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Pharmacological Evaluation and Phytochemical Profiling of Dioscorea Bulbifera L. Tuber

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KEYWORDS

Dioscorea bulbifera L. tuber; antioxidant; antidiabetic; anti-inflammatory; GC-MS; HPTLC.

ABSTRACT:

Introduction: *Dioscorea bulbifera* L. (*D. bulbifera*) (Family: Dioscoreaceae), locally known as 'Baonla,' is naturally grown in the forest-encircled area of Nayagram, Jhargram, India. There is no previously published report on the phytochemical and pharmacological potential of *D. bulbifera* tuber.

Objective: The present experimental study was performed to evaluate the phytochemical and pharmacological investigation of the *D. bulbifera* tuber.

Methods: Gas-chromatography-Mass-spectroscopy (GC-MS) and high-performance thin-layer chromatography (HPTLC) were used for phytochemical screening, The antioxidant potential of the tuber-extracts was examined by using assays such as DPPH, metal chelating, and reducing power assay. Furthermore, the anti-diabetic efficacy was tested by targeting the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase. The protein denaturation assay was utilized to assess the plant's anti-inflammatory properties.

Results: *D. bulbifera* tuber methanolic extract showed a significantly higher total phenolic content of 105.58±123 mg GAE/g, and the aqueous extract was found to have appreciably higher total flavonoid content of 306.85±20.10 mg QRE/g. Among all the tuber-extracts, the methanolic extract has shown good antioxidant activity by different assays such as DPPH ((IC_{50}: 36.97±1.74 µg/ml), metal chelating (IC_{50}: 97.32±7.9 µg/ml), and reducing power assay, maximum antidiabetic (α-amylase: IC_{50}: 81.75±4.5 µg/mL and α-glucosidase: IC_{50}: 168.17±7.27 µg/mL), and anti-inflammatory (IC_{50}:149.33±4.94 µg/mL) potential as compared to their respective standard. Numerous biologically active substances were found by GC-MS analysis; among the most abundant ones were 2-hydroxy-gamma-butyrolactone (19.9%) and 13(Z)-Docosenoic acid methyl ester (20.22%) in the methanolic extract of *D. bulbifera* tuber. HPTLC analysis identified and quantified four phenolic acids (gallic acid, caffeic acid, 4-hydroxybenzoic acid, and t-cinnamic acid) and two flavonoids (quercetin and myricetin) with the validated method.

Conclusion: *D. bulbifera* tuber has the potential to be used therapeutically to treat oxidative stress, diabetes, and inflammatory diseases.

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1. Introduction

Nature provides various types of herbs to treat different diseases for humanity. Due to their low toxicity and cost-effectiveness, traditional medicinal plants have been used in many countries to treat various diseases since ancient times. For the phytoconstituents, medicinal plants became more interested in developing herbal drugs. According to the World Health Organization (WHO), almost 80% of the world's population chooses or depends on medicinal plants for their primary healthcare needs [1]. The medicinal plant consists of different phytoconstituents (phenols, flavonoids, terpenoids, etc.) with various medicinal values such as antioxidant, anticancer, antimicrobial, and anti-inflammatory activities. It also has potential ant-diabetic activity due to the presence of secondary metabolites like kaempferol, apigenin, myricetin, quercetin, etc., which have an effect against different types of enzymes that are responsible for the disbalance of glucose levels in the human body [2].

Oxidative stress can be defined as a disproportion between the generation of reactive oxygen species (ROS) and antioxidant defense, which may lead to unembellished damages at cellular levels. ROS can be produced in the body as a by-product of various biochemical progressions or due to the revelation of electromagnetic radiation [3]. Various studies suggested that polyphenols are supposed to have oxidation-scavenging properties due to numerous hydroxyl groups prevailing in their structure. The probable mechanism of their antioxidant property is repression of ROS generation either by inhibiting the enzyme constructing ROS or by enlightening the protection aptitude of antioxidant defenses [4].

Inflammation is the complex response of the tissue that involves leukocyte cells such as macrophages, neutrophils, and lymphocytes; these cells release few mediators, signaling molecules, and specialized substances such as vasoactive amines and peptides, eicosanoids, proinflammatory cytokines, and acute phase proteins, which mediate the inflammatory process [5]. Chronic disease pathogenesis and progression largely depend on this fundamental immune response. Cellular oxidative stress also causes discomfort, edema, redness, and heat, increasing the generation of free radicals, which in turn causes chronic inflammation [6].

The most prevalent endocrine illness, diabetes mellitus (DM), is often known as "sugar" and typically develops when there is an inadequate or absent supply of the hormone insulin or, less frequently, when the activity of the hormone is impaired (insulin resistance). A chronic condition of the metabolism of proteins, lipids, and carbohydrates is known as diabetes mellitus. One of the main characteristics of diabetes mellitus is a reduced or defective insulin secretory response, which leads to a decreased ability to use glucose or carbs and, therefore, to hyperglycemia [7]. The International Diabetes Federation (IDF) estimates that 40.9 million people in India have diabetes today, and by 2025, that number is expected to rise to 69.9 million [8]. The World Health Organization has advocated using medicinal plants to treat DM and has called for more significant scientific efforts to assess the hypoglycemic qualities of various plant species.

