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Effects of *Dioscorea bulbifera* nutritional potential on *in vitro* anti-inflammatory and anti-obesity properties: Tamilnadu's unconventional food plant

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Abstract
Objective: Obesity-induced chronic inflammation has been combated with dietary methods that include structured lipids such as medium-chain fatty acids and diacylglycerols. Obesity is indeed a multifaceted condition that results to a variety of life-threatening disorders. It is quickly becoming one of the most potentially serious issues, with significant economic repercussions.
Methods: Obesity is on the rise at an alarming rate, so finding natural products that can help people lose weight while having the fewest adverse effects has been more important. One of the most commonly investigated techniques for determining the potential efficacy of anti-obesity agents is the inhibition of digestive enzymes. Using two complementary methods: ABTS radical scavenging activity and iron reducing power tests, complete aqueous extracts of Dioscorea bulbifera were evaluated for inhibitory activity against pancreatic lipase, lipoxygenase, and protein denaturation, as well as for anti-oxidant potential, using two complementary methods: ABTS radical scavenging activity and iron reducing power assays.
Results: The total phenolic and flavonoid content of Dioscorea bulbifera aqueous extracts was also calculated quantitatively. The results revealed that Dioscorea bulbifera aqueous extracts were the most active plants Inhibition of lipase 64.23%, lipoxygenase inhibition 81.23% and albumin denaturation 82.31%.
Conclusion: As a result, the current study reveals that aqueous extracts of Dioscorea bulbifera could be useful in the treatment of obesity.

Keywords: *anti-inflammatory*, *anti-obesity*, *Dioscorea bulbifera*

Introduction

Plants have long been used as a base for medicine, and they are an important part of the health-care system. In India, there are over 45,000 plant species, with a distinct hotspot in the Eastern Himalayas, Western Ghats, and Andaman and Nicobar Islands. Although there are 3000 plants with therapeutic potential that have been securely recorded, traditional medicine practitioners handle around 6000 plants. India is the world's leading producer of medicinal plants and is appropriately referred to as the "Botanical Garden of the World" (Kantati *et al*., 2016) [8]. Herbal plants seem to be the greatest bioresources of traditional and modern medical medications, nutraceuticals, food development, folk medicine, pharmaceutical intermediates, and chemical creatures for synthetic drugs. For thousands of years, medicinal plants have been employed in traditional treatments for a variety of human illness, and they continue to be an important restorative assistance in the recovery of different ailments (Davidson-Hunt, 2000) [5].

Because phytochemical molecules exist in herbal plants, they are beneficial for both healing and curing human ailments. Phytochemicals are naturally occurring compounds found in herbal plants, leaves, vegetables, and roots that act as a resistance mechanism and provide protection against a variety of ailments. There are two types of phytochemicals: major and minor compounds. Primary constituents include chlorophyll, proteins, and common sugars, whereas secondary constituents include terpenoids, alkaloids, flavonoids, steroids, saponins, and phenolic compounds (Hamilton, 2004) [7]. Plant secondary metabolites known as phenolic compounds are one of the most abundant and widely distributed categories of secondary metabolites. The biological actions of phenolic compounds, which are antioxidants and free radical scavengers, have been the subject of a number of studies. Flavonoids are polyphenols that are widely distributed in plants and have a variety of biological benefits, including antioxidant, anti-inflammatory, and anti-cancer characteristics (Cox and Balick, 1996). The food industry is becoming increasingly interested in crude extracts of herbs, spices, and other plant materials that are high in phenolics and flavonoids because they prevent oxidative lipid breakdown and hence increase the quality and nutritional content of food. Nutrition is the study of food and the nutrients and other substances found in it, as well as how their action, interaction, and balance affect health and disease. The assessment of edible fruit and vegetable proximate and nutrient content is critical in determining their nutritional importance. As a result, a variety of therapeutic plant species are also used as food. Because many medical plant species are also consumed

as food in addition to their medicinal effects, determining their nutritional significance can aid in determining their value (Marwat*et al*., 2008).

