 70% ethanolic extract of *D. bulbifera* tubes are attempted to evaluate for the cardio-protective activity and hypolipidemic activity in High fat diet-induced hyperlipidaemia along with the phytochemical evaluation of the same extract by the GC-MS analysis. **Results:** The results showed that 50 mg kg^{-1} b.wt., of *D. bulbifera* extract treatment for seven days decreases the level of cholesterol and LDL significantly ($p<0.05$). Likewise, 30 days of treatment with 50 mg kg^{-1} b.wt., of *D. bulbifera* extract exhibits cardioprotective activity in high-fat diet-induced hyperlipidemic rats by increasing HDL cholesterol ($p<0.05$) and decreasing the total cholesterol, LDL and triglycerides ($p<0.05$). GC-MS analysis of methanolic fraction from 70% ethanolic extract of *D. bulbifera* showed the presence of fatty acids in higher concentrations. **Conclusion:** The *D. bulbifera* exhibits hypolipidemic and cardio-protective activity in high-fat diet-induced diseased animals.

Key words: *Dioscorea bulbifera*, GC-MS, high fat diet, lipid profile, phytoconstituents

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hyperlipidemia causes about 17 M deaths worldwide every year[1]. It is also an important factor in the development of cardiovascular disease and atherosclerosis. Atherosclerosis is a chronic inflammatory disease initiated by multiple factors, with the solid contribution of endothelial damage related to lipid peroxidation. This endothelial dysfunction leads to the permeation of Low Density Lipoproteins (LDL) in the intima layer, resulting in cardio artery diseases[2,3]. A plant-based diet rich in leaves, seeds, fruits, tubers, vegetables and legumes and low in saturated fat is an effective prescription for anyone with more severe atherosclerosis. The anti-hyperlipidemic activity of herbs plays an important role in the reduction of cardiovascular diseases, which is the top disease that causes mortality all over the world. Lipid-lowering activities of medicinal plant for phytochemistry research and drug development for such a disease are now focused all over the world. However, there are few herbs available that provide some bioactive effect for persons with the above disease. The anti-hyperlipidaemia property in medicinal plant plays a key role to reduce hypercholesterolemia. Thus, there is an increasing search for lipid-lowering agents from natural origin. The *D. bulbifera* is a glabrous non-spiny climber of 10-20 feet high with bulbils 1-8 cm in size[4]. Traditionally it is used as purgative, de-flatulent, aphrodisiac, rejuvenating, anti-tubercular, general debility, diabetic disorders and skin disorders[5]. Phytochemical screening of *D. bulbifera* shows compounds such as phenolics, tannins, carbohydrates, vitamin C and vitamin D[6]. Phytochemical studies reveal that the tubers of *Dioscorea* species possess high amounts of polyphenolic compounds[7]. The *D. bulbifera* is used as a source of raw material for the synthesis of cortisone and sex hormone[8]. *Dioscorea* species have been reported to have anti-oxidative, anti-fungal, anti-mutagenic, hypoglycaemic and immunomodulatory effects. In this review, an attempt has been made to give an overview of the anti-hyperlipidemic activity in traditional medicinal plants. *Dioscorea bulbifera* L. (Dioscoreaceae) is found commonly in India. The present study was designed to evaluate the 70% ethanolic extract of *D. bulbifera* for the cardio-protective activity and hypolipidemic activity in high-fat diet-induced hyperlipidemia along with the phytochemical evaluation of the same extract by GC-MS analysis. The antihyperlipidemic activity of the traditional medicinal plants in these communities is more helpful for the development of new drug lead molecules used in the protection against hypercholesterolemia.

MATERIALS AND METHODS

Study area: The tubers of *Dioscorea bulbifera* was collected in January, 2019 from the Thovalai, Tamil Nadu, India. The tubers material was dried (45-50°C), crushed and macerated in ethanol: water (80:20 v/v) at room temperature for seven days. After this period, the extract was filtered, concentrated in a rotary vacuum evaporator and lyophilized. The lyophilized extract was stored at 4°C.

Collection and identification of plant: Tubers of *D. bulbifera* were collected from the Thovalai, Trichy district. The plant material was identified and authenticated at the Department of Pharmacognosy, Centre for Advanced Research in Indian System of Medicine, (CARISM), SASTRA University, Thanjavur, Tamil nadu, India. The herbarium and voucher sample was prepared and deposited in the Department of Pharmacognosy and Phytopharmacy, Sastra University (Voucher No. DB-0062) Thanjavur.

Chemicals and reagents: High-fat diet (western food) was obtained from Sigma-Aldrich, St. Louis, USA. Other chemicals used were of analytical grade.

