ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 11, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

ASSESSMENT OF TOTAL PHENOLIC CONTENT (TPC), TOTAL FLAVONOID CONTENT (TFC) AND ANTIDIARRHEAL AND ANTIOXIDANT ACTIVITIES OF DIOSCOREA BULBIFERA TUBER EXTRACT

MD. ASHRAFUL ISLAM, DIPANKAR CHAKRABORTY, SREEBASH CHANDRA BHATTACHARJEE, MD. HASAN ALI, MD. FARHAD SARKER, MD. SAMRAT MOHAY MENUL ISLAM

BCSIR Chattogram Laboratories, Bangladesh Council of Scientific and Industrial Research, Bangladesh.
***Corresponding author: Ashraful Islam; Email: ash.bcsir@gmail.com**

Received: 03 July 2024, Revised and Accepted: 08 October 2024

ABSTRACT

Objective: This study aims to conduct a comprehensive phytochemical screening of the tuber of *Dioscorea bulbifera* extract, evaluate its antioxidant capacity, and assess its anti-diarrheal activity.

Methods: In this research, we reported the phytochemical screening, antidiarrheal and antioxidant activity of methanol, acetone, and dichloromethane (DCM) extracts. The qualitative phytochemical assessment of the bioactive extracts of the tuber of *Dioscorea bulbifera* was done. Quantitative analysis of total phenolic contents (TPC) and total flavonoid contents (TFC) were also determined. The scavenging activity of *D. bulbifera* was determined by DPPH assay. The antidiarrheal activity was experimented by the castor oil-induced diarrhea method.

Results: TPC of Acetone, Methanol & DCM extract of *D. bulbifera* was 537.2, 352.7, and 79.12 µgGAE/ml, respectively, and TFC of those extracts were 2.96, 1.08, and 0.35 mgQE/g, respectively. The Acetone extract of *D. bulbifera* (IC_{50} 27.99 µg/ml) showed the highest antioxidant activity. Antidiarrheal properties of the three extracts were tested at 150 mg/kg, 300 mg/kg, and 600 mg/kg per body weight (bw) in mice. The methanol and DCM extracts showed an important antidiarrheal activity by curbing 69.56% and 86.95% of diarrheal faces at the doses of 600 and 300 mg/kg/bw, respectively.

Conclusion: Since the current study implies that the plant extracts gave significant results for Antidiarrheal, Antioxidant, and Phytochemical analysis, the tuber of *D. bulbifera* may contain therapeutic potential compounds, such as flavonoids, terpenoids, alkaloids, tannins, and saponins. These results moderate more investigation for the potential discovery of new natural bioactive compounds from this medicinal plant.

Keywords: *Dioscorea bulbifera*, Phytochemical, Total Phenolic Contents, Total Flavonoid Contents, antioxidant, Antidiarrheal.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i11.51957. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

In recent years, it has become a booming interest in exploring natural sources for novel therapeutic agents, driven by the need for effective treatments with minimal side effects. Among these sources, *Dioscorea bulbifera*, commonly known as air potato, has emerged as a promising candidate due to its rich pharmacological properties. This perennial vine, native to Asia and Africa, has been traditionally used for various medicinal purposes, prompting scientific investigations into its bioactive constituents and therapeutic potential [1-3]. *Dioscorea bulbifera* has different properties such as antioxidant potential [4-9], total phenolic content [10-12], antibacterial properties [13-17], and its contributions to health and medicine [18]. First and foremost, *Dioscorea bulbifera* has demonstrated significant antioxidant activity, making it a compelling subject of study in the field of natural antioxidants. In our body, harmful free radicals are neutralized by antioxidants, so it potentially mitigating oxidative stress and contributing to overall well-being [19]. The exploration of *Dioscorea bulbifera's* antioxidant capabilities provides valuable information of its potential as a functional food or drug. The total phenolic content (TPC) and total flavonoid content (TFC) of *Dioscorea bulbifera* have been the focus of numerous studies due to their association with various health benefits. Phenolic compounds and flavonoids are known for their antioxidant, anti-inflammatory, and anticancer activity to make them valuable targets for drug discovery and nutraceutical development [20,21]. Investigating the total phenolic content of *Dioscorea bulbifera* sheds light on the abundance of these bioactive compounds, further establishing its potential as a natural source of health-promoting substances. Furthermore, the plant exhibits promising antibacterial properties, making it a subject of interest in the pursuit of novel antimicrobial agents [22]. As antibiotic resistance becomes an increasingly pressing global concern, the search for alternative antimicrobial sources, such as *Dioscorea bulbifera*, becomes crucial. Studies focusing on its antibacterial properties provide a foundation for exploring its potential applications in the field of infectious disease management. In addition to its antibacterial prowess, *Dioscorea bulbifera* has been investigated for its antidiarrheal properties, suggesting potential benefits in gastrointestinal health. This facet of its pharmacological profile opens avenues for research into natural remedies for gastrointestinal disorders, providing a holistic perspective on its medicinal value. In summary, *Dioscorea bulbifera* emerges as a plant of significant interest, encompassing a spectrum of bioactive properties ranging from antioxidant, TPC, and TFC to antidiarrheal potentials. The exploration of these facets not only contributes to our understanding of the plant's therapeutic value but also holds promise for the development of new pharmaceuticals or dietary interventions in the realm of health and wellness.

