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Review Paper

A Multifaceted Medicinal Plant: Dioscorea bulbifera L. and Its Potential in Antimicrobial, Anti-Inflammatory, Anticancer, and Anti-diabetic Therapy

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ABSTRACT

Since ancient history, Dioscorea bulbifera L. has been recognized and publicized as a detoxification, anti-inflammatory and hemostatic agent due to which it can boast of ancient Chinese pharmacopoeia evidence dating back centuries-a seventh century text "Tang Bencao." D. bulbifera is a valuable remedy for a broad array of clinical conditions including infection, goiter and malignancy, thus having a wide application in the Asian and African regions mostly.(1) This review aims to be comprehensive regarding the pharmacology, phytochemistry and toxicology of D. bulbifera, revealing its therapeutic prospects for modern medicine.(2)The bioactive compounds extracted using methanol and ethyl acetate demonstrated significant antimicrobial effects against pathogens like Pseudomonas aeruginosa and Mycobacterium tuberculosis, highlighting their potential in combating these microorganisms.(3) Meanwhile, significant anti-inflammatory and analgesic effects were presented by D. bulbifera in different pain models, implying that the plant could be natural for alternative pain relief.(4) The petroleum ether extract also showed remarkable results in anti-tumor research it has powerful tumor-inhibiting activity, while some of its active compounds kill a variety of cancer lines, among them liver and cervical cancers.(5) Additionally, D. bulbifera exhibited a notable capacity to suppress the activity of α-amylase and α-glucosidase enzymes, highlighting its antidiabetic potential, with methanol extracts proving to be more potent.(6) This review emphasizes D. bulbifera with great therapeutic potential multipurpose; among them include antimicrobial, anti-inflammatory, anti-tumor and anti-diabetic, thus encouraging further modern pharmacological applications.

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Introduction

Dioscorea bulbifera, often referred to as air potato or air yam, is a climbing plant that produces tubers and is part of the Dioscoreaceae family.The Dioscorea bulbifera is placed first time in the world in Tang Bencao" , which is the first pharmacopoeia. In this country, it has been traditionally known under the names "Huangdu" and "Huangyaozi".[7] It is found in nearly all regions of the world: from tropical Africa to northern Australia, the Indian subcontinent, Florida in the United States, China, Cambodia, Thailand, Malaysia, and Laos (Coursey 1967; Wilkin 2001).[8] In China, it emerges naturally in the greater half of the southern part of the country: specifically Henan, Anhui, Jiangsu, Fujian, Guangdong, Guangxi, Shaanxi, Gansu, Sichuan, Guizhou, Yunnan, and Tibet (Editorial Board of Chinese Materia Medica, 1999).[9]Traditionally, D. bulbifera prepared different types of medicine. It is a weakly odorous plant with bitter and salty tastes.[10]As per the ancient Indian medical theory, the plant treats pathogenic heat detoxifies poison, dissolves phlegm, softens mass, and cool blood to stop bleeding, which is why it has been used for ages.[11]Based on historical records in traditional Chinese medicine, this plant has been used for treating conditions like hemoptysis, epistaxis, pharyngitis, goiter, pyogenic skin infections, scrofula, trauma, orchitis, and cancer (Ting et al., 1985). In the last few decades D. bulbifera becomes the focus of qualitative and quantitative studies due to very numerous clinical therapeutic aspects.[2]



Figure 1: Dioscorea bulbifera

Advantages

- **High Yield Potential** Produces both aerial bulbils and underground tubers, resulting in significant biomass and food production.

- **Rapid Growth**
  Grows quickly and vigorously, useful for covering large areas and providing fast ground cover or shade.
- **Low Input Requirements**
  Thrives in poor soils with minimal fertilizer, irrigation, or maintenance, making it suitable for resource-limited farming.
- **Nutritional Value**
  Certain edible varieties are rich in carbohydrates, serving as a valuable food source in traditional diets.
- **Ethnomedicinal Uses**
  Used in traditional medicine to treat ulcers, infections, inflammation, and other ailments.
- **Bioactive Compounds**
  Contains antioxidant and antimicrobial properties, offering potential for pharmaceutical research.
- **Soil Conservation**
  Its dense growth helps prevent soil erosion and can improve soil structure in degraded lands.
- **Industrial Applications**
  The starch content and biomass are being explored for use in bioenergy, bioplastics, and other green technologies.
- **Climate Resilience**
  Performs well in variable climates, supporting sustainable and adaptive agricultural practices.
- **Caution – Invasive Potential**
  While beneficial, wild types are invasive in many regions and can displace native vegetation if not properly managed.[12]

Disadvantages

- **Invasive Nature**
  In many regions, especially in the southeastern United States, air potato is highly invasive and can aggressively overtake native vegetation, disrupting local ecosystems.
- **Toxicity in Wild Varieties**
  Many wild forms contain toxic compounds such as *dioscorine*, making them unsafe for human consumption without proper processing—or not at all.
- **Threat to Biodiversity**
  Its rapid and dense growth can smother native plants, reduce biodiversity, and alter the structure of plant communities.
- **Difficult to Control**
  Once established, air potato can be challenging to remove due to its prolific bulbils and underground tubers, which regenerate easily.
- **Not Universally Edible**
  Only selected cultivated varieties

are safe for food use. Distinguishing between edible and toxic types requires expertise.