Preparation of extract: The collected plant material was dried under shade for 15 days. The raw material was pulverized and used for the extraction. Extraction was carried out using 70% ethanol by cold percolation method. The extract was concentrated using a rotary vacuum evaporator at 40°C. The concentrated extract was preserved in the refrigerator. The yield of extract was calculated as 2.73%.

GC-MS analysis: The methanolic fraction separated from 70% ethanolic extract was injected for GC-MS analysis. The extract of *D. bulbifera* (10 mg) was dissolved in methanol. The sample was analysed by GC-MS on 500 Perkin Elmer using the following experimental conditions: Column type-elite-5 (5% diphenyl 95% dimethyl polysiloxane), column dimension 30 m×0.32 mm), carrier gas-helium 1 mL min^{-1}, column temperature from 50°C up to 285°C at the rate of 10°C min^{-1} and 5 min hold, at 285°C, injector and detector temperature 290°C, injection mode split, volume injected: 0.5 µL of a solution prepared from 2 mg/100 mL in methanol. The total run time was 30 min. The mass spectrum was taken using Mass detector-turbo mass gold- Perkin Elmer. Transfer line temperature 230°C, source temperature 230°C, scan range is from 40-450 amu, ionization technique-Electron ionization

technique. The component identification was confirmed by comparing the mass spectra of compounds with available NIST and Wiley mass spectral libraries. The quantitative composition was obtained by peak area normalization[8].

Experimental animals: Male Wistar albino rats weighing 140-160 g were allowed to have a standard pelleted diet M/s Hindustan Lever Foods, Bangalore, India and water *ad libitum*. They were housed under standard environmental conditions. All the animal experiments were performed after getting clearance from Animal ethical clearance (Clearance No. 7/SASTRA/IAEC/RPP).

Acute toxicity: For evaluation of acute toxicity, the Albino Wistar rats strain was taken. All the animals were kept in an identical situation and fed a laboratory diet as per the prescribed dose. All the animals were adults with an average age of 6 months. To study the safety of the *D. bulbifera* extract, the acute toxicity studies were initially carried out using 70% ethanolic extracts obtained after successive extraction with solvents of increasing polarity. The animals were divided into 4 groups of each containing 10 animals. The methanolic extract of *D. bulbifera* as a suspension in 5% Tween 80 were prepared and administered orally at a single dose ranging from 400-1000 mg kg^{-1} b.wt., of the rats.

Hypolipidemic activity: Animals were divided into 4 groups of 10 rats each. Group 1 animals were considered normal and were fed with a standard diet. Group 2 animals were treated High Fat Diet (HFD), group 3 animals treated high fat-diet along 70% ethanolic extract of *D. bulbifera* at 50 mg kg^{-1} and group 4 animals treated with a high-fat diet along with standard drug d-fenfluramine at 10 mg kg^{-1} b.wt., for 30 days. After 30 days all animals were fasting continued up to 24 hrs. The blood was collected from the 7th day and 30th day by retro-orbital puncture under volatile (ether) anaesthesia. Plasma total cholesterol[9,10] triglycerides[10,11] and LDL[12,13] was analyzed.

Statistical analysis: The values have been expressed as Mean±SD. Analyses were done using students t-test by SPSS software.

RESULTS AND DISCUSSION

GC-MS analysis of *D. bulbifera* extract: GC-MS analysis showed the presence of maltol, 9,12 octadecadienoic acid methyl ester and oleic acid methyl ester as the major compound. Followed by this hexadecanoic acid, methyl ester and isomaltol (derivatives of flavone and isoflavone) as the major compounds. Other compounds are mentioned in Table 1 and the Gas chromatogram of the same are mentioned in Fig. 1. GC-MS analysis of methanol soluble compounds of *D. bulbifera* 70% ethanolic extract has indicated the presence of fatty acids like octadecadienoic acid methyl ester and hexadecadienoic acid ethyl ester which are not reported earlier. The hexadecadienoic acid-diethyl dimethyl ester with a peak area of 27.97% was found to be present in a higher concentration. The cardioprotective activity of this compound has not been reported earlier. The

