ORIGINAL RESEARCH

Estimation of phytoconstituents and their Antibacterial Activity of Dioscorea bulbiferatuber extracts

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ABSTRACT
India, with an abundance of medicinal plants, holds a strong knowledge of herbal system where Ayurveda is the oldest medicinal system. The medicinal plants play vital role in disease prevention since they are rich source of phytochemicals. Phytochemicals are non- nutritive substances that have numerous health benefits. *D. bulbifera* is one such useful medicinal plant that belong to the family of *Dioscoreaceae* and is commonly known as Varahi Kanda, Kadu Kanda, Ratalu, Yam and Air potato. It has numerous traditional uses and its medicinal benefits have been reported in the Indian traditional systems of medicine. The present work encompasses phytochemical screening and evaluation of antibacterial and antioxidant activity of *D. bulbifera tubers*. The dried and powdered sample of tubers was subjected to Soxhlation using four different solvents in increasing order of their polarity. The phytochemical screening revealed the presence of variety of phytochemicals such as, alkaloids, phenols and tannins, flavonoids, glycosides etc. The phenol and flavonoid content of *D. bulbifera* was calculated by colorimetric method. The phenol content was found in great amount in ethanol extract, also the flavonoid content was maximum in ethanol extract. The antibacterial activity was evaluated by disc- diffusion method. *D. bulbifera* exhibited good antibacterial and antioxidant potential. The antioxidant potential, in terms of DPPH scavenging potential, was found maximum in ethanol extract, followed by water and chloroform extract. Maximum antibacterial activity was reported in chloroform extract against *Klebsiella* and in ethanol extract against *E. coli*.
Keywords: antibacterial, phytochemical, screening, soxhlet

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INTRODUCTION

Uttarakhand is a region with a great wealth of medicinal herbs and plants. Most of these medicinal plants are traditionally used to treat various ailments due to the presence of phytochemicals.These are basically plant metabolites synthesized in all part of plant body by itself and have some definite physiological action on animals [1]. Plants are major source of secondary metabolites such as saponins, sapogenins, carotenoids, tannins alkaloids and flavonoids which are formed as products of primary metabolism and produced for defence against predators [2]. Metabolites such as tannins, alkaloids and flavonoids, are known to be the brain behind the healing potentials of plants [3].*D. bulbifera,* the Air potatois a tribal plant, which belongs to the family *Dioscoreaceae* assigned to the order dioscorales. It is native to tropical Asia and sub-Saharan Africa. *D.bulbifera* possess potential therapeutic uses. It is found throughout India particularly in warmer places and is known as Yam or Air potato. Many tests prove that it contains flavonoids, saponin, steroids, cardiac glycosides, and terpenoids [4].It is also known as Varahi in Sanskrit, Kaachil in Malayalam, Dukkar Kand in Marathi and Gethi in Uttarakhand [5]. *D. bulbifera* is a staple food for various tribal group. It is a monocot, herbaceous, tuber-yielding climber. The tubers and bulbils are edible as it has starch, low fat, fibres and minerals which provide it nutritional value.The commercially viable phyto-steroidal sapogenin diosgenin are also found in this plant. Due to present of secondary metabolites, it is used to treat diabetes, cancer, microbial infections, cardiac problems, digestive abnormalities, syphilis, typhoid, goitre and throat infection [6].Tubers are used in leprosy, asthma, cough, cold, tuberculosis, contraceptive, constipation, indigestion, abdominal pain, muscular pain, bone fracture, dysentery, sore throat, struma, wounds, boils, cuts, injury, carbuncle, and tumour and also used as refrigerant to reduce

body heat during summer. Fresh tuber decoction cures laryngitis in children, insect bite, ring worm, goitre, and fever. Root powder is used as component of local medicine for tuberculosis. It maintains kidney function. Also used in diseases of lungs, spleen, diarrhoea, improving digestion and metabolism. Bulbils cure typhoid of children [7].

MATERIALS AND METHODS

The current study was carried out at Post Graduate Laboratory of Department of Biochemistry and Biotechnology at Sardar Bhagwan Singh University, Balawala, Dehradun (Uttarakhand), during the month of December 2022 to June 2023.