- **Limited Commercial Value** Despite high yields, air potato lacks widespread commercial cultivation due to edibility concerns and regulatory restrictions in many countries.
- **Legal Restrictions** In several regions (e.g., Florida, USA), its cultivation, transport, or sale is banned or regulated due to its invasive status.
- **Pest and Disease Risk** Dense growth may harbor pests or diseases that could affect nearby crops or natural vegetation.[13]

Biological activities

1. Antimicrobial activity

1.1Material Methods

1.1.1Extraction and isolation

The bulbils of D. bulbifera L. var sativa, after being air-dried (2 kg), were ground into a fine powder and subjected to three separate 24-hour extractions using methanol.[14] The resulting methanolic extract was concentrated under reduced pressure, yielding a dark residue (DBB; 90 g).[15] From this, 80 g was taken and mixed with 150 ml of water, followed by sequential partitioning with ethyl acetate (EtOAc) and n-butanol. The EtOAc and n-butanol fractions were then dried under reduced pressure, producing 35 g and 23 g of extracts, respectively.[16] Notably, the n-butanol extract lacked antimicrobial properties, unlike the EtOAc extract.[17] A portion of the EtOAc extract was further processed using silica column chromatography with Merck 60 [0.040-0.063 mm; 56 g], employing a hexane-EtOAc gradient of increasing polarity as the mobile phase.[18] This procedure resulted in five primary sub-fractions, labeled A through E, which were evaluated for antimicrobial activity.[17] Only sub-fraction C (DBB2), eluted with a Hexane-EtOAc ratio ranging from 4-6 to 3-7, demonstrated activity. A total of 4.8 g of DBB2 was further fractionated and purified using column chromatography over silica gel and Sephadex LH-20, leading to the isolation of compound 1 {White needles; [α]D 21 = -64.6 (c = 0.025, DMSO); 60 mg; Rf = 0.78, CH2Cl2-MeOH 95-5; C21H22O8 m/z 403 [M+H]+ ; m.p. = 252-253°C}, compound 2 {White needles; [α]D 21 = +52 (c = 0.010, pyridine); 71 mg; Rf = 0.44, CH2Cl2-MeOH 95-5; C20H20O8, m/z 387 [M-H]- ; m.p. = 312-313 °C}, compound 3 {White gum; [α]D 21 = +56.2°(c = 0.8, CH2Cl2); 32 mg; Rf = 0.37, CH2Cl2-MeOH 95-5; C21H22O7, 358 [M-CO]+ }, compound 4 {White needles; [α] D21 = - 5o (c = 0.6, CH2Cl2); 58 mg; Rf = 0.56, CH2Cl2-MeOH 95-5; C21H24O8; m/z 439 [M+Cl];

m.p. = 265-266 °C}, compound 5 {White crystals; [α]D 20 = - 46o (c = 0.9, CD3OD); 64 mg; Rf = 0.41, CH2Cl2-MeOH 95-5; C23H26O10; m/z 485 [M+Na]+ ; m.p. = 199-200 °C}, compound 6 {Yellow amorphous powder; 28 mg; Rf = 0.48, CH2Cl2-MeOH 95-5; C15H12O3, m/z 241 [M+H]+ }.[19]

1.1.2General procedure

Melting points of the compounds were measured using a Gallenkamp apparatus. The Perkin-Elmer 241 polarimeter was used to determine optical rotation values. Infrared spectra (IR) were recorded using a Shimadzu FTIR-8400S spectrometer on KBr-pelleted samples.[20] Electrospray ionization mass spectrometry (ESIMS) analyses were using a Hewlett Packard HP-1100 LC-MSD system and a Bruker FTMS4.7T BIOAPEXII mass spectrometer.Proton (^1H) NMR was obtained at 400 MHz and carbon-13 (^13C) at 100 MHz, both in deuterated DMSO and pyridine with a Varian Mercury Plus spectrometer. Chemical shifts (δ) are expressed in ppm with tetramethylsilane (TMS) as the internal standard and coupling constants (J) are expressed in Hz.(21) Column chromatography was performed with Merck silica gel 60 (particle size 0.040–0.063 mm) and Sephadex LH-20.(23) Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 pre-coated plates, utilizing hexane:ethyl acetate (7:3) and ethyl acetate:methanol (98:2) as the developing solvent systems. The spots from the chromatography analysis were viewed under UV light (254/365 nm) and then identified by spraying with 50% sulfuric acid, then heating at 110 °C for 10 minutes.[21]