Table 1: GC-MS details of *D. bulbifera* extract

Peak name	Retention time	Peak area (%)
Glycerin ($C_3H_8O_3$; MW: 92)	3.36	1.2958
2-Furanmethanol ($C_5H_6O_2$; MW: 98)	5.22	5.4114
1,2-Cyclopentanedione ($C_5H_6O_2$; MW: 98)	6.57	1.3641
Butanedioic acid, 2-hydroxy-2-methyl-, dimethyl ester ($C_7H_{12}O_5$; MW: 176)	7.35	1.0719
Isomaltol ($C_6H_6O_3$; MW: 126)	7.60	2.2491
5-cis-Methyl-1R,3-cis-cyclohexanediol ($C_7H_{14}O_2$; MW: 130)	8.53	0.7872
3-Acetylthymine ($C_7H_8N_2O_3$; MW: 168)	9.59	1.7138
1-Butanol, 3-methyl-, formate ($C_6H_{12}O_2$; MW: 116)	10.00	10.8687
Maltol ($C_6H_6O_3$; MW: 126)	10.30	14.1262
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- ($C_6H_8O_4$; MW: 144)	10.92	1.9263
2(3H)-Furanone, dihydro-4-hydroxy- ($C_4H^6O_3$; MW: 102)	11.10	2.6141
Heptanediamide, N,N'-di-benzoyloxy- ($C_{21}H_{22}N_2O_6$; MW: 398)	11.21	0.9567
6-Acetyl-α-d-mannose ($C_8H_{14}O_7$; MW: 222)	12.40	0.4625
3,5-Heptadienal, 2-ethylidene-6-methyl- ($C_{10}H_{14}O$; MW: 150)	13.98	0.4873
Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$; MW: 270)	22.94	10.8359
9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$; MW: 294)	25.04	12.7210
Oleic acid, methyl ester ($C_{19}H_{36}O_2$; MW: 296)	25.11	13.2264
Palmitic acid α-monoglyceride ($C_{19}H_{38}O_4$; MW: 330)	26.80	2.8107
2,4-Hexadienedioic acid, 3,4-diethyl-, dimethyl ester ($C_{12}H_{18}O_4$; MW: 226)	27.97	10.0874
1-[2-(5-Hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl] ethanone ($C_{14}H_{24}O_2$; MW: 224)	28.06	4.9834

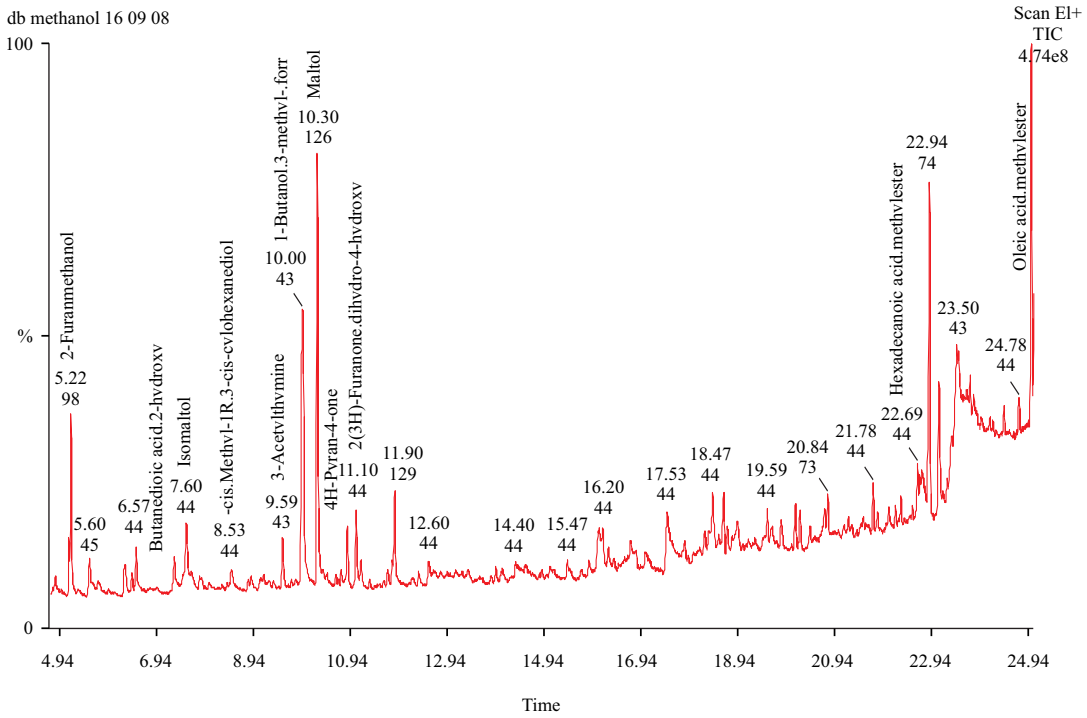

Fig. 1: GC-MS chromatogram of *D. bulbifera* extract

Table 2: Acute toxicity study of *D. bulbifera*

Dose level (mg kg^{-1})	Sample size	Mortality observed	
		After 24 hrs	After 72 hrs
400	10	0/10	0/10
600	10	0/10	0/10
800	10	0/10	0/10
1000	10	0/10	0/10

observed cardioprotective activity in the present study might be due to the presence of anyone compound mentioned in Table 1 or the action might be due to the synergistic action of all the mentioned (Table 1) compounds[14,15].