METHODS

Sample preparation and extraction

A total of 20 kg Tuber of *Dioscorea bulbifera* were collected from Laxmipur-Kholabaria, Natore Sadar, Natore and Ramgarh, Khagrachori. The collected plant material was identified and authenticated by the Bangladesh National Herbarium (BNH), which plant Identification & Accession number is DACB 94821. At first, the Tuber of *Dioscorea bulbifera* was washed by distilled water and cut into small pieces.

Then it was dried at 30°C and ground to powder. This powder was extracted successively with Methanol, Acetone, and Dichloromethane (DCM) by cold extraction method. Solvents were evaporated by rotary evaporation at 50°C and the residue was freeze-dried. The dried extract was considered as the crude extract.

Phytochemical analysis

The extracts underwent phytochemical analysis to detect the presence of tannins, saponins, terpenoids, phenols, alkaloids, and flavonoids using wet chemical reactions.

Quantitative analysis of total phenolic content (TPC)

The total phenolic amount of *Dioscorea bulbifera* was destined by Folin-Ciocalteu's method. Shortly, 100 mg plant extract of methanol, acetone, and dichloromethane (DCM) extracts was taken in 50 ml VF and filled up to mark. In the test tube 500 µL methanol, acetone, and DCM extract were taken (duplicates) separately. In each test tube added 2 ml of 10% Folin-Ciocalteu's reagent and 2.5 ml of 7.5% Na_2CO_3. The same procedure was followed for Gallic acid as standard. The concentration of Gallic acid was 200, 100, 50, 25, 12.5 and 6.25 µg/ml. Absorbance was taken by UV Spectrometer at 765 nm wavelength.

Quantitative analysis of total flavonoid contents (TFC)

$AlCl_3$ complex forming test was applied to quantify the TFC of the extracts [23]. For determination of flavonoid content, quercetin was used as a standard. From the standard quercetin solutions, different conc. (8, 4, 2, 1 and 0.5 mg/ml) were prepared in methanol. 200 µl of each of the standard solution was added to 1ml of dis. H_2O and then mixed with 150 µl of 5% Sodium nitrite. After 5 min, 200 µl of 15% $AlCl_3$ solution was added and stood for 2 min. Then 500 µl solution of 1N NaOH was mixed orderly. The absorbance of the mixture was taken at 510 nm by UV-spectrometer. The same procedure was repeated for the extract of tuber of *Dioscorea bulbifera* and TFC was calculated as quercetin equivalents (mg QE/g). All the processes were done in duplicate.