1.1.3 Antimicrobial assays

Chloramphenicol (98%) was used as a standard antibiotic for screening for activity against Gram-negative bacterial strains. For detection of microbial growth, the colorimetric indicator p-iodonitrotetrazolium chloride (INT, 97%) was used. Ciprofloxacin (≥98%) was used as a reference drug for activity against Mycobacterium smegmatis, and isoniazid (≥99%) was used for activity against Mycobacterium tuberculosis. Finally, phenylalanine arginine β-naphthylamide (PAβN, ≥98%) was used as an efflux pump inhibitor to assess its role in increasing antibiotic susceptibility.[24]

Both K. pneumoniae and E. aerogenes were investigated, as well as these three strains of organisms, such as P. aeruginosa, Escherichia coli, and Mycobacteria: the 4 Mycobacteria are M. smegmatis, a drug-susceptible strain of M. tuberculosis H37Rv obtained from the American Type Culture Collection, and two clinical strains of M. tuberculosis MTCS1, MTCS2.[17] M. smegmatis would be cultured on medium Brook 7H11 agar, and incubated overnight.

The plating of M. tuberculosis was performed on Löwenstein-Jensen medium and incubated at 37°C for 3-4 weeks. MIC and MBC determination of test samples was performed with Middlebrook 7H9 broth on M. smegmatis and M. tuberculosis. All Gram-negative bacteria were activated under Nutrient Agar.[25]

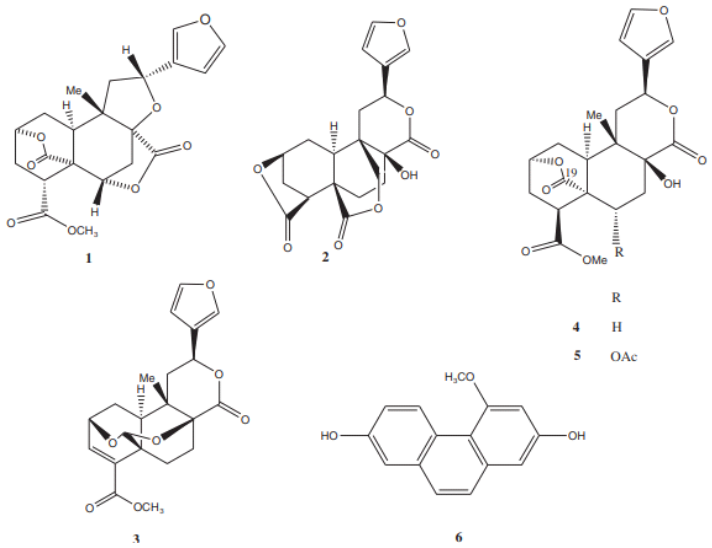

Figure 2:Structures of the compounds isolated from the bulbils of Dioscorea bulbifera

2.Anti inflammatory activity

2.1Materials and methods

2.1.1Plant collection and extraction

Dioscorea bulbifera var. sativa bulbs were collected in February. The plant materials were authenticated against a previously general specimen (voucher no. 22211/SRF/CAM) at the Cameroon National Herbarium. After collection, the bulbs were thoroughly dried and ground into a fine powder. A total of 12 kg of this powder was extracted twice using a methanol-water mixture (8:2), each extraction lasting 24 hours. The combined extracts were then concentrated under reduced pressure (rp) a dark brown residue, that yielded 530 grams in total for an overall yield of 4.42%.[4]

2.1.2Animals

In this study, Swiss mice weighing between 25 and 35 grams, both male and female, and aged 6 to 8 weeks, were utilized. Female mice were employed in the neuropathic pain model, while male mice were used for studying inflammatory pain.[26] The animals were sourced from the Department of Pharmacology at the Universidade Federal de Santa Catarina (UFSC) in Florianópolis, Brazil. They were kept in an

environment with optimal lighting, temperature, and humidity conditions (a 12-hour light-dark cycle, 22 ± 2 ◦C, 60–80% humidity), with unrestricted access to food and water.[27] All experimental procedures adhered to the "Principles of Laboratory Animal Care" as outlined in NIH Publication Nos. 85-23. The Swiss mice, weighing 25-35 gm, of both sexes, aged 6-8 weeks, were carefully evaluated for the purpose of the present investigation.[28] Female mice were used in the model of neuropathic pain; males were used for inflammatory pain.[29] These animals were obtained from the Department of Pharmacology of the Universidade Federal de Santa Catarina (UFSC, Florianópolis, Brazil), and the animals were housed under conditions of optimal light, temperature, and humidity (12-hour light-dark cycle, 22 ± 2 ◦C, 60-80% humidity) with food and water provided ad libitum.[30] All procedures used in the present study adhered to the "Principles of Laboratory Animal Care" from NIH Publication Nos. 85-23 and were approved by the Animal Ethics Committee of the UFSC.[31] The least number of animals possible were used along with the least painful stimulus capable of showing consistent effects were approved by the Animal Ethics Committee of the Universidade Federal de Santa Catarina.[32] The number of animals and the intensity of noxious stimuli used were the minimum necessary to demonstrate the consistent effects.