SAMPLE COLLECTION AND AUTHENTICATION

D. bulbifera plant was collected from Pithoragarh, Uttarakhand in the month of January 2023 and subjected to authentication from Department of Botany, Dayanand Brijendra Swarup (P.G.) College, Dehradun.

SOXHLET EXTRACTION

The plant material (200g) was washed with distilled water to remove soil debris, cut to small pieces and then shade dried for 15-20 days. The dried sample was crushed into fine powder by electric blender along with 800ml of each solvent, and then subjected to Soxhlation using different solvents in increasing order of polarity (petroleum ether < chloroform ethanol < water) [8]. The final products thus obtained was kept in an air-tight container and stored at 4C in the refrigerator for further studies [9].

QUALITATIVE PHTOCHEMICAL ANALYSIS

All the extracts were subjected to preliminary phytochemical screening, following the standard methods for the detection of various phytochemicals. Different extracts of *D. bulbifera* were subjected to phytochemical screening for the following phytochemicals- alkaloids, flavonoids, carbohydrates, steroids, phenols, tannins, saponins, terpenoids, glycosides, proteins, and amino acids [10-11].

QUANTITATIVE ANALYSIS

Estimation of Total Phenol Content: Different concentrations of each extract were separately mixed with 1ml of Folin- Ciocalteau phenol reagent and 0.8ml of aqueous 20% Na_2CO_3 solution and allowed to stand for 15 minutes. Then, absorbance was measured at 765nm using UV- visible spectrophotometer. The sample concentration was calculated with the help of standard plot of gallic acid (25-250 ug/ml) and TPC is expressed in terms of ug of the gallic acid equivalent (GAE) per mg of the dry mass [12].

Estimation of Total Flavonoid Content: Different concentrations of each extract were separately mixed with 0.75ml of ethanol, "0.05ml of 10% $AlCl_3$, 0.05ml of 1M potassium acetate and 1.4ml of distilled water and allowed to stand for 30 minutes. The absorbance at 415nm was measured using UV- visible spectrophotometer and the sample concentration was calculated using standard plot of quercetin (10-100 µg/ml). The TFC is expressed in µg of quercetin equivalent (QE) per mg of the dry mass [13].

Evaluation Of Antibacterial Activity: In- vitro antibacterial activity was determined by disc diffusion method. The bacterial strains used in the study are *Klebsiella spp.* and *E. coli.* They were obtained from the Microbiology Department of Sardar Bhagwan Singh University, Balawala (Uttarakhand) and identified by staining and morphological characteristics. The nutrient agar medium was prepared and then autoclaved at 121℃ for 15 minutes. The agar was then poured into 10 petri plates and allowed to solidify. Meanwhile, plant extracts were separately dissolved in Dimethyl Sulfoxide (DMSO) to obtain different concentrations i.e., 200µl/ml, 400µl/ml, 600µl/ml, 800µl/ml and 1000µl/ml. The solidified plates were inoculated with 100 µl of suspension culture of both the bacterial strains. Later, the paper discs were saturated with each extract of different concentrations, and then placed on respective section of each plate. The plates were incubated in upright position at 37"C for 24 hours. The diameter of zone of inhibition was measured in millimetres (mm). Any zone of inhibition around the well indicated the presence of antibacterial activity [14-15].

Evaluation Of Antioxidative Activity:

Dpph Radical Scavenging Activity: 2,2-diphenylpicrylhydrazyl (DPPH) solution was prepared by mixing 15mg of DPPH in 10ml of ethanol. 75µl of this solution was diluted with ethanol to obtain a final volume of 3ml. The absorbance was taken immediately at 517nm for control reading. Later, 75µl of DPPH solution was added to the tubes containing different concentrations of each sample i.e., 100 µl, 300 µl, 500 µl and 750 µl. The mixture was then diluted to 3ml with ethanol. Decrease in absorbance then measured at 517nm. Ascorbic acid is used as a reference. The results are expressed as a percentage inhibition of DPPH is calculated from the following formula-