In the present experimental study, *D. bulbifera* could not produce any lethality at the oral maximum dose of 1000 mg kg^{-1} b.wt./day. All the rats were healthy and active at the time of completion of the experiment reported in Table 2.

Hypolipidemic activity of *D. bulbifera*: Rats treated with High Fat Diet (HFD) increased cholesterol, LDL-c, triglycerides level and decreased HDL-c levels. When the 70% ethanolic extract of *D. bulbifera* was given orally continuously for 1 month the rising trend of cholesterol, LDL-c and triglycerides were significantly less than the normal control group. The LDL-c showed a significant decrease under the influence of *D. bulbifera*, when d-fenfluramine along the cholesterol diet was administered. The HFD was observed to increase the level of lipid profile like total cholesterol, LDL and TGL in plasma ($p<0.05$), Table 3 results revealed that the *D. bulbifera* extract-treated group of animals showed a significant reduction ($p<0.001$) in cholesterol level. On treating animals with *D. bulbifera* extract at the dose of 50 mg kg^{-1} b.wt. Table 4 results showed that the HDL level (*p 0.05) increases in the treatment group with *D. bulbifera* tubers extract. Likewise, the HDL level was seen to be decreased in diseased animals and increased in treatment ($p<0.05$, Table 4). Similarly, in Table 4 and 5 results displaced the significant reduction in LDL level and Triglycerides. On treating animals with *D. bulbifera* extract at the dose of 50 mg kg^{-1} b.wt., bad cholesterol such as LDL and triglycerides were found to be decreased significantly ($p<0.05$) in Table 5 and 6. Moreover, Dwivedi[13] has explained that the high-fat diet causes an increase in the level of myocardial lipids. It increases the LDL cholesterol level in the blood, which in turn, leads to the build-up of harmful deposits in the arteries, thus, favouring coronary heart disease. Moreover, HFD promotes lipolysis in the myocardium[14]. Lipolysis results in the release of a large amount of free fatty acids and triacylglycerol. The decrement of TGL observed in the present study reveals that *D. bulbifera* extracts exhibit hypolipidemic activity.

The hypolipidemic activity of *D. bulbifera* tubers extract can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and

Table 3: Role of 70% ethanolic extract of *D. bulbifera* on total cholesterol level among the experimental animals

	Total cholesterol level (mg dL^{-1})		
Groups	Initial	After 7 days	After 1 month
Normal control	64.32±7.89	63.80±6.52	64.70±8.42
High fat diet (HFD)	61.64±7.32	895.42±49.75**	480.82±40.72*
HFD+*D. bulbifera*	62.32±4.98	840.44±90.85*	328.50±38.20**
HFD+d-fenfluramine	66.40±5.85	860.52±78.85*	280.50±16.80*

Data were expressed as Mean±SD, n = 10, **p<0.001, *p<0.05

Table 4: Role of 70 % ethanolic extract of *D .bulbifera* on HDL-c level among experimental animals

	HDL-c level (mg dL^{-1})		
Groups	Initial	After 7 days	After 1 month
Normal control	18.50±4.33	19.32±4.85	18.37±5.85
High-fat diet (HFD)	19.32±3.85	21.82±5.32*	21.85±4.85*
HFD+*D. bulbifera*	17.20±2.88	18.60±3.85*	26.20±6.85*
HFD+d-fenfluramine	16.80±3.75	17.32±4.85*	18.55±5.85**

Data were expressed as Mean±SD, n = 10, **p<0.001, *p<0.05, HDL: High-Density Lipoprotein

Table 5: Role of 70% ethanolic extract of *D. bulbifera* on LDL-c level among experimental animals

	LDL-c level (mg dL^{-1})		
Groups	Initial	After 7 days	After 1 month
Normal control	21.85±4.78	22.75±5.72	23.22±6.85
High fat diet (HFD)	22.75±5.32	498.50±80.32**	260.40±65.32**
HFD+*D. bulbifera*	24.85±4.85	510.50±78.53*	88.55±12.10**
HFD+d-fenfluramine	23.42±4.85	330.80±81.32*	94.85±16.85**

Data were expressed as Mean±SD, n = 10, **p<0.001, *p<0.05, LDL: Low-Density Lipoprotein