Determination of antioxidant activities

The antioxidant properties of the tuber extract of *Dioscorea bulbifera* were tested using the 1, 1- diphenyl-2-picryl hydrazyl (DPPH) method. First, 8 mg of DPPH was added in 200 mL of methanol for preparation of the stock solution. In a test tube, 3 mL DPPH solution was combined with 2 mL of different concentrated Methanol, Acetone, and Dichloromethane (DCM) extract. 3 mL of DPPH solution was added with 2 mL of methanol which was treated as a control. Then all test tubes were kept in a dark place for about 25 min. The absorbance was determined at 517 nm wavelength. Antioxidant of Ascorbic acid (48, 24, 12, 6, 3 µg/mL) was determined as a standard. The below equation was used to determine the % of antioxidants or % of Inhibition:

$$\% \text{ of Inhibition/Scavenging activity/Inhibition} = [(A_x - A_y) \div A_x] \times 100$$

Where, A_x = Absorbance of control; A_y = Absorbance of sample.

Test for antidiarrheal activity

Experimental animals

Swiss albino mice whose weight was 20–25 g and age was 5–7 weeks, were used for this experiment. The tested mice were collected from the Animal House under the Division of Pharmacology, BCSIR Chattogram Laboratories. I was taken permission from the ethical committee of BCSIR.

Castor oil-induced diarrhea in mice

The experiment procedure was followed by Mekonnen *et al*. 2018. Before testing, all mice were fasted for about 20 h. The mice for the castor oil-induced diarrheal test were divided into eleven groups. In each group, three mice were taken. Group 1 was given distilled water (1 mL/kg) orally, which acted as a negative control group and Group 2 took loperamide (5 mg/kg) as a positive control group. Groups 3-11 received Methanol, Dichloromethane, and Acetone extracts of tuber of *Dioscorea bulbifera.* The doses of these three extracts were 150, 300, and 600 mg/kg/bw, respectively. After 1 h, each group received castor oil by 1 mL orally. Then they were placed in cages with white papers in the bottom and observed for 4 h for the count of diarrheal faces number. The total number of feces of the control group was considered as 100% defecation. The activity was revealed as % of inhibition of diarrhea. The percent of inhibition of defecation and % inhibition of diarrhea was determined by using the following equation:

% Inhibition of defecation = $[(A_c - B_s)/A_c] \times 100$; where, A_c = average No. of defecation caused by negative control and B_s = average No. of defecation caused by drug or extract.

% Inhibition of diarrhea = $[(X - Y)/X] \times 100$; where, X = average No. of diarrheal faces in the negative control and Y = average No. of diarrheal faces in the drug or extract.

RESULTS AND DISCUSSION

Table 1 shows that different phytochemical constituents such as Tannins, Terpenoids, Alkaloids, Flavonoids, Saponins, and Phenols were present in the various solvent extracts of tuber *Dioscorea bulbifera*. Phenolic and Flavonoid phenolic compounds were present in most of the solvent extract. Tannins were not found in any extract. The comprehensive phytochemical profile of *Dioscorea bulbifera* not only underscores its medicinal value but also presents significant opportunities for drug discovery. Isolating and characterizing these compounds can lead to the development of novel pharmacological agents with enhanced efficacy and safety profiles.

In this research, a quantitative study was run for TPC and TFC (Table 2). The methanol extract was high TPC (352.7 µg GAE/ml) and the Acetone extract was high TFC (2.96 mg QE/g) (Fig. 3).

Antioxidant properties of *Dioscorea bulbifera* extract, i.e., %Inhibition and IC_{50} were determined as shown in Table 3. Ascorbic acid was used as a standard for antioxidants which IC_{50} was 9.60 µg/mL. In acetone extract antioxidant properties were high (IC_{50} 27.99 µg/mL) and in DCM extract it was low (IC_{50} 4739.27 µg/mL). The antioxidant properties of *Dioscorea bulbifera* are primarily attributed to its high content of phenolic compounds, flavonoids, and essential vitamins. These antioxidants can play a critical role in scavenging free radicals, thus protecting cells from oxidative damage. Extensive *in vitro* and *in vivo* studies have demonstrated the tuber's ability to prevent oxidative stress-related conditions such as cardiovascular diseases, diabetes, and certain types of cancer [24]. The regular inclusion of *Dioscorea bulbifera* in the diet could provide substantial health benefits by mitigating oxidative stress and promoting cellular health.