2. Objectives

Dioscorea bulbifera L. (Family: Dioscoreaceae), locally known as ‘Baonla,’ is naturally grown in the forest-encircled area of Nayagram, Jhargram, India. The rhizomatous tuber of *D. bulbifera* is used as food by the local tribal peoples [9, 10]. The tuber of *D. bulbifera*, considered food by the forest border people in this lateritic region of the Jhargram district in West Bengal, has been cut into pieces and then boiled. Phytochemical analysis of *D. bulbifera* produced results suggesting the presence of several different substances, such as proteins, lipids, sterols, alkaloids, polyphenols and tannins, flavonoids, and saponins. Depending on from which region they originated, these molecules may be different [11]. The latest findings have identified seven clerodane diterpenoids, known as Bafoudiosbulbins (A to G), sixteen Diosbulbins (A-P), and nearly 150 different types of medicinal extracts from *D. bulbifera's* bulbs, tubers, leaves, and rhizomes [12, 13]. *Dioscorea bulbifera* contains a substantial amount of the steroid diosgenin. Various bio-functional compounds found in this plant are used to treat several kinds of physiological and microbiological ailments [14]. This plant is traditionally used against various diseases like oxidative stress, inflammation, diabetes mellitus, and other gastrointestinal complications [15-17]. *D. bulbifera* possesses medicinal properties that include antibacterial, antioxidant, anti-inflammatory, anthelmintic, antihyperglycemic, antineoplastic, and beneficial effects

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on skin conditions and metabolic problems [14, 17, 18]. Hence, the present study aims to explore the antioxidant, anti-diabetic, and anti-inflammatory properties of different extracts of *D. bulbifera* L. tuber. This study also explores phytochemical profiles through GC-MS and HPTLC analysis. So, the evaluation of this plant with such adeptness is influential for the development of new drug discovery for the treatment of inflammation and diabetes.

3. Materials and Methods

3.1 Plant collection and extraction

D. bulbifera L. was procured from Jhargram (22.45° N, 86.98° E), West Bengal, India, and underwent authentication at the Botanical Survey of India in Shibpur, Howrah, and specimen number (CNH/Tech.II/2021/49) was assigned. A 100gm of plant powder was used to make a thimble, and successive extraction was carried out in the Soxhlet apparatus using solvent n-hexane, chloroform, ethyl acetate, methanol, and water. A rotary vacuum evaporator was used to concentrate the extract, and it was stored at 4 °C for future analysis. High-performance liquid chromatography (HPLC) grade methanol was used as the stock solution to create standard solutions (1 mg/mL). 10–12 mg of dried extract, diluted in 1 mL of HPLC-grade methanol, was then centrifuged to obtain the supernatant for the HPTLC analysis. Everything from the standards to the samples was carefully maintained at 4 °C to avoid deterioration.

3.2 Total phenolic content (TFC)

The TPC of *D. bulbifera* tuber different extracts was performed by using the Folin-Ciocalteu method [19]. 0.4 ml samples of gallic acid were mixed with Folin-Ciocalteu reagent (10% w/v, 0.4 ml), followed by 4 ml 7% Na_2CO_3. The final mixture was incubated in a dark place at room temperature for 90 minutes. Finally, the absorbance was measured at 730 nm with the help of a UV-VIS spectrophotometer (UV-1780, Shimadzu). The final result was expressed as mg Gallic Acid Equivalent (GAE)/gm of dry extract.

3.3 Total flavonoid content (TFC)

The previous method was used to estimate the TFC of the different extracts[20]. 0.2 ml of a 10% (w/v) methanolic solution of aluminium chloride ($AlCl_3$), 0.2 ml of a 1M potassium acetate solution, and 5.6 ml of distilled water were added to 1 ml of standard (quercetin) or sample to volume makeup. The mixture was then allowed to stand at room temperature in a dark place for 30 minutes. Total flavonoid content was express as quercetin equivalent per gram (QRE/g) of dry weight.

3.4 Antioxidant activity

3.4.1 Free radical scavenging activity (DPPH)

To evaluate antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed [21]. The stock solution was prepared in 1mg/ml methanol for extraction and standardization. Diluted solution (10-100 µg/ml) was added to 1 ml DPPH (0.3mM) solution. After 30 minutes of incubation at a dark place, the absorbance was measured at 517 nm against the blank solution. The DPPH scavenging activity was calculated as a percentage (%) using the following equation.

Percentage (%) scavenging = $[(A_0-A_1)/A_0] \times 100$

Where A0 = absorbance of control and A_1 = absorbance of the test sample.

3.4.2 Metal chelation assay

This method primarily assesses the impact of ferrous ion chelation on various extracts. 0.1 ml of extract was mixed with 0.1 ml of ferrous chloride (5 mM). Then, the mixture was mixed with a 5 mM ferrozine solution to start the reaction. Then the mixture was allowed to sit at room temperature for 10 minutes, and absorbance was measured at 562 nm [20].

Metal chelating activity = $[(A_0 - A_1)/A_0] \times 100$

where A_0 = absorbance of the control and A_1 = absorbance of the test sample.