Table 6: Role of 70% ethanolic extract of *D. bulbifera* on triglycerides level among experimental animals

	Triglycerides level (mg dL^{-1})		
Groups	Initial	After 7 days	After 1 month
Normal control	24.85±6.70	30.32±7.85	31.40±6.52
High fat diet (HFD)	28.45±8.12	340.70±64.80**	180.50±12.32**
HFD+*D. bulbifera*	26.32±4.80	160.55±69.85**	128.50±10.85**
HFD+d-fenfluramine	29.70±4.85	97.50±6.80**	70.85±9.30*

Data were expressed as Mean±SD, n = 10, **p<0.001, *p<0.05

absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism[16-18], as observed in hyperlipidemic rats treated with *D. bulbifera*, which showed a significant reduction in the levels of total cholesterol and triglycerides and increases the HDL level. Other molecules able to decrease the serum level of cholesterol are flavonoids and phenolic acid[19], also present in *D. bulbifera*. Interestingly, *D. bulbifera* was able to decrease both serum level of cholesterol (**p>0.001, Table 3) and total triglycerides (**p>0.001, Table 6). Some mechanisms of high-fat diet-induced hypertriglyceridemia are insulin resistance in rats[20]. Although cholesterol in the diet alters the activity of several enzymes and regulates hepatic carbohydrate metabolism, leading to hepatic insulin resistance[21] and hypertriglyceridemia, the mechanisms by which an excess of carbohydrates produces these effects are unknown[21].

Feeding rats with a high dosage of fatty materials (>60% of total calories in diet) affects lipid metabolism and causes hyperlipidemia[22]. Insulin resistance, hyperinsulinemia and mild hypertension, which are features associated with obesity-related hypertension. Carbohydrates' feeding evokes significant alterations particularly in liver TGL[23] metabolism and is reported to be atherogenic due to the induction of lipogenic enzymes in the liver. In the current study, the administration of the high-fat diet for 30 days has significantly increased the glucose, insulin and triglyceride levels like an earlier study[24]. Administering *D. bulbifera* (50 mg kg^{-1}) has prevented the development of hyperlipidaemia. Lipid changes observed in high-fat-diet treated rats are noted to have elevated levels of cholesterol, TGL, LDL and HDL-C. Accumulation of cholesterol, TGL and LDL is observed in tissues. These findings are consistent with the results of another study[25]. The increased conversion of carbon from a

high carbohydrate diet into glycerol-3-phosphate might be responsible for the elevated level of TGL in high cholesterol diet-administered animals[26].

Feeding a high-fat diet to hyperlipidemic rats produces an increase in activity of HMG-CoA reductase and the addition of fructose to cultured rat hepatocytes increases HMG-CoA reductase by approximately 3-fold[27]. The *D. bulbifera* extract might be an inhibitor of HMG CoA reductase, which is responsible for the observed decrease in the level of lipid profile. The ascorbic acid concentration of *D. bulbifera* is reported in the previous study[28]. Vitamin C can inhibit the formation of ox-LDL in the *in vitro* condition. Even if ascorbic acid is water-soluble and is not incorporated in LDL particles, it has been proposed that this Vitamin may prevent LDL particle oxidation by scavenging free radicals and other reactive species in an aqueous milieu[29].

The concentration of flavonoids and polyphenolic compounds of *D. bulbifera* according to Arai *et al.*[30] has noted that the intake of flavonoids inversely correlates with the plasma total cholesterol and LDL cholesterol concentrations. Polyphenols reduce the susceptibility of LDL to oxidation in *in vitro*[31].

CONCLUSION

This study showed that *D. bulbifera* extract is a rich source of phytoconstituents such as flavonoids, tannins, alkaloids, terpenoids and vitamin C. Among the different extracts of *D. bulbifera*, 70% ethanolic extract is a loaded source of secondary metabolites. The pharmacological evaluation of *D. bulbifera* has proved that 70% ethanolic extract of *D. bulbifera* exhibits hypolipidemic activity by decreasing the level of total cholesterol, LDL, TGL and increases the level of HDL. In pharmaceutical companies, 70% ethanolic extract of *D. bulbifera* can be used as a potent hypolipidemic and antioxidant activity.

SIGNIFICANCE STATEMENTS

The hypolipidemic activity of natural *D. bulbifera* can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism as observed in hyperlipidemic rats. The results presented that *D. bulbifera* tubers reduce oxidative stress by free radical scavenging and protect against lipid peroxidation and are also able to manage hyperlipidemia by decreasing serum level of cholesterol and triglycerides and increased serum level of HDL, similarly to conventional drugs.

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