Table 1: Phytochemical analysis of different extracts of D. *bulbifera* tubers

Solvent Extract	Tannins	Saponins	Alkaloids	Flavonoids	Terpenoids	Phenols
Methanol	-	-	+	+	+	+
Acetone	-	+	+	+	+	+
DCM	-	-	-	+	-	+

N.B. DCM: Dichloromethane, +: Present, -: Not present

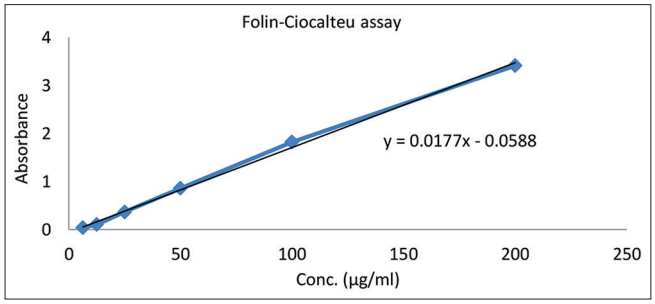

Fig. 1: Calibration curve of Gallic acid

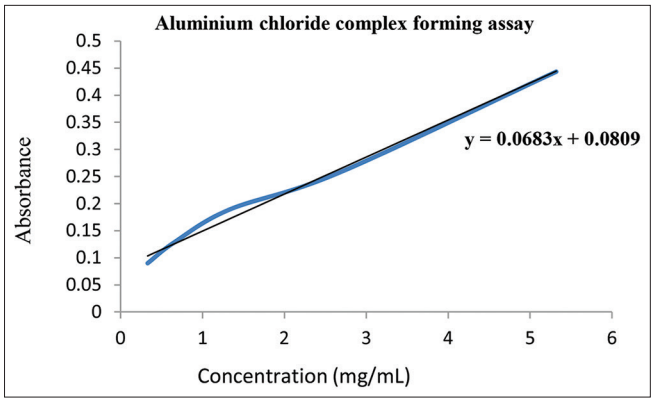

Fig. 2: Calibration curve of Quercetin

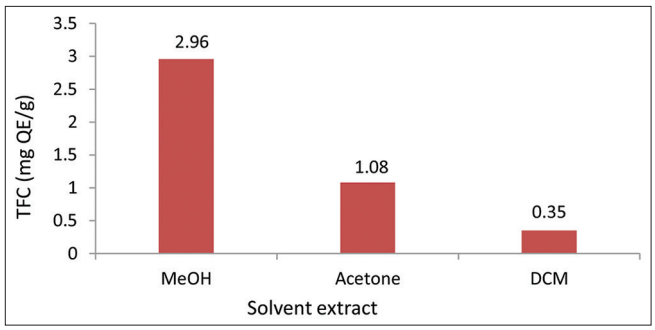

Fig. 3: Total Flavonoid content of *D. bulbifera* tuber extracts

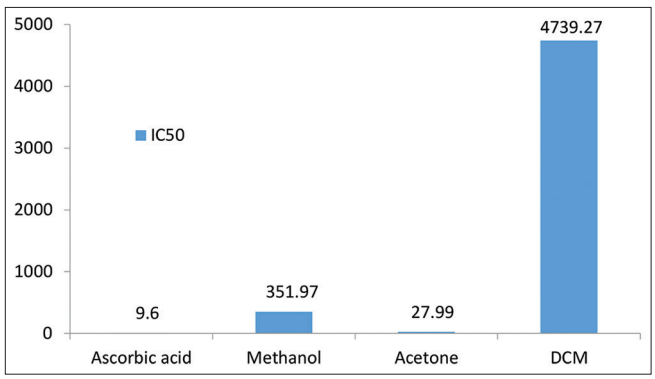

Fig. 4: IC_{50} of Ascorbic acid & different solvent extract of *D. bulbifera* tuber

The effect of different solvent extract of the tuber of *D. bulbifera* on Castor Oil-Induced Diarrhea in mice was reported in Table 4. Dichloromethane extract (300 mg/kg) was the highest antidiarrheal activity and methanol extract (150 mg/kg) was the lowest antidiarrheal activity. Diarrheal diseases are a major global health concern, particularly in developing countries where access to medical care may be limited [25]. *Dioscorea bulbifera* has demonstrated significant antidiarrheal activity, which is supported by both traditional uses and scientific research.