3.4.3 Ferric-reducing power assay

The previous method [22] was used to measure the reducing power of the different extracts of *D. bulbifera*. The primary purpose of this assay is to determine how efficiently Fe (III) can be reduced to Fe (II). Ferric-reducing power assay is performed by mixing 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide with varying concentrations of sample or standard (50–200 µg/ml). After incubating for 25–30 minutes at 50° C in a water bath, 2.5 ml 10%

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trichloroacetic acid (TCA) was added, and then the mixture was centrifuged for 10 min at 1000 g. Collect the supernatant and mix 2.5 ml of distilled water with 1% ferric chloride (0.5 ml). The final solution was appropriately mixed with the help of vortex, and absorbance was measured at 700nm, where the rise in absorbance suggested a greater sample reduction capacity.

3.5 In vitro antidiabetic activity

3.5.1 α-Amylase Inhibition Assay

D. bulbifera extract was evaluated for alpha-amylase assay to determine the antidiabetic activity followed by a previously established method[23]. Diluted extract solution (100-1000 µg/ml) was prepared in phosphate buffer solution (pH 6.9), from which 500 µl of each extract was withdrawn and alpha-amylase solution (13U/ml), incubated the mixture for 20 min at 37° C. Again, in the above mixture, 500 µl starch solution (1%) was added and incubated for 20 min at 37° C. The following reaction was discontinued by adding 1ml, 3,5-Dinitrosalicylic acid (DNSA). With the help of a UV-VIS spectrophotometer, the optical density of the above mixture was measured at 500 nm. Acarbose, an alpha-amylase inhibitor, was used in this experiment as a positive control. Three separate runs of this experiment were conducted to ensure reproducible results. The percentage inhibition activity was calculated by using the formula:

$$\% \text{ inhibitory activity} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 = absorbance of the control (100% enzyme activity) and A_1 = absorbance of the sample. Then, the half-maximal inhibitory concentration (IC_{50}) was calculated to measure the effectiveness of a compound.

3.5.2 α-Glucosidase Inhibition Assay

α-Glucosidase is one of the important assays for determining anti-diabetic activity, which is performed following the previously established method [2]. At first, the stock solution for extract or standard (acarbose) in sodium phosphate buffer (pH 6.8), then dilution (100-800 µg/ml) was done. From each dilution, withdraw 100 µl of solution and add 400 µl of alpha-glucosidase (1 U/ml) solution, which is kept for incubation at 37 °C for 20 min. In the above mixture, 70 µl, 5 mM para-nitrophenyl-beta glucopyranoside (PNPG) was added, made with buffer solution, and incubated at 37 °C for 60 min. 2.5 ml of Na_2CO_3(0.1 M) solution was added to discontinue the reaction. Thus, absorbance was measured at 400 nm using phosphate buffer as a blank and 100% enzyme activity solution as a control. The following equation calculates the percentage of inhibition for alpha-glucosidase activity.

$$\% \text{ inhibitory activity} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 = absorbance of the control (100% enzyme activity) and A_1 = absorbance of the sample. The half maximal inhibitory concentration (IC50), which measures how effectively a substance inhibits biological or biochemical function, was used to express the inhibitory activity.

3.6 Anti-inflammatory assay

Following the previous protocol, *D. bulbifera* extract was screened for an in vitro anti-inflammatory assay [23]. Upon mixing 100 µl of extracts or standard (50-550 µl) with freshly made BSA solution (0.45 ml, 0.5% w/v), the mixture was incubated at 37°C for 20 minutes. Following the incubation procedure, the mixture was heated to 57° C for five minutes, cooled for twenty minutes, and then 2.5 ml of phosphate-buffered saline (pH 6.3 to 6.4) was added. The pH was adjusted by glacial acetic acid, and the final solution mixture was measured at 255 nm using a UV-VIS spectrophotometer. The percentage inhibition of precipitation (protein denaturation) was calculated as:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 = absorbance of the control (100% Protein denaturation) and A_1 = absorbance of the sample.

3.7 GC-MS analysis

GC-MS analysis was carried out in a combined Trace 1300 GC couple and Thermo TSQ800 Triple Quadrupole MS, which is fitted with HP5MS fused silica capillary column (30×0.25 mm inner diameter and 1ME film thickness), to trace the compounds present in methanolic extract. 99.99% Helium (carrier gas) was maintained at 0.5 mL of injection volume, 1 ml/min of flow rate, and 250°C of temperature. At an electron energy of 70 eV, the mass spectra were scanned every 0.5 seconds, covering the range of 40 to 550 (m/z). The National Institute Standard and Technology Library, which has 62,000 patterns, was used to determine the components associated with the peaks.

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3.8 HPTLC analysis

The previously established method did the instrumental parameters and analysis of HPTLC [24]. The plate was developed in a Twin Trough Chamber (CAMAG) for antioxidants up to a length of 90 mm at 25±5° C using a pre-saturated mobile phase of chloroform: ethyl acetate: formic acid (5:4:1 v/v/v). After drying of plates, chromatograms were documented using TLC UV Cabinet 4 (CAMA in a wavelength of 254 nm, 366 nm, and 416 nm, and then the plate was scanned in a TLC scanner 4 controlled using VisionCATS software (CAMAG; slit width 6×0.45 nm) to recorded the chromatograms.