Inflammation is a typical defensive response to tissue injury that entails a complicated web of enzyme activation, mediator release, fluid extravasations, cell migration, tissue disintegration, and repair. It's a complicated process that's often linked to pain and includes things like increased vascular permeability, increased protein denaturation, and membrane changes (Umapathy *et al*., 2010). Inflammation is triggered by harmful stimuli such as bacteria, irritants, or damaged cells in the vascular tissue. Inflammation is the organism's protective response to damaging stimuli and the start of the tissue's healing process. Inflammation, on the other hand, if left untreated, can lead to disorders including vasomotor rhinorrhoea, rheumatoid arthritis, and atherosclerosis. Because of their adverse effects and effectiveness, current medicines such as opiates and non-steroidal anti-inflammatory drugs (NSAIDS) are not useful in all situations of inflammatory illnesses (Ahmadiani *et al*., 1998). *Dioscorea bulbifera* is an indigenous plant that belongs to the Dioscoreaceae family, which is part of the dioscorales order. It's a tuberous-root climbing plant. Dioscorea is a vast genus of annual twinning herbs that can be found across the world's moist tropics and warm temperate zones. It's also referred to as air yam, air potato, or bulbil-bearing yam. It is known in India as Gonth, Kolkand, and Varaheekand. When boiled or cooked, the tuber is edible. A single dose of one teaspoon of tuber powder and water administered orally heals stomach pain (Gao *et al*, 2007) [6]. *Dioscorea bulbifera* has traditionally been used to reduce the glycemic index, thereby providing better protection against diabetes and obesity (Punjani *et al*., 2007) [10]. It is commonly used in traditional Indian and Chinese medicine to treat sore throats, goitres, gastric cancer, and rectum carcinoma (Chandra Subhash *et al*., 2012) [2]. This tuber contains plant reserves, namely starch, and is frequently consumed by humans. Many of the plants are stored as secondary metabolites in the tuber, which are generally referred to as anti-nutritional agents. Most studies have not found high levels of flavonoids, terpenoids, cholesterol, or alkaloids. Because of the presence of diosgenin, a steroidal medicine, *Dioscorea bulbifera* has been revered by herbalists for ages. It is indeed employed as a starting point for the production of hormones and corticosteroids, which help men become more fertile. Saponins have a natural inclination to repel germs, making them a promising therapy option for fungal and yeast infections.

Materials and Methods

Plant collection

Fresh vegetable marked *Dioscorea bulbifera* was collected in K.K. Nagar (June 2017). Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamilnadu, validated the plant.

Extraction

For 1 minute, the aerial bulbils of *Dioscorea bulbifera* were crushed in a blender to a paste-like consistency. The homogenised sample was first freeze dried to minimise moisture content for a more efficient extraction process, and then the filter solution utilised for further research was boiled with water.

Phytochemical Screening

Standard techniques were used to detect the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins, and polyphenols in the aerial bulbils of *Dioscorea bulbifera* decoction (Harborne 1973; Trease and Evans 1983).

Pancreatic Lipase Inhibitory Activity

The lipase inhibition activity of *Dioscorea bulbifera* aerial bulbils aqueous extracts was tested using Khan *et al*.'s (2010) technique. The activity of porcine pancreatic lipase was evaluated using p-nitrophenyl butyrate (NPB) as a substrate in this assay. In a 0.1 mM potassium phosphate buffer (pH 6.0), a lipase solution (1 mg/mL) was produced. 1 ml of formulated decoction of cumin seed, flax seed, and ragiwere are pre-incubated with 1 ml of lipase for 10 minutes at 37°C to assess lipase inhibitory action. After that, 0.1 mL NPB substrate was added to start the reaction. The amount of p-nitrophenol produced in the reaction was measured at 405 nm using a UV-Visible spectrophotometer after 15 minutes at 37°C, and the percentage of inhibitory activity was measured.