1.1.3 Behavioural testing

Withdrawal response to delta-hindpaw

Assessment of mechanical hypersensitivity was performed on the individual mouse for the indoor environment by placing it in a clear Plexiglas box (size = 9 cm x 7 cm x 11 cm) on an elevated wire mesh with the contact surface of ventral component against the right hindpaw allowing access point from the above. After 10 applications, each 3 s using the von Frey hair of 0.4 g (VFH, Stoelting, Chicago, IL, USA), withdrawal response frequency was recorded. Thus, the animal haved tested on acclimatization prior to the behavioral testing for 1 h and evaluated levels of mechanical hypersensitivity at various time points as specified later. [33]

Induction of mechanical hypernociception by complete Freund's adjuvant (CFA)

Animals received a treatment orally with vehicle (10 mL/kg),Dioscorea bulbifera extract, administered at doses of 250 mg/kg or 500 mg/kg, and gabapentin (70 mg/kg) was used as a positive control. The mild restraint was applied one hour after treatment and all animals received an intraplantar (i.pl.) injection of 20 µL of complete Freund’s adjuvant (CFA) (1 mg/mL of heat killed Mycobacterium tuberculosis in a mixture of paraffin oil

85% and mannide monooleate 15%) according to Kassuya et al. (2003). The mechanical nociceptive response was evaluated from the von Frey filament at 1, 2, 4, 6, 8, and 24 hours after CFA injection. After the 24-hour hypernociception assessment, a subsequent dose of the assigned treatment was given, and administration continued daily until the nociceptive behavior of treated animals resembled that of the vehicle control group. Thereafter, treatments were maintained once daily, and pain responses were recorded every 24 hours up to 14 days following CFA injection.[34]

Neuropathic pain, induced by the partial ligation of the sciatic nerve (PLSN)

In 2005, to evaluate behaviours resembling neurological pain, the manner described by Quintao et al.Following the mechanical basal pain behavioural assessments, mice were administered intraperitoneal injections of chloral hydrate (7%, 8 ml/kg).PLSN was performed by ligating 1/3 to 1/2 of the sciatic nerve using an 8-0 silk suture (Ethicon, Edinburgh). Five days later, a second assessment of mechanical basal pain behaviour was made to ensure that pain was being induced. Animals were administered either vehicle (10 ml/kg), gabapentin (70 mg/kg), or Dioscorea bulbifera (500 mg/kg) treatment, orally, and after treatment was administered, mechanical hypersensitivity of the right hindpaw, was made at 1, 2, 4, 6, 8 and 24 hours after treatment. After the 24-hour reading, a b.i.d. dosing schedule commenced, with hypernociception being assessed the next day, before and two hours after the first administration on that day, to check for duration of efficacy and the tolerability of each drug.[35]

PGE2 induced hypernociception

The baseline nociceptive response was assessed using a 0.4 g von Frey filament, as described before. Animals were then administered orally vehicle (10 mL/kg) or Dioscorea bulbifera extract (500 mg/kg). One hour following treatment, 100 ng LPS per paw intraplantar injection was given, diluted in 20 µL saline.The development of hypernociception was subsequently monitored at 1, 2, 4, 6, 8, and 24 hours after LPS administration.[36]

Mechanical hypernociception induced by PGE2

The experimental procedure, based on the method described by Kassuya et al. (2007), involved evaluating the analgesic effects of Dioscorea bulbifera extract. Initially, the baseline response to von Frey hair stimulation was assessed. Animals were then administered either a vehicle (10 mL/kg), D. bulbifera extract (500 mg/kg), or dipyrone (120 mg/kg) orally. After one hour, prostaglandin E2 (PGE_2) was injected subcutaneously into one paw at a dose of 0.1 nmol. Pain sensitivity was assessed

using von Frey hair stimulation at 1, 2, 4, 6, and 8 hours following PGE_2 administration. This timeline aimed to determine whether the treatments acted directly on PGE_2 receptors or influenced downstream signaling pathways.To further explore the extract's mechanism of action, hypernociception tests were conducted at the same time intervals. Animals received pretreatment with either L-NAME (30 mg/kg, intraperitoneally), a nitric oxide synthase inhibitor, or glibenclamide (5 mg/kg, intraperitoneally), which blocks ATP-sensitive potassium channels. These agents were used to assess the involvement of the NO–cGMP–potassium channel pathway in the analgesic effect of D. bulbifera. The antagonists or their respective control vehicles were administered 30 minutes prior to the oral dosing of D. bulbifera extract or the reference drug, dipyrone (120 mg/kg), to evaluate changes in the extract's efficacy.[36]