Contemporary scientific studies have illuminated its potential as an antidiarrheal agent, its robust antioxidant capabilities, and its rich phytochemical profile, marking it as a promising candidate for advancements in food and drug discovery.

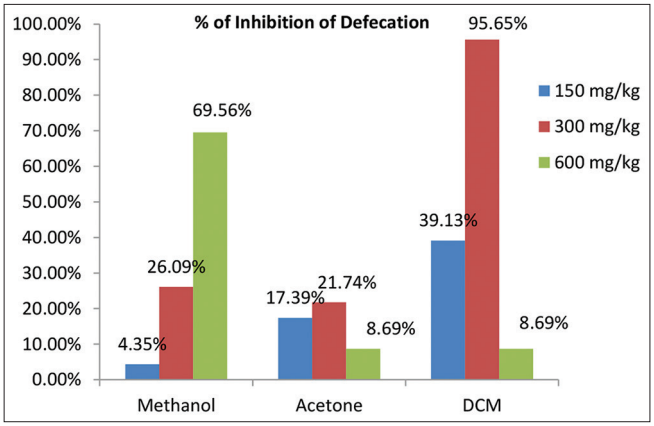

Fig. 5: % Inhibition of Defecation of Methanol, Acetone & DCM extract of the tuber of *Dioscorea bulbifera* on Castor Oil-Induced Diarrhea in mice

Table 2: Quantitative analysis of total Phenolic content (TCP) and Total Flavonoid content (TFC)

Solvent extract	TPC (µg GAE/ml)	TFC (mg QE/g)
Acetone	537.2	2.96
Methanol	352.7	1.08
DCM	79.12	0.35

GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents

Table 3: Scavenging activity of various organic solvent extracts of *Dioscorea bulbifera* tuber

Solvent extract	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Ascorbic acid	48	97.82165	9.60
	24	88.15521	
	12	79.10143	
	6	36.96392	
	3	17.97141	
Methanol	1045.5	83.39006	351.97
	522.75	72.70252	
	261.375	53.64193	
	130.69	38.25732	
	65.34	17.72634	
Acetone	560.75	86.30361	27.99
	280.375	78.82914	
	140.1875	62.66848	
	70.09	44.37032	
DCM	3852	46.22192	4739.27
	1926	28.11436	
	963	38.25732	
	481.5	29.27161	
	240.75	18.99251	

Table 4: Effect of different solvent extract of *D. bulbifera* on Castor Oil-Induced Diarrhea in mice

Sample	Conc. (mg/kg)	No. of mice	Onset of diarrhea (min)	Time (h)	No. of dry faces	total no. of dry faces after 4 h	No. of wet faces	total no. of wet faces after 4 h	Total No. of faces	Mean weight of total faces	% Inhibition of defecation	% Inhibition of diarrhea
LOP	5	3	60	1	3	3	2	12	15	0.83 g	34.78	40
				2	0		8					
				3	0		2					
				4	0		0					
DW	1 ml	2	26	1	2	3	7	20	23	1.13 g	100	100
				2	1		4					
				3	0		4					
				4	0		5					
Meth.	150	3	40	1	3	3	8	19	22	0.99 g	4.35	5
				2	0		3					
				3	0		5					
				4	0		3					
	300	2	56	1	6	6	1	11	17	0.46 g	26.09	45
				2	0		3					
				3	0		2					
				4	0		5					
	600	2	169	1	1	3	0	4	7	0.51 g	69.56	80
				2	2		0					
				3	0		1					
				4	0		3					
Ace.	150	3	10	1	2	2	4	17	19	1.51 g	17.39	15
				2	0		3					
				3	0		3					
				4	0		7					
	300	2	47	1	3	6	3	12	18	0.56 g	21.74	40
				2	3		6					
				3	0		1					
				4	0		2					
	600	3	18	1	6	8	6	13	21	0.96 g	8.69	35
				2	2		4					
				3	0		2					
				4	0		1					
DCM	150	2	20	1	5	6	5	8	14	0.71 g	39.13	60
				2	1		2					
				3	0		1					
				4	0		0					
	300	2	190	1	0	1	0	2	3	0.2 g	86.95	90
				2	1		0					
				3	0		0					
				4	0		2					
	600	3	27	1	1	5	5	16	21	1.31 g	8.69	20
				2	4		7					
				3	0		2					
				4	0		2					