3.8.1 Method Validation

Following the International Conference on Harmonization guidelines for validating analytical methods allowed for establishing a viable HPTLC method [25]. The standard calibration curve was drawn by plotting the concentration versus peak area. Assess the limit of detection (LOD) and limit of quantification (LOQ) using the S/N method; LOQ is evaluated with an S/N ratio of 10 and LOD with an S/N ratio of 3. For the precision of methods, intraday, interday, and RSD were also evaluated by a previously established method[21]. A recovery test was implemented to maintain the accuracy of the method. The antioxidant was identified and quantified at 254nm using CAMAG TLC scanner 4 with VisionCATS software.

3.9 Statistical analysis

Version 3.05 of GraphPadInStat from GraphPad Software, USA, was used to conduct the statistical analysis. For all data, mean ± standard deviation (SD) has been used.

4. Results and discussion

4.1 Total phenolic and flavonoid content

The results of TPC and TFC of *D. bulbifera* L. tuber showed (Fig. 1) that the methanolic extract (105.58±123 mg GAE/g) exhibited significantly high ($p < 0.05$) TPC where the least amount of TPC was found in n-hexane extract (129.49 ±1 .43 mg GAE/g) (Fig. 1. A). On the other hand, TFC was found to be significantly high in the aqueous extract (306.85±20.10 mg QRE/g), while other extracts showed moderate TFC contents (Fig. 1. B). The previous report found that the health advantage of dietary plant polyphenols is because of the antioxidant effect, which mainly neutralizes free radicals. Long-term digestion of plant polyphenols has medicinal value against cardiovascular diseases, diabetes, cancer, neurological illness, etc. [26]. As phenolic compounds consist of hydroxyl groups, they dissolve more readily in polar organic solvents [27]. Our research indicated that *D. bulbifera* has more phenolic and flavonoid content in a polar solvent (methanol and aqueous), a factor in deploying the antioxidant effect.

4.2 Antioxidant activity

4.2.1 Free radical scavenging activity (DPPH)

The DPPH antioxidant assay is performed worldwide to quantify free radical scavenging capacity. Mainly, DPPH radicals receive a proton or electron from antioxidant chemicals that subsequently transform into the stable diamagnetic molecule DPPH-H (DPPH to DPPH2), which changes their color from violet to yellow[23]. Figure 1. C shows the DPPH radical scavenging activity of different extracts of *D. bulbifera* compared to ascorbic acid as standard.

Significantly high radical scavenging activity was shown by methanolic extract (IC_{50}: 56.94±4.49 µg/ml; $p < 0.05$) as compared to ascorbic acid (IC_{50}: 36.97±1.74 µg/ml; $p < 0.05$) (Fig.1.C). The antioxidant capacity of a plant is due to the presence of phenolic and flavonoids [28]. Our study supports the previously published work where methanolic extract exhibits the highest DPPH radical scavenging activity over plant tuber [29].

4.2.2 Metal chelation assay

Redox-active metal catalysis is linked to the technique of metal ion chelating, which stops the production of reactive oxygen species. It has previously been documented that transition metal ions facilitate oxidative damage, which may cause neurodegenerative illnesses such as Parkinson's and Alzheimer's. With its mild chelating action in vitro, the plant extract shows therapeutic promise for treating diseases [20]. Figure 1. C shows the metal-chelating activity of different plant extracts of *D. bulbifera*. The ferrozine-Fe^{2+} complex forms in the presence of extract in this case, and the ability of the methanolic extract (IC50: 97.32±7.9 µg/ml) to chelate iron was compared with EDTA (IC50: 71.29±5.8 µg/ml). Thus, the study emphasizes that metal-chelating activity present in *D. bulbifera* tubers

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can give protection against oxidative damage resulting from metal-catalyzed decomposition reactions.