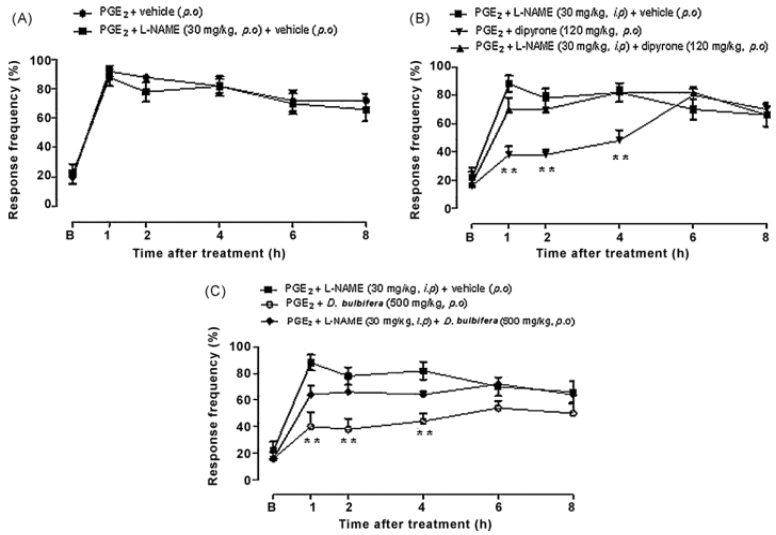

Figure 3: Effect of L-NAME, Dipyrone, and Dioscorea bulbifera Extract on PGE_2-Induced Hyperalgesia Over Time

This illustration presents the impact of inhibiting synthase with l-NAME (30 mg/kg, i.p.) on mechanical antinociceptive efficacies of dipyrone (120 mg/kg, p.o) and methanol extract of the bulbs of Dioscorea bulbifera (500 mg/kg, p.o) in mechanical hypernociception through i.pl. injection of PGE2 (0.1 nmol/paw) induced. The injections of PGE2 were conducted 30 min before l-NAME and 1 h before extract or dipyrone. n = 8-10/group, *p < 0.05 significantly different from control (l-NAME + vehicle). B, response baseline for mechanical stimulation.[33]

3. Anti-tumor activities

3.1 Materials and methodas

D. bulbifera mainly finds application in treating various disorders of the thyroid gland and different types of cancers.[37] Clinical therapeutic anti-tumor activities were studied with the extracts of D. bulbifera dissolved in polar and nonpolar solvents.[38] Less polar organic solvent extraction of the components led to a significant anti-tumor activity in inhibiting the growth of various tumor cells and survivability of tumor-bearing mice[39]. The activities were well marked even against human liver, colon, and other cancer cell lines. To evaluate the extracts based on different solvent systems for their anticancer potency, tubers of D. bulbifera were sequentially extracted with petroleum ether, ethanol, and water.[37] Mouse in vivo HepA cell line assays were used for screening the extracts. The results indicated that the petroleum ether extract possessed the strongest activity against ascitic formation, while the ethanol extract produced a moderate effect, and the water extract, on the contrary, appeared to have a promoting effect on tumor growth.[39] Active compounds obtained in the petroleum ether extraction appeared to be the most effective against tumor cell growth and should therefore be the active compounds responsible for the clinical antitumor activity of D. bulbifera. 10 In vitro experiments proved that the D. bulbifera decoction was able to inhibit cell growth in a dosage- and time-dependent manner in human squamous cell carcinoma cell line SiHa, human cervical cancer cells Hela, and human hepatoma cells HepG2. Among these three types, the SiHa cells were the most sensitive to the extract, while HepG2 cells were the least sensitive. In another in vitro research, the anti-tumor effect of D. bulbifera was investigated with soft agar colony assays for anti-tumor activity. [38]

Studies on Dioscorea bulbifera have shown that its 75% ethanol rhizome extracts, along with several isolated monomeric compounds, exhibit strong inhibitory effects on tumor-promoting activity in 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced JB6 mouse epidermal cells (Gao et al., 2007). Active compounds identified from D. bulbifera included diosbulbin B (43), caryatin (73), catechin (74), 3,5-dimethoxykaempferol (71), myricetin (75), and its glycosides such as myricetin-3-O-β-D-galactopyranoside (76) and myricetin-3-O-β-D-glucopyranoside (77), as well as hyperoside (78), methyl-O-α-D-fructofuranoside (120), and butyl-O-α-D-fructofuranoside (121). Among the flavonoids, kaempferol-3,5-dimethyl ether (83) displayed the most potent inhibitory activity with an IC_{50} of 0.64 μg/mL. Furthermore, Komori reported that diosbulbin A (42) significantly suppressed the growth of solid sarcoma 180 tumors.