Lipoxygenase Inhibition Assay

With minor modifications, a spectrophotometric assay for determining LOX activity was utilised as described (Kemal *et al*., 1987). The experiment was performed with soybean 15-lipoxygenase (15-LOX). The loss of soybean 15-LOX activity (5 µg) was measured in inhibition experiments using 0.2 µM linoleic acid (Sigma) as the substrate produced in a solubilized condition (Kemal *et al*., 1987) in 0.2M borate buffer (pH 9.0). Inhibition tests were conducted using a UV-Vis spectrophotometer in the presence of various doses of aerial bulbils aqueous extracts of *Dioscorea bulbifera* (25, 50, 75, 100 µg/mL) and a reference component, quercetin (Beckman Coulter, DU 730 Life Sciences). IC50, which represents the concentration needed to inhibit 50% of LOX activity, was also determined. Equation used to compute the hydroperoxide content and lipoxygenase activity is, Specific activity (LOX) =ΔA. V/ε.l.c

Wherein, ΔA represents the value of absorbance increase per min, V is the volume of incubation mixture, ε is the extinction coefficient for linoleic acid (25 x 10^{-3} mol/l/cm), l is the length of the cuvette (1 cm) and c is the concentration of enzyme in mg (0.005). The figures are the average of three separate experiments.

Inhibition of Albumin Denaturation

In a PBS solution, 0.2 percent (w/v) egg albumin was made (pH 6.4). A total of 50 mL of aerial bulbils aqueous extracts of *Dioscorea bulbifera* standard were added to 5 mL of this stock solution at various concentrations. The test tubes were heated for 5 minutes at 72°C before being cooled. At 660 nm, the absorbance of these solutions was measured.

ABTS Radical Scavenging Activity Assay

According to Re *et al*., (1999), an equivalent amount of ABTS stock solution (7.4 mM) was added to a 2.6 mM potassium persulfate solution and maintained at room temperature in the dark for 16 hours to generate a dark coloured solution containing the ABTS radical cation. The ABTS radical cation was diluted to an initial absorbance of 0.7 ±0.02 at 734 nm before usage. The free radical scavenging activity of Rheum rhizome extract was tested by adding 10 µL of the extract to 290 µL of ABTS working solution at five different final concentrations (0.25, 0.5, 1.0, 2.5, and 5.0 mg/mL). ABST radical scavenging activity was measured in mg of trolox equivalent (TE) per mL of *Dioscorea bulbifera* aerial bulbils aqueous extracts.

Ferric Reducing Antioxidant Power Assay

Benzie and Strain described a method for measuring reducing power based on Fe(III) to Fe(II) conversion (1996). In a 10:1:1 ratio, 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM ferric chloride (FeSO4•6H2O) solution were combined to make the FRAP working reagent. 10µL aerial bulbils aqueous extracts of *Dioscorea bulbifera* (25, 50, 75, 100 µg/mL) were mixed with 10µL distilled water, then 100 µL of prewarmed FRAP working reagent were added. At a wavelength of 593 nm, the optical density of the mixture was measured. The usage of ferrous sulphate (FeSO4•7H2O) to reduce power was described.

Result and Discussion

Phytochemical screening

The phytochemical screening of *Dioscorea bulbifera* aerial bulbils aqueous extracts revealed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides, and saponin, as well as the absence of glycosides and tannin (Table -1).

Table 1: Phytochemical screenings of from the aerial *bulbils* aqueous extracts *of Dioscorea bulbifera*

Sl. No.	Phytochemical Constituents	Observation	Aerial *bulbils* aqueous extracts *of Dioscorea bulbifera*
1	Alkaloids		
	Dragendorff's test	Orange /red precipitate	+
	Mayers test	Cream precipitate	+
2.	Flavonoids		
	Alkalai Reagent	Intense yellow colour	+
	Lead aceate test	Precipitate formed	+
3.	Glycosides		
	Keller-Killiani test	Pink colour (Ammonia layers)	+
4.	Tannin		
	$FeCl_3$ test	Blue-black colour	+
5.	Saponins		
	Frothing test	Foam	-
6.	Terpenoids		
	Salkowski test	Reddish brown colour ring formed in interface	-
7.	Polyphenols		
	-Ferrozine test	Raddish blue	+
8.	Anthocyanin		
	-Ammonia test	Pink color in ammonia layer	+