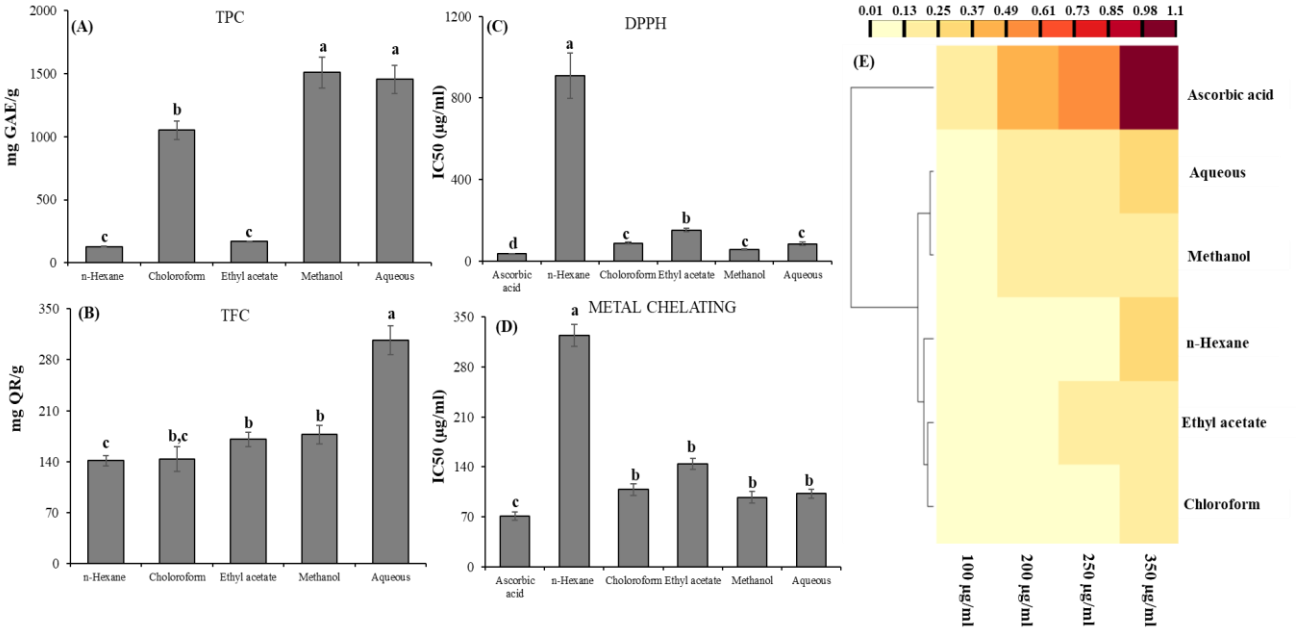

Figure. 1 TPC (A), TFC (B), DPPH assay (c), metal chelating assay (d), and reducing power assay (E) of *D. bulbifera* tuber extracts with different solvents. Values are expressed as Mean ± SD (n = 6). Values with different letters are significantly different ($p < 0.05$), as shown by the Tukey test.

4.2.3 Ferric-reducing power assay

The $Fe^{3+/}$ferricyanide complex is reduced to its ferrous form by the sample's antioxidants in the reducing power test, which is detected by the production of Perl's Prussian blue at 700 nm. The value of increasing absorbance with increasing sample concentration shows more reducing power ability. To determine the samples' reduction capability, this assay relies on monitoring the conversion of Fe^{3+} to $Fe^{2+,}$ made possible by their presence. The heat map in Figure 1. E visually adorns the concentration-dependent ferric reduction activity of all *D. bulbifera* extracts. This heat map showed that the formation of good clustered with aqueous extract and moderate to less cluster with methanol, n-hexane, ethyl acetate, and chloroform extract, respectively, with the standard ascorbic acid (Fig. 1. E). Heatmap provides a crystal-clear image of how absorbance increased as sample concentration increased. This appearance mainly indicates the hydrogen-donating capacity to stabilize the molecules by taking hydrogen ions from the samples. Our research found similarities with earlier studies that found strong antioxidant activity in an aqueous extract of this plant [30].

4.3 Antidiabetic activity

4.3.1 α-amylase and α-glucosidase inhibition assay

In inhibiting the activities of α-amylase and α-glucosidase, postprandial blood sugar concentration decreases. The breakdown of dietary starch is the main source of blood glucose, and the enzyme α-amylase is required for the process to start. Human pancreatic and salivary glands produce α-amylase, which can break down starch into smaller oligosaccharides. On the other hand, the α-glucosidase is found in the brush-border surface membrane of the intestinal cell, which transfers oligosaccharides to monosaccharides. These monosaccharides are absorbed by the intestinal epithelia, increasing the blood glucose level. Consequently, inhibiting the two hydrolytic enzymes may decrease the digestive tract's glucose uptake into blood vessels, limiting the rise in postprandial blood levels. Nowadays, inhibition of α-amylase and α-glucosidase is considered

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one of the convenient ways to treat diabetes mellitus [31].

The inhibitory effect of *D. bulbifera* extract on α-amylase and α-glucosidase is presented in Figure 2. Enzyme inhibition appears to be dose-dependent, while the extract's concentration influences the level of inhibition. Notably, *D. bulbifera* methanolic extract (IC_{50}: 81.75±4.5 µg/mL) showed significantly high ($p < 0.05$) amylase inhibitory activity compared to the standard acarbose (IC_{50}: 51.74±1.05 µg/mL) (Fig. 2. A). On the other hand, with α-amylase, *D. bulbifera* methanolic extract (IC_{50}: 168.17±7.27 µg/mL) showed significant high ($p < 0.05$) glucosidase inhibitory activity as compared with the acarbose (IC_{50}: 121.34±7.51 µg/mL) as standard (Fig. 2. B). The findings imply that *D. bulbifera* tuber has the ability to inhibit the α-amylase and α-glucosidase enzymes and prevent the absorption of glucose, hence regulating blood glucose levels, when extracted using polar solvents.