Additionally, D. bulbifera extracts exhibited high cytotoxicity against Bel-7402 human liver cancer cells, showing inhibition rates of 99.1% and 92.6% by pennogenin glycosides—specifically, pennogenin-3-O-α-L-rhamnopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (34) and pennogenin-3-O-α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (35), respectively.[39]

4. Antidiabetic activity

4.1 Material and Methods

4.1.1 Chemicals

All the chemicals and reagents obtained were of A.R. grade. Sigma Aldrich provided α-glucosidase and 4-nitrophenyl α-D-glucopyranoside, USA. DNSA [dinitrosalicylic acid] was obtained from SRL Pvt. Ltd.[37] (Mumbai, India). Petroleum ether, ethyl acetate, methanol, ethanol, dipotassium hydrogen phosphate (K2HPO4), and potassium dihydrogen phosphate (KH2PO4) were purchased from Qualigens, Mumbai, India. Methanol, sodium potassium tartarate, and sodium hydroxide (NaOH) were also included. Pure porcine pancreatic α-amylase and sodium chloride (NaCl) were obtained from HiMedia Laboratories, Mumbai, India. Obtained acarbose from Bayer Pharmaceuticals Pvt. Ltd. (Mumbai, India).[39]

4.1.2 Plant Material

Fresh leaves, stems, and flowers of Gmelina glauca, along with bulbs of Dioscorea bulbifera, were collected in January from the Western Ghats region of Maharashtra, India. The plant species were taxonomically identified and authenticated by a botanist at the National Research Institute of Ayurvedic Sciences, under the Central Council for Research in Ayurveda and Siddha (Ministry of AYUSH, Government of India), located at Nehru Garden, Kothrud, Pune – 411038. Voucher specimens were deposited with identification numbers 327 for G. glauca and 860 for D. bulbifera.[39]

The leaves, stems, and flowers of Gmelina glauca were shade-dried at ambient temperature, while the bulbs of Dioscorea bulbifera were cut into small pieces and dried similarly. Once dried, all plant materials were ground into a coarse powder using a dry grinder.[10] A portion of 100 grams from each powdered sample was extracted sequentially in a Soxhlet apparatus using petroleum ether, ethyl acetate, and methanol at 80°C, yielding approximately 2%, 8%, and 8%, respectively. An additional 100 grams of each powdered sample was subjected to cold maceration using 70% ethanol in distilled water. The petroleum ether, ethyl acetate, and methanol extracts were concentrated under reduced pressure at

4°C using a rotary evaporator, while the ethanol extracts were freeze-dried (lyophilized) and stored in airtight containers at 4°C. For enzyme inhibition assays, 20 mg of each dried extract was dissolved in 2 mL of 10% dimethyl sulfoxide in distilled water. A 1:20 dilution of these reconstituted extracts was used for both α-amylase and α-glucosidase inhibition studies.[39]

4.1.3 *α*-Amylase Inhibition Assay

Amylase activity was measured using a modified version of the Bernfeld method (1955), with starch serving as the substrate. Porcine pancreatic α-amylase (50 µg/mL, with an optical density of 0.4 at 280 nm) was incubated with test samples at a concentration of 1 mg/mL under identical conditions for 10 minutes at 37°C. The reaction used 1% starch as the substrate, and a reaction mixture without any extract served as the control. The amount of reducing sugars released was quantified using the dinitrosalicylic acid (DNSA) method by measuring absorbance at 540 nm.[40] Enzyme activity was expressed in micromoles of maltose produced per minute. One unit of α-amylase activity was defined as the enzyme quantity that releases 1 µM of maltose under the assay conditions. The percentage inhibition of each sample was compared against the standard inhibitor, acarbose, and the inhibitory effect was calculated using the standard formula.[41]

$$\% \text{ Inhibition} = \frac{(\text{A540}_{\text{Control}} - \text{A540}_{\text{Test}})}{\text{A540}_{\text{Control}}} \times 100.$$