$$\% \text{ Radical inhibition} = \frac{(A0-A1)}{A0} \times 100$$

Where, A_0- Absorbance of blank
A_1- Absorbance of sample

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RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Test performed	Petroleum ether	Chloroform	Ethanol	Water
Mayer's test	+	+	+	+
Hager's test	+	+	+	+
Wagner's test	+	+	+	+
Alkaline reagent test	-	-	+	+
Ammonia test	-	-	+	+
Ferric chloride test	-	-	+	+
Molisch's test	-	-	+	+
Fehling's test	-	-	+	+
Benedict's test	-	-	+	+
Salkowski test	-	-	-	-
Vanillin- HCl test	-	-	+	+
Foam height test	-	-	-	-
Bontrager's test	+	+	+	+
Legal test	+	+	+	+
Millon's test	-	-	-	+
Biuret's test	-	-	-	+
Ninhydrin test	-	-	-	+

Table 1. Results of Qualitative Phytochemical Analysis of different extracts of Dioscorea bulbifera. A minus (-) sign indicates a negative result for the test while a plus (+) sign indicates a positive result.

Various phytochemicals such as alkaloids, phenols, tannins, carbohydrates, proteins, flavonoids, saponins etc. were found in floral extracts of *D. bulbifera.* Different extracts displayed different confirmatory test for phytochemicals. The ethanol extract displayed positive results for most of the phytochemicals whereas water extract displayed positive results for few phytochemicals.

ESTIMATION OF TOTAL PHENOL CONTENT

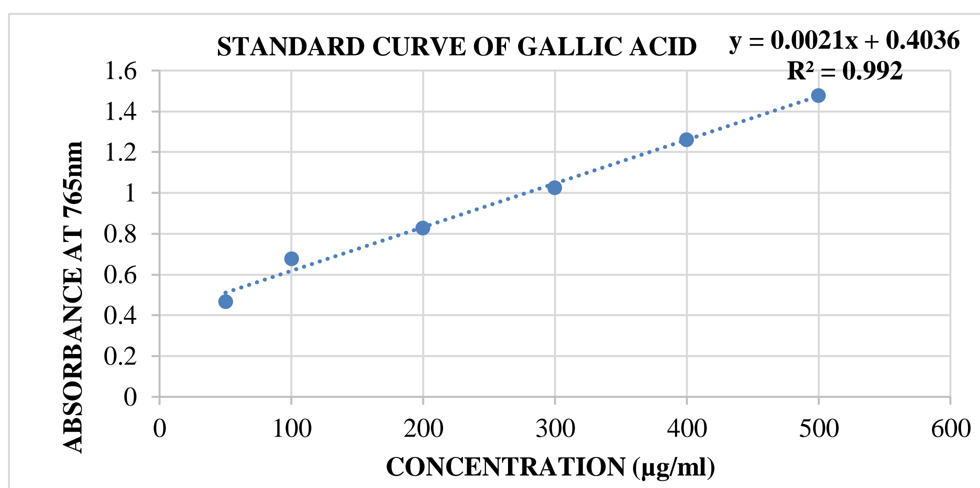

Figure 1. Standard Curve of Gallic Acid for Estimation of Total Phenol Content

Concentration of extract (mg/ml)	Phenol Content of Extracts (µg of GAE/mg)	
	Ethanol	Water
100	193.28	159.27
300	247.94	235.53
500	485.23	380.08

Table 2. Total phenol content of ethanol and water extract of *D. bulbifera.*

The standard curve was obtained using various concentrations of gallic acid. Based on this curve, phenol content of *Dioscorea bulbifera* extracts was calculated in ethanol and water extract of different concentrations (100mg/ml, 300mg/ml, and 500mg/ml). The phenol content of both the extracts is depicted in Table 2. The results suggested that the ethanol extract has more phenol content than water extract.