LOP: Loperamide, DW: Distilled water, Meth. : Methanol, Ace. : Acetone, DCM: Dichloromethane

CONCLUSION

Dioscorea bulbifera stands out as a multipurpose plant with significant potential in both the food and pharmaceutical industries. Its antidiarrheal and antioxidant properties, coupled with its rich phytochemical content, make it a powerhouse of health benefits. Ongoing research and development could unlock further applications, contributing to advancements in food technology and drug discovery. Integrating *Dioscorea bulbifera* into mainstream health practices could offer natural, effective solutions for a range of health issues, promoting wellness and disease prevention.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to Dr. Dipankar Chakraborty, Principal Scientific Officer at BCSIR Chattogram laboratories, for his invaluable guidance and insightful feedback throughout the course of this research. We are also grateful to Dr. Mohammad Mostafa and Dr. Sreebash Chandra Bhattacharjee for their technical assistance and support in conducting experiments and analyzing data. Without their expertise and dedication, this research would not have been possible. Furthermore, we acknowledge the financial support provided by the Bangladesh Council of Scientific and Industrial Research (BCSIR), Ministry of Science and Technology, Bangladesh. This support has been crucial in funding the equipment and materials necessary for this study. Finally, we extend our thanks to our families for their unwavering support and encouragement throughout this endeavor.

CREDIT AUTHOR STATEMENT

Author Contributions: Md. Ashraful Islam and Dr. Dipankar Chakraborty conceived and designed the study. Md. Hasan Ali and Md. Farhad Sarkar conducted the experiments. Dr. Dipankar Chakraborty and Md. Ashraful Islam analyzed the data. Dr. Sreebash Chandra Bhattacharjee and Md. Samrat Mohay Menul Islam contributed to data interpretation. Md. Ashraful Islam and Md. Hasan Ali wrote the original draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version for submission.

CONFLICTS OF INTEREST

It is ensured that the authors have no conflicts of interest.

FUNDING

I collected funds from an R &D project of the 2022-2023 fiscal year, which was donated by the Bangladesh Council of Scientific and Industrial Research (BCSIR), Ministry of Science and Technology, Bangladesh.