4.1.4 Glucosidase Inhibition Assay with Murine Pancreatic, Liver, and Small Intestinal Extracts

Mice had been bred in the National Toxicology Centre, Pune. They weighed about 20 gm at 10 weeks and were male Swiss mice. The whole experiment was performed according to the guidelines of Institutional Animal Ethical Committee. The mouse was starved for 12 hours. The pancreas, liver, and small intestinal tissues were excised and homogenized in 10 mM ice cold phosphate buffer containing 6 mM NaCl (1 : 10 dilutions; w/v) and appropriate amount of protease inhibitors. Tissue homogenates were centrifuged for 10 min 10,000 r.p.m. supernatant was taken as a source of enzyme and diluted to get an absorbance of 0.4 (at 280 nm). Enzyme inhibition assay was performed as already stated above. Percentage inhibition of the samples against pancreatic, small intestinal, and hepatic glucosidases was assessed. So it was appropriate under any criteria of institute ethical committee.[42]

Ten-week-old Swiss male mice, each weighing approximately 20 grams, were sourced from the National Toxicology

Centre in Pune. All experimental protocols followed the regulations established by the Institutional Animal Ethical Committee. Prior to tissue collection, the animals were fasted for 12 hours. The pancreas, liver, and small intestine were isolated and homogenized in an ice-cold 10 mM phosphate buffer containing 6 mM NaCl, using a 1:10 (w/v) ratio, along with appropriate protease inhibitors. The homogenates were centrifuged at 10,000 rpm for 10 minutes, and the resulting supernatant served as the enzyme source. This supernatant was further diluted until the absorbance reached 0.4 at 280 nm. Enzyme inhibition assays were then performed as described earlier, and the percentage inhibition of glucosidase activity from pancreatic, intestinal, and hepatic tissues was determined.[42)]

These were 20 gm, 10-week-old Swiss males acquired from National Toxicology Centre, Pune.[(39)] All procedures were performed according to the guidelines of the Institutional Animal Ethical Committee. The mouse was starved for a duration of 12 hours. Pancreas, liver, and small intestine tissues were excised and homogenized using 10 mM from cold phosphate buffer containing 6 mM NaCl (1 : 10 dilutions; w/v) and appropriate amounts of protease inhibitors. Tissue homogenates were centrifuged, taking 10 minutes at 10 000 r.p.m. and the supernatant was taken as a source of enzyme and diluted to obtain an absorbance of 0.4 (at 280 nm). Then the enzyme inhibition assay was performed as stated above. Percentage inhibition of the samples against pancreatic, small intestinal, and liver glucosidases was then computed.

Practical: Swiss male mice, aged ten weeks and weighing approximately 20 grams, were obtained from the National Toxicology Centre, Pune. All experimental procedures were conducted in compliance with the guidelines of the Institutional Animal Ethical Committee. Prior to the experiment, the mice were subjected to a 12-hour fasting period. Following euthanasia, the pancreas, liver, and small intestine were carefully dissected and homogenized in ice-cold phosphate buffer (10 mM) containing 6 mM NaCl, using a 1:10 weight-to-volume ratio along with suitable protease inhibitors.[(6)] The resulting homogenates were centrifuged at 10,000 rpm for 10 minutes, and the supernatants were collected as enzyme sources. These were then diluted to achieve an absorbance of 0.4 at 280 nm. Enzyme inhibition assays were performed as previously described, and the percentage inhibition of glucosidase activity from pancreatic,

intestinal, and hepatic tissues was determined for each sample.

4.1.5 *α*-Glucosidase Inhibition Assay

An α-glucosidase inhibition assay was conducted using extracts from the leaves, stems, and flowers of Gmelina glauca, as well as the bulbs of Dioscorea bulbifera, following the method outlined by Sanap et al. (2010) with minor modifications. Each plant extract was combined with α-glucosidase enzyme (0.1 U/mL) and incubated at 37°C for one hour. The enzymatic reaction was initiated by adding 2 mM p-nitrophenyl-α-D-glucopyranoside in a 100 mM phosphate buffer (pH 6.8). After a further 10-minute incubation at the same temperature, the reaction was terminated using sodium carbonate.The formation of p-nitrophenol, which imparts a yellow coloration, was quantified using a Shimadzu UV-1601 spectrophotometer at 420 nm. Enzyme activity was defined as the amount of enzyme required to hydrolyze 1 µM of substrate per minute under the assay conditions.[39]

The glucosidase inhibition test was carried out on extracts from the leaves, stems, and flowers of Gmelina glauca, as well as the bulbs of Dioscorea bulbifera, following the method described by Sanap et al., 2010. In simple terms, each plant extract was mixed with α-glucosidase enzyme at a concentration of 0.1 unit/mL and incubated at 37°C for an hour to allow interaction.To kickstart the enzyme's activity, a compound called p-nitrophenyl-α-D-glucopyranoside (at 2 mM concentration) was added in a phosphate buffer (100 mM, pH 6.8). After letting the reaction proceed for 10 minutes at 37°C, it was stopped by adding 2 mL of 0.1 M sodium carbonate (Na_2CO_3).The amount of enzyme activity was then measured using a Shimadzu UV-1601 spectrophotometer. It detects the release of p-nitrophenol—a yellow-colored product that appears when the enzyme breaks down the substrate—by reading its absorbance at 420 nm. For reference, one unit of α-glucosidase activity corresponds to the amount of enzyme that breaks down 1 µM of p-nitrophenyl glucopyranoside per minute under these test conditions.[42]