+ Positive result; - Negative result

TLC Profile of Aerial *Bulbils* Aqueous Extracts *of Dioscorea Bulbifera*

The anti-obesity and anti-inflammatory compound was extracted efficiently using an aqueous decoction loaded on Pre-coated TLC plates (60 F2 54 Merck) and developed with a solvent system of petroleum ether, chloroform, and methanol in the ratio of 1:0.5:0.1. UV 240nm and 360nm were used to examine the produced plate (Fig-1)

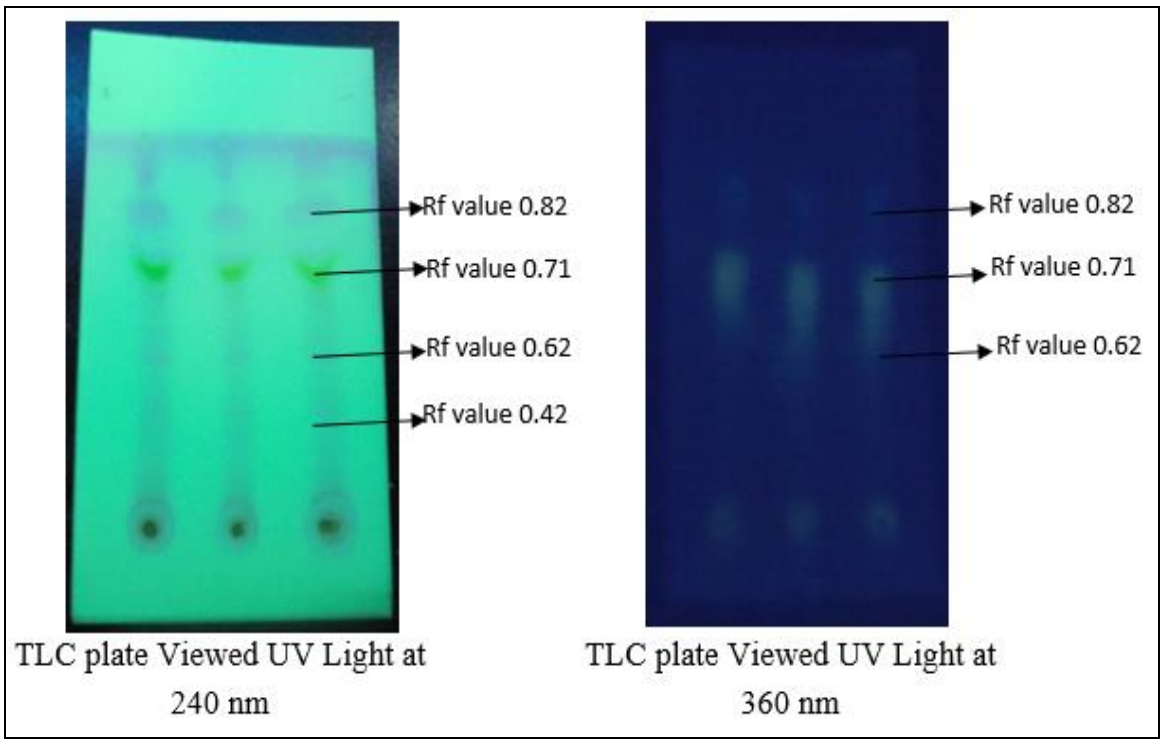

Fig 1: TLC profile of decoction from the aerial bulbils aqueous extracts of *Dioscorea bulbifera*