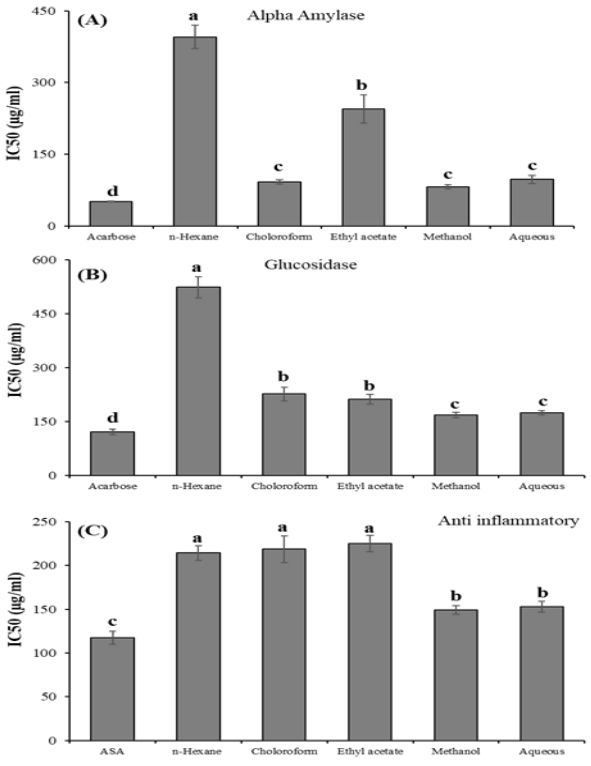

Figure 2. IC50 (µg/ml) of α-amylase (A), α-glucosidase (B), and anti-inflammatory (C) assay of different *D. bulbifera* tuber extracts and standard. Data are expressed as mean±SD. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

4.4 Anti-inflammatory assay

The protein denaturation method is used to evaluate the anti-inflammatory properties of different extracts of *D. bulbifera*. This assay involves the denaturation of bovine serum albumin using heat. Type III hypersensitivity reactions, linked to several disorders like glomerulonephritis, lupus, etc., are linked to denatured proteins[20]. The graph elaborates on the anti-inflammatory properties of various *D. bulbifera* tuber extracts, shown in Fig. 2. C. Among the extracts, the methanolic extract (IC_{50}:149.33±4.94 µg/mL) showed significantly stronger ($p < 0.05$) inhibition compared to other extracts, and here acetylsalicylate (IC_{50}:117.52±7.61 µg/mL) used as a standard. It has been demonstrated by earlier studies that non-steroidal anti-inflammatory medicines (NSAIDs) work by blocking COX enzymes, inhibiting the synthesis of natural prostaglandins, and reducing protein denaturation. The ability to detect anti-inflammatory potential is successfully demonstrated by this assay[26].

4.5 GC-MS analysis

The GC-MS study of the methanolic extract of *D. bulbifera* tuber revealed sixteen compounds having a range of phytochemical activities. The chemical components with their retention time, name, area, molecular formula, molecular weight, reported activities, and structure are shown in Table 1, while the chromatogram is displayed in Fig. 3. A. The GC-MS study performed on the methanol fraction of *D. bulbifera* tuber revealed the presence

4.6 Phytochemical analysis

Figure 4. B demonstrates the presence of phytochemicals in different extracts of *D. bulbifera* tuber through HPTLC analysis. A validated HPTLC method was used to identify and quantify the phytochemicals. Table 2 shows the perfect validation data of identified phytochemicals for quantification. Four phenolic acids and two flavonoids were identified from the plant. n-Hexane extract did not show the presence of any phytochemical. Gallic acid (hR_f=0.251), caffeic acid (hR_f=0.478), 4-hydroxybenzoic acid (hR_f=0.619), t-cinnamic acid (hR_f=0.75), quercetin (hR_f=0.517), and myricetin (hR_f=0.333) were identified and quantified in

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the rest of the extracts. Methanolic extract showed the highest content of gallic acid (0.43±0.02 µg/ml), 4-hydroxybenzoic acid (0.62±0.02 µg/ml), t-cinnamic acid (0.35±0.01 µg/ml), and quercetin (1.71±0.03 µg/ml) (Table 3). Aqueous extract showed the highest content of caffeic acid (0.81±0.02 µg/ml) and myricetin