4.1.6 Development of HPLC Fingerprints

Shimadzu liquid chromatographic system LC-6AD with automated gradient controller, detector, and photospectrophotometer, SPD-M20A Photo Diode Array UV-Vis, coupled with Phenomenex Luna 5 µ C-18 column (250 × 4.6 mm ID) was used. 1 mL of HPLC grade methanol was added to 1 mg of sample and sonicated for 10 min followed by

centrifugation at 3000 rpm for 15 min. The final volume of the sample was made up to 10 mL with methanol and filtered using a 0.22-micron filter (Millipore). 20 µL of sample was injected for development of the chromatogram for 55 min. A binary gradient with mobile phase consisting of: (solvent A) water-acetic acid (0.5% v/v) and (solvent B) acetonitrile: water-acetic acid (99.5–0.5) = 80: 20 (v/v). The flow-rate was maintained constant at 1 mL/min and the solvent gradient elution program was as follows: 0–10 min, 90% A, 10% B; 10–20 min, 90–80% A, 10-20% B; 20-30 min, 80-60% A, 20-40% B; 30-40 min, 60-40% A, 40-60% B; 40-45 min, 40-30% A, 60-70% B; and 45-50 min, 30-90% A, 70-10% B.[43]

Statistical Analysis

Apart from the numbers that are given as means ± SEM, with n = 3, the data was analyzed by ANOVA and two-tailed Student's test ($P < 0.05$).

4.1.7Murine Pancreatic Glucosidases Inhibitory Activity of Plant Extracts

In this study, murine pancreatic enzyme activity measured at 0.24 U/min was used as the baseline, representing 100% enzyme activity. The petroleum ether extract from the bulb of Dioscorea bulbifera showed notable inhibition of pancreatic glucosidase activity, reducing it by 22.23%. In comparison, Gmelina glauca extracts showed lower levels of inhibition—16.18% for the stem, 15.92% for the leaf, and 16.37% for the flower.When ethyl acetate was used as the solvent, D. bulbifera again showed strong inhibitory activity at 23.59%, while G. glauca extracts from different plant parts produced similar but slightly lower effects.The trend continued with methanol and 70% ethanol extracts: D. bulbifera inhibited enzyme activity by 26% with methanol and 18.13% with 70% ethanol. Notably, the methanol extract of D. bulbifera showed a statistically significant difference ($p < 0.05$) when compared to the other extracts, highlighting its stronger potential as an enzyme inhibitor.(Table 1).[6][39]

Table 1. Percent murine pancreatic glucosidase inhibition by plant extracts.

Acarbose [40.89 ± 1.03]	% Glucosidase inhibitory activity			
Petroleum ether	Ethyl acetate	Methanol	Ethanol [70%]	
G. glauca				
Stem	16.81 ± 0.25	19.32 ± 1.64	16.37 ± 1.35	13.12 ± 1.96
Leaf	15.92 ± 1.9	19.76 ± 1.94	18.76 ± 1.88	14.3 ± 1.03
Flower	16.37 ± 2.6	13.71 ± 2.11	18.87 ± 0.89	16.07 ± 2.48
D. bulbifera				
Bulb	22.23 ± 2.71	23.59 ± 0.71	26.1 ± 0.87	18.13 ± 0.15

Conclusion:

Traditionally used for detoxifying, anti-inflammatory, and hemostatic properties, Dioscorea bulbifera L. is a medicinal plant known best in Asia and Africa. This review examined the plant's diverse pharmacological potentials: antimicrobial, anti-inflammatory, anti-tumor, and antidiabetic. Active compounds from D. bulbifera have undergone significant in vitro and in vivo evaluation, suggesting a good source for generating new therapeutic entities. This plant has been shown to inhibit the key enzymes α-amylase and α-glucosidase, which may be useful in the treatment of diabetes, while anti-inflammatory and analgesic properties could be applied in the management of pain. D. bulbifera exhibits anti-tumor activity through bioactive compounds like diosbulbins and flavonoid derivatives, which may be useful in cancer treatment.

To date, most of this research has supported the efficacy of D. bulbifera; however, clinical trials and toxicity studies are still needed to establish the therapeutic effectiveness and safety of the plant. The formulation of modern medicines from bioactive compounds from D. bulbifera could create new natural alternatives for considerable human diseases. With diverse pharmacological effects, D. bulbifera is an excellent candidate to develop for integration into modern medicine; however, further study is needed to achieve this aim.

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