REFERENCES

1. Adeniran AA, Sonibare MA. *In vitro* antioxidant activity, brine shrimp lethality and assessment of bioactive constituents of three wild *Dioscorea* species. J Food Meas Char. 2017;11(2):685-95. doi: 10.1007/s11694-016-9438-5
2. Kuncari ES. Nutrition value and phytochemical screening of gembolo (*Dioscorea bulbifera* L.) bulbils and tubers from Bogor, west java. J Ilmu Pertanian Indones. 2023;28(1):18-25. doi: 10.18343/jipi.28.1.18
3. Adejumobi II, Agre PA, Onautshu DO, Adheka JG, Bambanota MG, Monzenga JL, *et al*. Diversity, trait preferences, management and utilization of yams landraces (*Dioscorea species*): An orphan crop in D R Congo. Sci Rep. 2022;12(1):2252. doi: 10.1038/s41598-022-06265-w, PMID 35145169
4. Chaniad P, Tewtrakul S, Sudsai T, Langyanai S, Kaewdana K. Anti-inflammatory, wound healing and antioxidant potential of compounds from *Dioscorea bulbifera* L. bulbils. PLos One. 2020;15(12):e0243632. doi: 10.1371/journal.pone.0243632, PMID 33306733
5. Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, *et al*. Antidiabetic and antioxidant properties of copper nanoparticles synthesized by medicinal plant *Dioscorea bulbifera*. J Nanomed Nanotechnol. 2015;S6:007.
6. Savi A, Calegari GC, Santos VA, Pereira EA, Teixeira SD. Chemical characterization and antioxidant of polysaccharide extracted from *Dioscorea bulbifera*. J King Saud Univ Sci. 2020;32(1):636-42. doi: 10.1016/j.jksus.2018.09.002
7. Kwon JB, Kim MS, Sohn HY. Evaluation of antimicrobial, antioxidant, and antithrombin activities of the rhizome of various *Dioscorea* species. Korean J Food Preserv. 2010;17(3):391-7.
8. Odeghe OB, Adikwu E, Ojiego CC. Phytochemical and antioxidant assessments of *Dioscorea bulbifera* stem tuber. Biomed Biotechnol Res J. 2020;4(4):305-11. doi: 10.4103/bbrj.bbrj_96_20
9. Akinyele KN, Emma-Okon BO, Fajobi AO, Morakinyo AE, Oyedapo OO. Studies of the anti-hyperglycemic and antioxidant activities of the extract of aerial yam (*Dioscorea bulbifera*). J Med Plants Res. 2021;5(10):503-14.
10. Bhandari MR, Kawabata J. Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea spp.*) tubers of Nepal. Food Chem. 2004;88(2):163-8. doi: 10.1016/j.foodchem.2003.12.027
11. Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB. Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. Int J Mol Sci. 2010;11(6):2362-72. doi: 10.3390/ijms11062362, PMID 20640157
12. Chunthorng-Orn J, Panthong S, Itharat A. Antimicrobial, antioxidant activities and total phenolic content of Thai medicinal plants used to treat HIV patients. J Med Assoc Thai. 2012;95 (Suppl 1):S154-8. PMID 23964459
13. Kuete V, BetrandTeponno R, Mbaveng AT, Tapondjou LA, Meyer JJ, Barboni L, *et al*. Antibacterial activities of the extracts, fractions and compounds from *Dioscorea bulbifera*. BMC Complement Altern Med. 2012;12(1):228. doi: 10.1186/1472-6882-12-228, PMID 23176193
14. Adeosun OM, Arotupin DJ, Toba OA, Adebayo AA. Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoe) tubers and peels against some pathogenic bacteria. J Phytopharmacol. 2016;5(1):20-6. doi: 10.31254/phyto.2016.5105
15. Dahiya P. Phytochemical investigation and antimicrobial properties of *Dioscorea bulbifera* tuber. Asian J Pharm Clin Res. 2017;10(12):317-9. doi: 10.22159/ajpcr.2017.v10i12.21530
16. Marandi RR, Britto SJ, George M, Minj E. Pharmacognostic, fluorescent, antibacterial and phytochemical analysis of tuber of *Dioscorea bulbifera* L. from Jharkhand. J Pharmacogn Phytochem. 2016;5(1):8-14.
17. Mohanty M, Choudhury R, Kumar S, Maggirwar R. Phytochemical screening and antibacterial activity of *Dioscorea bulbifera L*. fruits. Plant Arch. 2021;21(1):862-6. doi: 10.51470/PLANTARCHIVES.2021.v21.no1.119
18. Niyas FM. Medicinal uses of *Dioscorea bulbifera*-a review. Res J Pharm Technol. 2015;8(8):1059-62. doi: 10.5958/0974-360X.2015.00182.1
19. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr J. 2015;15:71.
20. Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Smith PT, *et al*. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J Agric Food Chem. 2011;59(23):12361-7. doi: 10.1021/jf203146e, PMID 22023309
21. Oueslati S, Ksouri R, Falleh H, Pichette A, Abdelly C, Legault J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. Food Chem. 2012;132(2):943-7. doi: 10.1016/j.foodchem.2011.11.072.
22. Ghosh S, Patil S, Ahire M, Kitture R, Kale S, Pardesi K, *et al*. Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. Int J Nanomedicine. 2012;7:483-96. doi: 10.2147/IJN.S24793, PMID 22334779
23. Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT. 2021;150:111932. doi: 10.1016/j.lwt.2021.111932
24. Fu TT, Shen L. Ergothioneine as a natural antioxidant against oxidative stress-related diseases. Front Pharmacol. 2022;13:850813. doi: 10.3389/fphar.2022.850813, PMID 35370675
25. Mokomane M, Kasvosve I, De Melo ED, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: Emerging strategies in prevention and management. Ther Adv Infect Dis. 2018;5(1):29-43. doi: 10.1177/2049936117744429, PMID 29344358