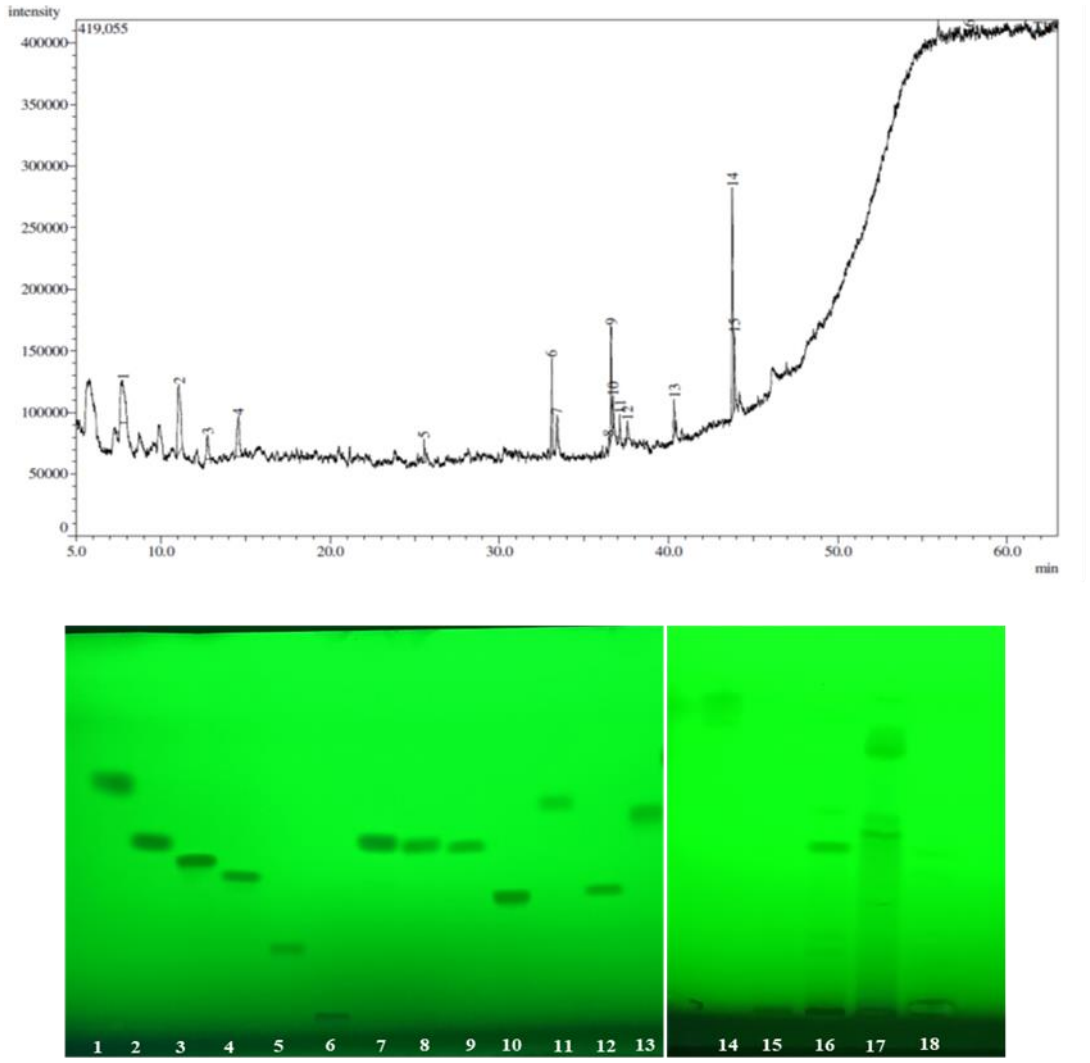

Figure. 3. (A) GC-MS of *D. bulbifera* tuber extracts. (B) HPTLC fingerprints of *D. bulbifera* tuber extracts. Mobile phase: chloroform, ethyl acetate, and formic acid (5:4:1); Wavelength: 254 nm. Track 1: t-cinnamic acid, track 2: 4-hydroxybenzoic acid, track 3: quercetin, track 4: caffeic acid, track 5: gallic acid, track 6: chlorogenic acid, track 7: vanillic acid, track 8: apigenin, track 9: p-coumaric acid, track 10: myricetin, track 11: naringenin, track 12: caffein, track 13: kaempferol, track 14: *n*-hexane extract, track 15: chloroform extract, track16: ethyl acetate extract, track 17: methanolic extract, and track 18: aqueous extract.

(0.62±0.02 µg/ml) (Table 3). Gallic acid has a wide range of therapeutical applications such as antioxidant, anticancer, antimicrobial, antibacterial, antidiabetic, antiviral, antifungal, anticholesterol, and antiulcer properties [32]. Antioxidant, anti-inflammatory, antidiabetic, and anticancer properties are exhibited by caffeic acid [33]. 4-Hydroxybenzoic acid exhibits antiviral, antimutagenic, anti-inflammatory, hypoglycemic, antioxidant, anti-platelet aggregating, and antibacterial properties [34]. Both quercetin and myricetin have antioxidant, anti-inflammatory, antidiabetic, etc activities [35, 36]. Figure 4 illustrates a

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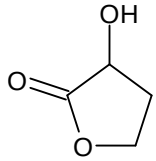
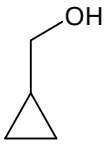
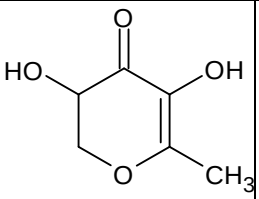
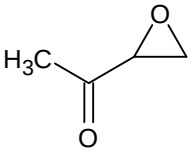
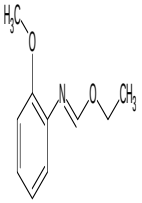
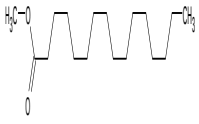
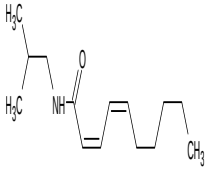
schematic diagram that depicts the mechanical role of *D. bulbifera* tuber. The pharmacological actions of *D. bulbifera* could be attributed to the presence of these phytochemicals.

This study was performed to evaluate the pharmacological properties of the n-hexane, chloroform, ethyl acetate, methanol, and aqueous extract of *D. bulbifera* tuber. Additionally, GC-MS and HPTLC

5. Conclusion

Table 1. Phytoconstituents identified by GC-MS analysis from methanolic extract *D. bulbifera tuber* extracts