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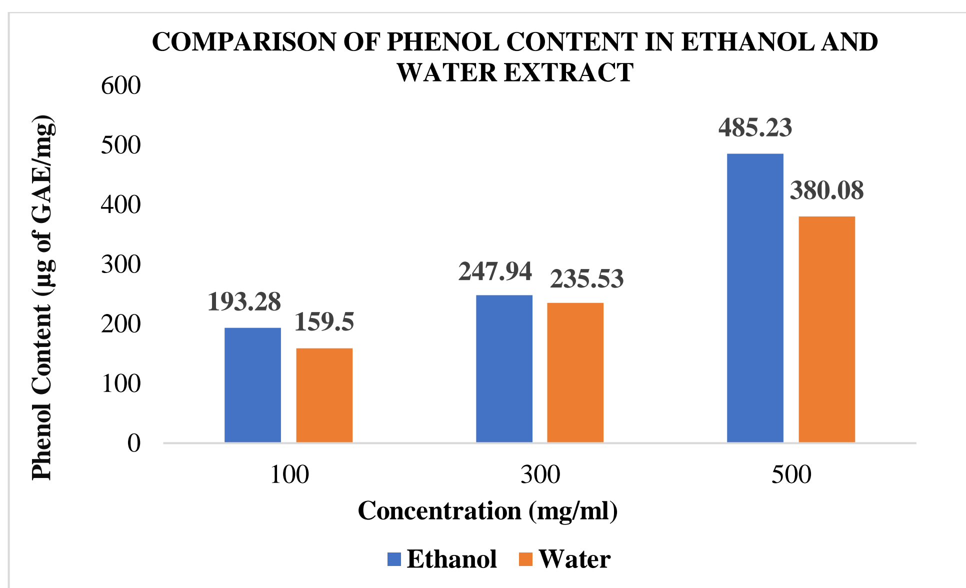

Figure 2. Comparison of Total Phenol Content in Ethanol and Water extract of *Dioscorea bulbifera*

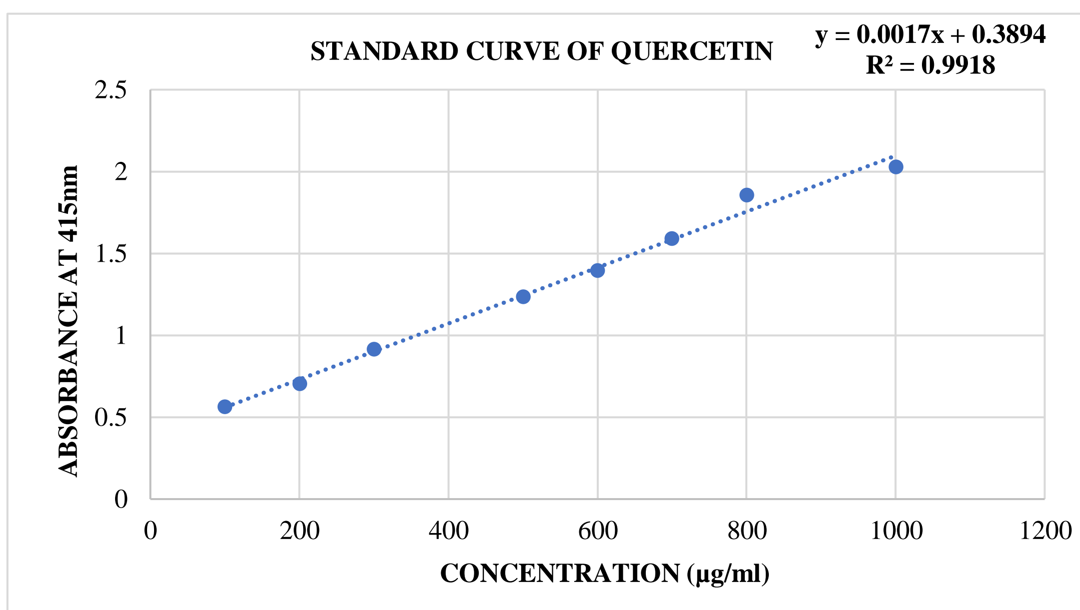

Figure 3. Standard Curve of Quercetin for Estimation of Total Flavonoid Content

Concentration of extract (mg/ml)	Flavonoid Content of Extracts (µg of QE/mg)	
	Ethanol	Water
200	271.35	170.53
400	527.28	365.87
600	818.73	448.68

Table 3. Total flavonoid content of ethanol and water extract of *Dioscorea bulbifera.*

The standard curve was obtained using various concentrations of quercetin. Based on this curve, flavonoid content of *Dioscorea bulbifera* extracts was calculated in ethanol and water extract of different concentrations (200mg/ml, 400mg/ml, and 600mg/ml). The flavonoid content of both the extracts is depicted in Table 3. The results suggested that the ethanol extract exhibited the highest flavonoid content of 818.73 mg/ml, whereas the water extract demonstrated the lowest flavonoid content of 448.68 mg/ml.