Anti-Obesity Activity Inhibition of Lipase Assay

Table 2 shows the percent lipase inhibition activity of aerial bulbils aqueous extracts of *Dioscorea bulbifera*. When compared to standard orlistate, aerial bulbils aqueous extracts of *Dioscorea bulbifera* showed considerably ($p < 0.05$) higher percent lipase inhibition and EC_{50}, whereas aerial bulbils aqueous extracts of *Dioscorea bulbifera* showed significantly lower percent lipase inhibition and EC_{50} 78.32 µl/ml. The aqueous extracts of aerial bulbils of *Dioscorea bulbifera* prevent the breakdown of dietary lipids into fatty acids. Flavonoids and alkaloids in aerial bulbils aqueous extracts of *Dioscorea bulbifera* were found to inhibit triglyceride breakdown and act as bioactive phytoconstituents. Flavonoid glycosides were the primary chemicals extracted from *Polygonum multiflorum*, according to Choi *et al*. (2018) [3], and they had good physiological activity. In obese people, kaempferitrin can lower the mRNA expression of fat synthesis genes and pro-inflammatory genes TLR4, TNF -α, and NLRP3, and boosting PPAR- α mRNA expression.

Table 2: Anti-obesity activity inhibition of lipase assay by aerial bulbils aqueous extracts of *Dioscorea bulbifera*

Different concentration of extract	Aerial bulbils aqueous extracts of *Dioscorea bulbifera*	Standard Orlistate
25 µl/ml	18.23±0.89	16.34±2.89
50 µl/ml	36.32±2.39	34.56±1.23
75 µl/ml	48.32±2.47	46.32±1.47
100 µl/ml	64.23±2.36	61.23±2.36
EC_{50} Value	78.32±1.45	83.12±0.78

The results are expressed as a percentage of Lipase formation that was suppressed compared to the control. The mean+SD of three experiments is shown by each value.

Anti-inflammatory properties of lipoxygenase inhibition by aerial bulbils aqueous extracts of *dioscorea bulbifera*

The anti-inflammatory effect of aerial bulbils aqueous extracts of *Dioscorea bulb*ifera was tested by inhibiting LOX using linoleic acid as a substrate. At 100 l/ml, the aerial bulbils aqueous extracts of *Dioscorea bulbifera* extract inhibited more than the other concentrations. At 100 µl/ml, the inhibition percentage was over 81.23 percent (Table-3).

At 100 µg/mL, the conventional diclofenac sodium showed a 73.21 percent inhibition. *Dioscorea bulbifera* aerial bulbils aqueous extracts had stronger inhibitory activity than the positive control. The addition of molecular oxygen to fatty acids with a cis, cis-1, 4-pentadiene system is catalysed by lipoxygenase. Unsaturated fatty acid hydroperoxides are formed as a result of this process. These compounds are then transformed into others that are important in the inflammatory process (Wang *et al*., 2019). As a result, substances capable of inhibiting that enzyme can be designated as antioxidants and anti-inflammatory agents.

Table 3: Inhibition activity of Lipoxygenase of aerial bulbils aqueous extracts of *Dioscorea bulb*ifera

Flavonoid Concentration	Aerial bulbils aqueous extracts of *Dioscorea bulbifera*	Standard Diclofenac sodium
25 µl/ml	22.36±2.46	18.32±1.63
50 µl/ml	40.23±1.78	37.32±1.78
75 µl/ml	57.32±2.89	54.23±1.46
100 µl/ml	81.23±0.23	73.21±0.89
EC_{50} Vlaue	56.32±1.78	69.32±2.45

The results are expressed as a percentage of Lipoxygenase inhibition compared to control. The mean+SD of five experiments is shown by each value.

Inhibition of protein denaturation

The effect of aerial bulbils aqueous extracts of *Dioscorea bulbifera* of remarkable action on protein denaturation inhibition was compared to that of the standard drug Diclofenac sodium. Denaturation of protein may cause the formation of auto antigen in some arthritic diseases. According to the findings of this study, aerial bulbils aqueous extracts of *Dioscorea bulbifera* are capable of reducing auto antigen production and inhibiting protein denaturation in rheumatic disease. The highest percentage inhibition of protein denaturation was found in aerial bulbils aqueous extracts of *Dioscorea bulbifera*, which was 59.32 percent at 100 µg/ml, which was similar to the percentage inhibition of diclofenac sodium, which was 57.23 percent (Table-4).