Peak no	RT	COMPOUND NAME	AREA%	Mol. Formula	M. W. (g/mol)	Reported bioactivity	Structure	Ref
1	7.736	2-Hydroxy-gamma-butyrolactone	13.9	C4H6O3	102	Antimicrobial		1
2	11.063	Cyclopropyl carbinol	16.18	C4H8O	72	Antibacterial		2
3	12.767	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	3.93	C6H8O4	144	Anticancer		3
4	14.577	1-(2-Oxiranyl)ethanone	8.29	C4H6O2	86	Not reported		NA
5	25.551	Ethyl N-(o-anisyl)formimidate	2.16	C10H13NO2	179	Anti-inflammatory		4
6	33.087	Methyl hexadecanoate	6.72	C17H34O2	270	Antioxidant		5
7	33.402	(E,E)-2,4-Decadienoic isobutylamide	2.26	C14H25NO	223	Antimicrobial		6

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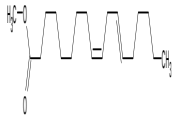
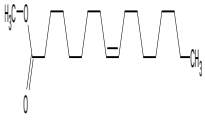
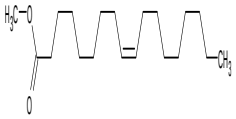
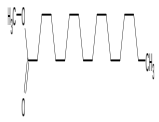
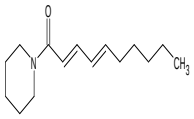
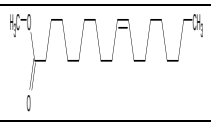
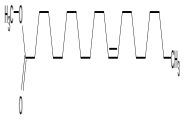
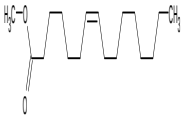
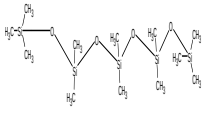
8	36.45	Methyl -9,12-octadecadienoate	1.02	C19H34O2	294	Anticancer		7
9	36.595	9-Octadecenoic acid (Z)-methyl ester	9.07	C19H36O2	296	Antimicrobial		8
10	36.725	9-Octadecenoic acid (Z)-, methyl ester	2.57	C19H36O2	296	Antimicrobial		8
11	37.102	Methyl stearate	1.36	C19H38O2	298	Antioxidant and antifungal		9
12	37.556	(2E,4E)-1-(Piperidin-1-yl)deca-2,4-dien-1-one	1.49	C15H25NO	235	Not reported		NA
13	40.318	Methyl cis-11-eicosenoate	2.31	C21H40O2	324	Antibacterial		10
14	43.759	13(Z)-Docosenoic Acid methyl ester	20.22	C23H44O2	352	Antimicrobial		11
15	43.867	cis-7-Hexadecenoic Acid methyl ester	6.831	C17H32O2	268	Antiinflamatory		12
16	56.624	Dodecamethylpentasiloxane	10.0	C12H36O4Si5	384	Antimicrobial		13

Table 2. Linearity, correlation coefficient, limits of detection, limits of quantification, repeatability, and precision of ten analytes.

Compound Name	Linear Range (µg/ml)	Linear Regression (R^2)	LOD (µg/ml)	LOQ (µg/ml)	% RSD				Recovery	
					Intra-day		Inter-day			
					R_f	Area	R_f	Area	%	% RSD
Gallic acid	1-10	0.99	0.7712	2.916	2.45	2.75	2.54	2.45	99.85	1.11
Caffeic acid	1-10	0.99	0.962	2.916	2.11	3.04	2.93	3.15	100.32	1.05
4-Hydroxybenzoic Acid	1-10	0.99	0.8604	2.6072	2.34	2.94	3.02	2.85	99.59	0.96
t-Cinnamic Acid	1-10	0.99	0.82	2.48	2.14	2.33	3.11	3.43	99.77	1.21

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Quercetin	1-10	0.99	0.8804	2.668	1.94	2.14	2.75	2.89	99.67	1.34
Myricetin	1-10	0.99	0.92	2.8	2.05	2.64	2.38	3.14	99.81	1.08

analyses were performed for phytochemical analysis. Notably, in *D. bulbifera* tuber, the polar solvent extracts showed significant antioxidant, anti-diabetic, and inflammatory properties. Generally, methanolic extracts exhibited strong effects since the majority of phytochemicals are found in polar solvents. It was discovered to have a significant amount of flavonoid and phenolic compounds through HPTLC. The antioxidants present in *D. bulbifera* tuber acting as a free radical scavenger were identified as gallic acid, caffeic acid, 4-hydroxybenzoic acid, t-cinnamic acid, quercetin, and myricetin exhibiting their potential as antioxidants to combat oxidative damage. The present study concludes that *D. bulbifera* tuber extract shows great potential as an antioxidant, antidiabetic, and anti-inflammatory through the inhibition of the free radical mechanism. Further studies will be needed to see the in vivo activities, and different formulation's effect and to explore mechanisms.

Table 3. Phytochemicals quantification (µg/ml) of *D. bulbifera tuber* extracts.

	Gallic acid	Caffeic acid	4-Hydroxy benzoic Acid	t-Cinnamic Acid	Quercetin	Myricetin
n-Hexane	ND	ND	ND	ND	ND	ND
Chloroform	0.04±0.01	ND	ND	ND	0.356±0.03	ND
Ethyl acetate	0.17±0.02	ND	0.26±0.03	0.065±0.01	1.11±0.04	ND
Methanolic	0.43±0.02	0.45±0.01	0.62±0.02	0.35±0.01	1.71±0.03	0.46±0.01
Aqueous	0.04±0.01	0.81±0.02	0.17±0.01	ND	ND	0.62±0.02

ND: Not Detected

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