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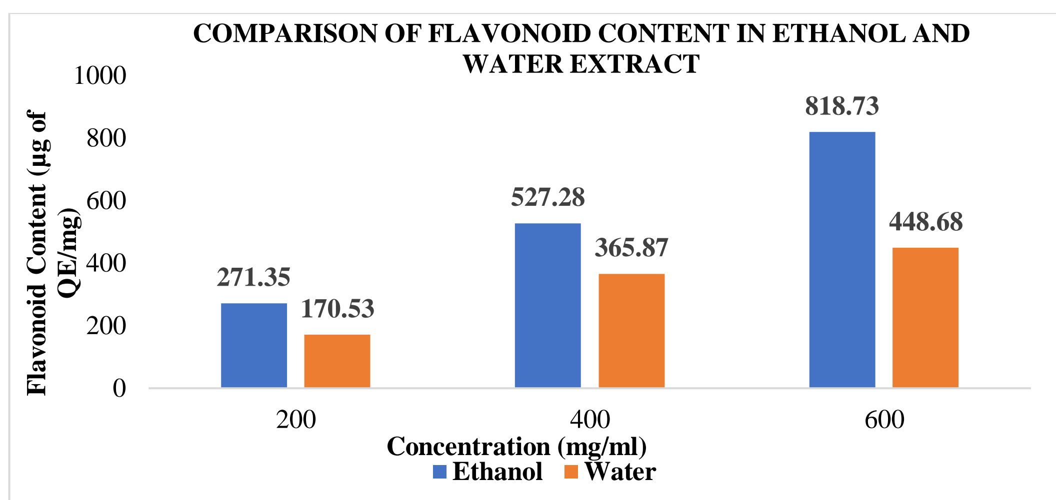

Figure 4. Comparison of Total Flavonoid Content in Ethanol and Water extract of *D. bulbifera.*

Evaluation Of Antibacterial Activity: The antibacterial activity of different extracts of *D. bulbifera* was evaluated against *Klebsiella spp.* and *Escherichia coli.* Different concentration (200µl/ml, 400µl/ml, 600µl/ml, 800µl/ml and 1000µl/ml) of each extracts displayed zone of inhibition (ZOI) of different sizes.The chloroform extract showed the highest antibacterial activity with a zone of inhibition measuring 17.99 mm, followed by the petroleum ether extract with a zone of inhibition of 17.2 mm against Klebsiella. Conversely, the ethanol extract displayed the greatest activity against Escherichia coli, resulting in a zone of inhibition measuring 11.4 mm.

Concentration of Extracts (µl)	Zone of Inhibition (mm)									
	Klebsiella spp.					*Escherichia coli*				
	200	400	600	800	1000	200	400	600	800	1000
Petroleum ether	10.1	12.9	15.7	16.2	17.2	5.3	5.6	6.1	6.2	6.4
Chloroform	11.7	13.5	16	17.2	17.9	5.1	5.7	6.2	6.8	7.3
Ethanol	8	9.3	10.2	12	13.2	9	9.7	10.2	10.7	11.4
Water	4.5	5.1	5.8	6.1	6.7	8	8.9	9.3	9.7	10.2

Table 4. Antibacterial activity of different extracts of *Dioscorea bulbifera againstKlebsiella spp.* and *Escherichia coli.*