Table 4: Inhibition activity of protein denaturation of aerial bulbils aqueous extracts of *Dioscorea bulbifera*

Flavonoid Concentration	Aerial bulbils aqueous extracts of *Dioscorea bulbifera*	Standard Diclofenac sodium
25 µl/ml	20.13±0.23	17.23±1.46
50 µl/ml	43.21±1.63	40.23±2.37
75 µl/ml	61.32±2.45	56.32±2.36
100 µl/ml	82.31±1.23	74.32±1.56
EC_{50} Vlaue	59.32±2.45	57.23±1.78

The results are expressed as a percentage of protein denaturation inhibition compared to control. The mean+SD of five experiments is shown by each value.

ABTS Radical Assay

The antiradical activity of aerial bulbils aqueous extracts of *Dioscorea bulbifera*, as a demonstration of nourishing food supply, was assessed in vitro using the ABTS assay, as well as the possibility of ABTS discolouration. The aerial bulbils aqueous extracts of *Dioscorea bulbifera*, as well as the assays used, showed significant differences. Table 5 shows the results of antioxidant activity obtained for the tested samples, as well as the Vitamin-C utilised as a standard. It is clear that aerial bulbils aqueous extracts of *Dioscorea bulbifera* displayed significant antioxidant activity, which was significantly greater than Vitamin C. However, the current study found that these activities were primarily due to the presence of polyphenol chemicals.

Table 5: Free radical-scavenging ability using ABTS assay of aerial bulbils aqueous extracts of *Dioscorea bulbifera*

Different concentration of extract	ABTS radicalactivity	
	Aerial bulbils aqueous extracts of *Dioscorea bulbifera*	Standard Vitamin-C
25 µl/ml	18.23±2.45	14.23±2.36
50 µl/ml	34.56±1.98	29.32±1.48
75 µl/ml	46.32±2.36	42.31±2.36
100 µl/ml	68.23±1.78	65.23±1.45
EC_{50} value	61.23	68.32

The results are expressed as a percentage of ABTS ability inhibited compared to control. The mean+SD of three experiments is shown by each value.

Reducing Power Assay

The premise behind the reducing power assay is that compounds having a reduction potential combine with potassium ferricyanide (Fe^{3+}) to generate potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric–ferrous complex with a maximum of 700 nm absorption. With increasing amounts of sample and standard concentrations, the reducing power of aerial bulbils aqueous extracts of *Dioscorea bulbifera* and standard increases. Several studies have indicated that phenolic compounds are key antioxidant elements in selected plants, and there are direct correlations between their antioxidant activity and total phenolic content, according to the findings.