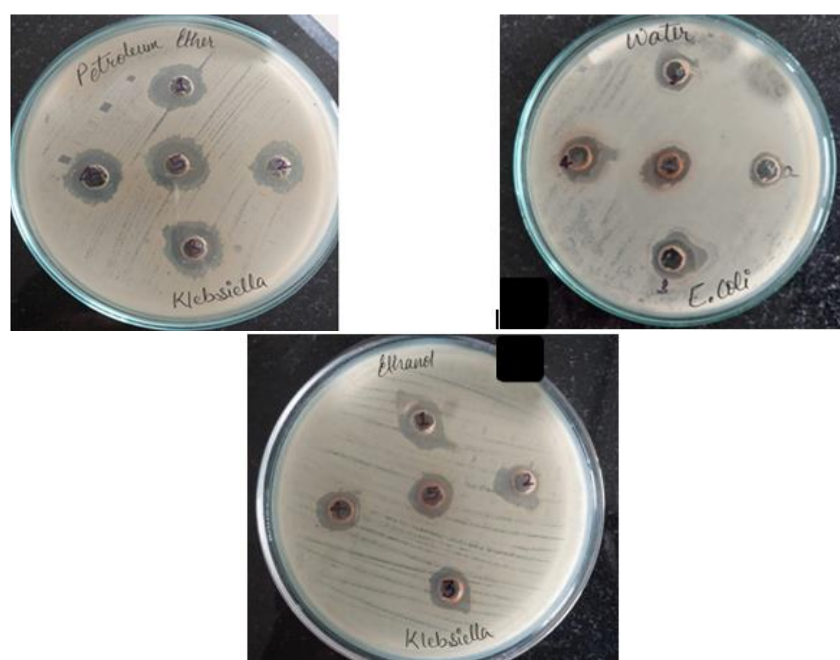

Figure 5. Antibacterial activity of *D.* bulbifera extracts against *Klebsiella spp.* (A) Petroleum ether extract and (B) Ethanol extract and against E. Coli (C) Water extract

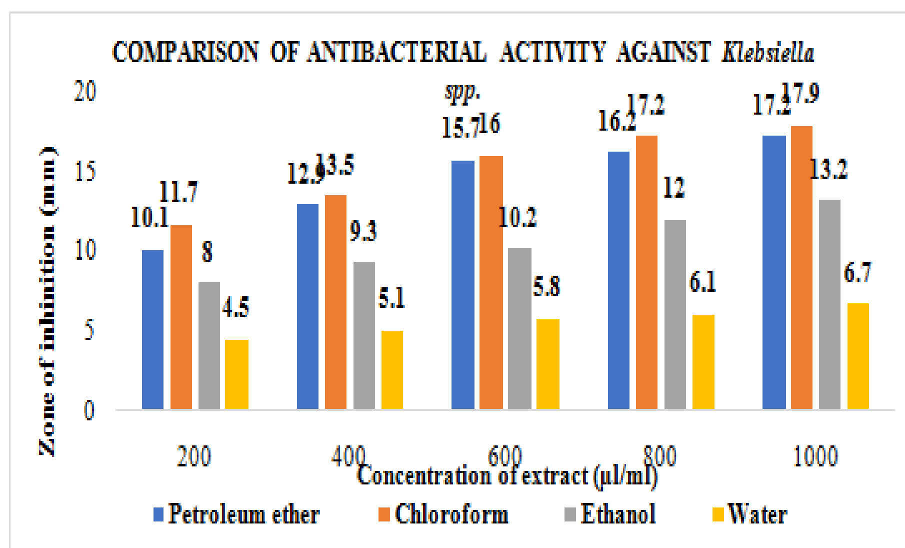

Figure 6. Graphical representation of comparative antibacterial activity of different extracts of *Dioscorea bulbifera againstKlebsiella spp*