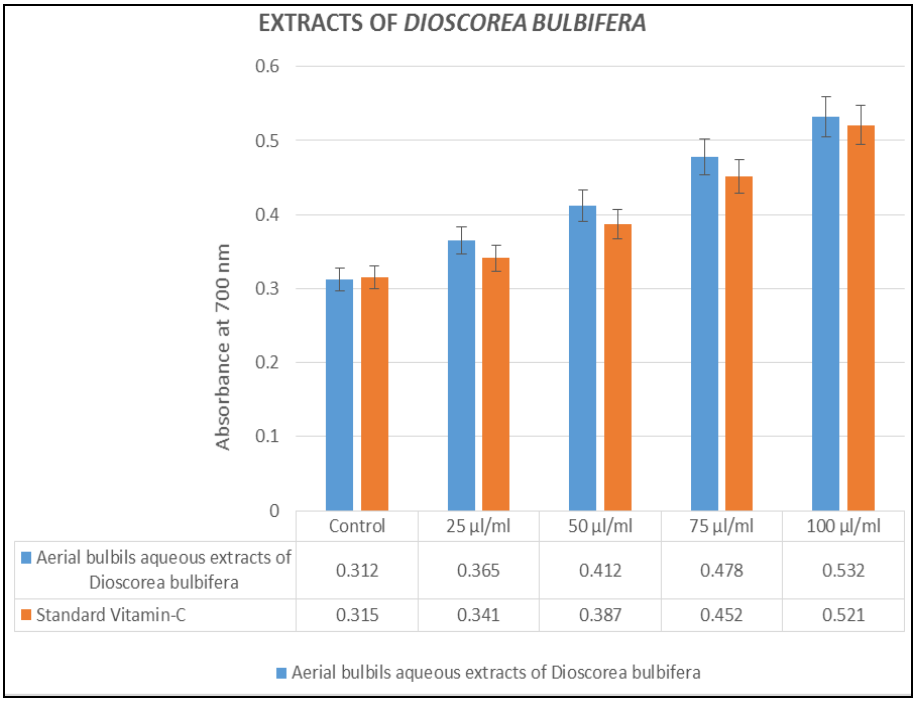

Fig 2: Reducing Power Assay of Aerial Bulbils Aqueous Extracts of Dioscorea Bulbifera

Conclusion

The outcomes of this study back up the plant's traditional use as a medicinal agent. The high phenolic content of aerial bulbils aqueous extracts of *Dioscorea bulbifera* confers stronger anti-inflammatory and anti-obesity activities. Furthermore, there was a strong link between TLC profile and the extract's anti-inflammatory and anti-obesity properties. These findings imply that the water infusion made from *Dioscorea bulbifera* aerial bulbils aqueous extracts is a good source of inflammatory metabolites. It will take more research to figure out what their active metabolites are.

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Reference

1. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," Analytical Biochemistry,1996:239(1):70-76.
2. Chandra Subhash, Saklani Sarla, Mishra P, Abhay, Bamrara Anoop. Nutritional Profile and Phytochemical Screening of Garhwal Himalaya Medicinal Plant Dioscorea bulbifera. Int. Research J. of Pharm, 2012.
3. Choi RY, Lee HI, Ham JR, Yee ST, Kang KY, Lee MK. Heshouwu (Polygonum multiflorum Thunb.) ethanol extract suppresses pre-adipocytes differentiation in 3T3-L1 cells and adiposity in obese mice. Biomed Pharmacother,2018:106:355-362.
4. Cox PA, Balick MJ. The ethnobotanical approach to drug discovery," Scientific American,1994:270(6):82-87.
5. Davidson-Hunt I. Ecological ethnobotany: sumbling toward new practices and paradigms. MASA J,2000:16:1-13.
6. Gao HL Wu, Kuroyangi M. "Seven compounds from D. bulbifera L.'' Natural Medicines,2007:55(5):277.
7. Hamilton AC. Medicinal plants, conservation and livelihoods," Biodiversity and Conservation,2004:13(8):1477-1517.
8. Kantati YT, Kodjo KM, Dogbeavou KS, Vaudry D, Leprince J, Gbeassor M. Ethnopharmacological survey of plant species used in folk medicine against central nervous system disorders in Togo.J Ethnopharmacol,2016:2(181)214-20.
9. Marwat SK, Khan MA, Ahmad M, Zafar M, Rehman F. Ethnomedicines for treatment of various diseases in D.I. Khan District," Sarhad Journal of Agriculture, 2008, 24. article 2.
10. Punjani BL. Some less known ethnomedicinal plants used by Maldaris of Sasan Gir forest n Junagardh district, Gujarat. Ethnobotany,2007:19(1-2):116-119.
11. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. "Antioxidant activity applying an improved ABTS radical cation decolorization assay," Free Radical Biology and Medicine,1999:26(9-10):1231-1237.

12. Wang J, Zhao Q. Kaempferitrin inhibits proliferation, induces apoptosis, and ameliorates inflammation in human rheumatoid arthritis fibroblast-like synoviocytes. Phytother Res,2019:33:1726-1735.
13. Ya Wu, Fang Tan, Tianyu Zhang, Binglin Xie, Lixian Ran, Xin Zhao. The anti-obesity effect of lotus leaves on high-fat-diet-induced obesity by modulating lipid metabolism in C57BL/6J mice. Applied Biological Chemistry, 2020, 63. Article number: 61