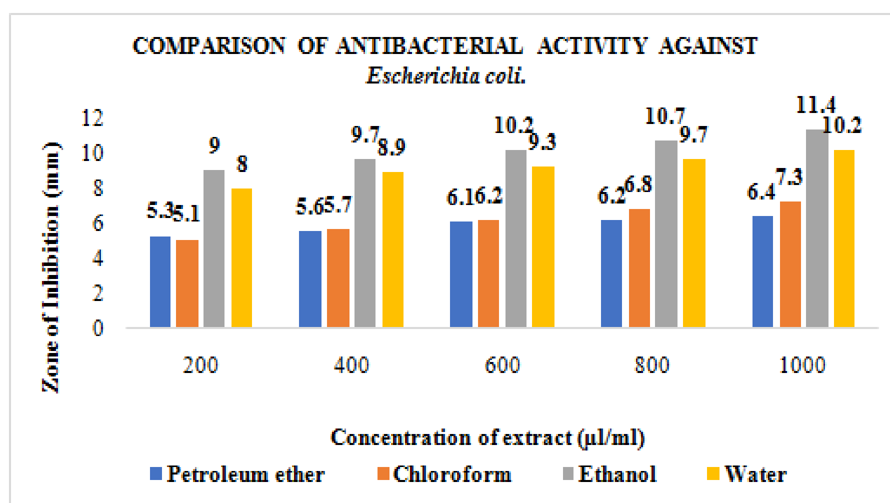

Figure 7. Graphical representation of comparative Antibacterial activity of different extracts of *Dioscorea bulbifera againstE. Coli.*

Evaluation Of Antioxidant Activity: The antioxidant activity of different extracts of *Dioscorea bulbifera* was evaluated by DPPH radical scavenging method, where ascorbic acid was used as a standard reference Table 5 reveals the antioxidant activity of different concentrations of each extract. Maximum activity was displayed by ethanol extract (79%), followed by water extract (76%). Meanwhile, minimum activity was displayed by petroleum ether extract (45%). Chloroform extract (68%) exhibited moderate scavenging activity.

Concentration of extract (mg/ml)	% Radial Inhibition				
	Standard	Petroleum ether	Chloroform	Ethanol	Water
100	57	17	41	48	46
300	78	31	57	65	63
500	92	45	68	79	76

Table 5. Antioxidant activity of *Dioscorea bulbifera* extracts in terms % radical inhibition.

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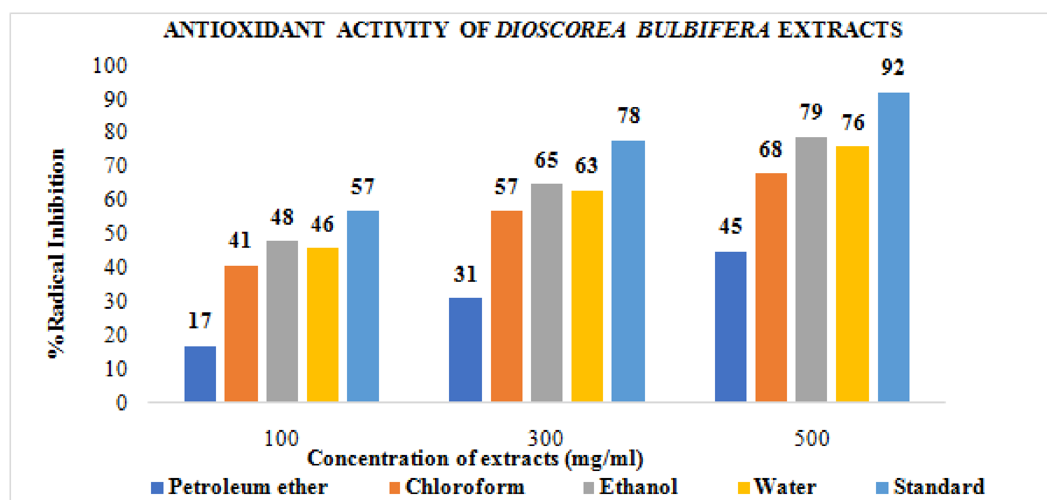

Figure 8. Graphical representation of comparative antioxidant activity of different extracts of *Dioscorea bulbifera* .

The increase in the concentration of the extracts led to a corresponding increase in the percent radical inhibition.

CONCLUSION

In the present study, a comprehensive analysis of *D. bulbifera* tuber extracts were carried out.And it revealed the presence of various essential phytochemicals. These compounds play pivotal roles in conferring biological activities to the plant and contribute to its overall therapeutic potential. The antimicrobial evaluation of *D. bulbifera* tuber extracts demonstrated promising inhibitory effects against a spectrum of microbial strains. Furthermore, the antioxidant activity screening showcased the radical scavenging potential of *D. bulbifera* tuber extracts. The presence of antioxidants, such as phenolics and flavonoids, underscored their contribution to the observed antioxidant activity. The cumulative results of qualitative, antimicrobial, and antioxidant analyses reinforce the medicinal significance of *D. bulbifera* tuber extracts.

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