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Antioxidant, Antibacterial Activities, GC-MS Analysis of Methanol Leaves Extract in Barleria Prionitis L

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Abstract :- *Barleria prionitis*, depending on the Acanthaceae family, a significant medicinal plant. To keep the feet from cracking during the monsoon season, the juice from the leaves is rubbed onto them. The current study's objectives were to assess the antioxidant, antibacterial properties of methanol extract of *B. prionitis* leaves to identify active compounds using GC-MS. Antioxidant activity was measured using the reduction test and by radical scavenging assay. The well permeation technique was employed to assess antibacterial activity. Antihemolytic activity was determined using the heat-induced hemolysis assay technique. For the GCMS analysis, the HP-5 column was utilised. The DPPH radical scavenging IC50, $ABTS^+$ radical cation scavenging IC50, superoxide radical scavenging IC50 of the B. prionitis methanol leaf extract were all 221.68 g/mL, 4.28 g/mL, and 86.88 g/mL, respectively. The RC50 of phosphomolybdenum reduction for the methanol leaves extract of *B. prionitis* was 44.31 g/L, but the RC50 of Fe3+ reduction 151.88 g/mL concentration. Value of IC50 for antihemolytic action was 91.98 g/mL. A maximal inhibition zone of 20 mm for *Staphylococcus aureus* was observed at a dosage of 500 g/mL, according to the antibacterial activity. With the aid of GCMS analysis, the antibacterial compound c-terpinen and antioxidant compounds including flavone, 3,4-dihydro-2-phenyl, and 6-methoxyflavone were obtained. The results indicated that there is strong antibacterial and antioxidant activity of B.prionitis leaves. The GCMS-identified flavone molecules might be in charge of antioxidant function. To isolate effective molecules to treat diseases, more research is required.

Key words: Barleria prionitis, Antioxidant activity, Antihemolytic activity, Antibacterial activity, DPPH, GC-MS.

INTRODUCTION

Barleria prionitis, often called porcupine flower, is a member of the Acanthaceae family. Porcupines are indigenous to Eastern Southern and Central Africa, Sri Lanka, India, and Sri Lanka[1]. An upright, thorny plant called a porcupine flower typically has one stem and can reach a height of 1.5 metres. Spine length is about 1.2 cm. Up to 5-9x2.5-4 cm, elliptic, pointed leaves with a fine point, with a wedge-shaped base, sparse puberulus, hairy margins, gland-dotted underside, and a leaf stalk up to 2 cm. Bracts are two and a half centimetres long, rectangular, and have a fine point at the top. Orange-yellow flowers are borne in cymes in the leaf axis. Tropical Africa and Asia[2] are the home to ova that measure 2.5mm and 2.5cm. Porcupine flower has a wide range of medical uses, including the treatment of fever, respiratory conditions, toothaches, joint pains, and a number of other maladies. A mouthwash consisting of root tissue is used to cure bleeding gums and stop toothaches. Traditional Indian medicine makes a variety of uses for the entire plant, including the leaves and roots. For instance, the leaves are frequently used to ease joint discomfort, toothache, and accelerate wound healing. Extracts from the plant are used in herbal cosmetics and hair products to promote healthy skin and hair because of its antibacterial characteristics. Children are fed the plant's bitter juice to cure catarrhal infections[3]. Boils and sores are treated with an infusion of the roots and leaves to reduce swelling. The leaves are chewed for fever, rheumatism, liver illness, indigestion with constipation, and jaundice since they are a diuretic and tonic[4].

Taxonomy

Domain of Taxonomy: Eukaryota

Spermatophyta is the phylum.

Dicotyledonae class

Scrophulariales, the order

The Acanthaceae family

Barleria prionitis is a species.

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Figure 1. Habitat of *Barleria prionitis*

MATERIALS AND METHODS

Extract Preparation

Leaf samples for *Barleria prionitis* were gathered in Maduravoyal, Chennai, Tamilnadu, India. The leaves were scrubbed, allowed to dry in the shade for 15 days, and then mechanically ground into a coarse powder. 100 mL of methanol were used to soak 20 g of powdered material for 72 hours. Following that, a rotary evaporator was used to filter and condense the supernatant, producing a greenish-mushy.

Phytochemical Test

B.prionitis ,a preliminary phytochemical analysis using specific reagents[5] as followed in standard methods[6].

Determination of phenols

To identify the compounds, the Folin-Ciocalteu reagent method was used with a few minor modifications[7]. In a mixture of 900 mL of H_2O, 1 mL of the Folin Ciocalteu reagent, 100 mL of methanol-extracted B. prionitis leaves (1 mg/mL) (1:10 diluted). 5 minutes later, 1 mL of Na_2CO_3 mixture (20%)was mixed. This was permitted to sit in the dark for 30 minutes and was calculated 765 nm by UV-VIS spectrophotometer, the equivalent gallic acid (g/mg),commonly used benchmark component.

Determination of flavonoids

The methanol leaf of B. prionitis[8] a slightly modified version of the chloride procedure. 500 L of one millilitre of leaf extract (1 mg/mL) and 0.5 millilitres of methanol were mixed. The mixture was then given 0.5 mL of a 10% $Alcl_3$ solution and 5% (w/v) sodium nitrite solution. After 50 L of 1 M NaOH solution added, 510 nm was assessed. Results expressed the g/mg of extract corresponding to quercetin.

Antioxidant activity

DPPH radical scavenging activity

1 mL of various leaf extract strengths (50-300 g/mL) combined 1 mL of 0.1 mM DPPH solution in methanol[9]. After that, it was kept in dark incubation for 30 minutes. As a control, each 1 mL of methanol, DPPH solution were utilised. UV-spectrophotometer was calibrated at 517 nm to assess the decline in absorbance. Because it was the reference standard, vitamin C was used. The following formula was used to determine the share of inhibition:

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide radical scavenging activity

A riboflavin-UV light-NBT system was implemented to provide superoxide radical scavenging activity10. The reaction mixture is subsequently replenished with increasing doses of the B. prionitis methanol leaf extract (20-120 g/mL), 1.5 mM riboflavin, 12 mM EDTA, and 50 mM NBT. Each reagent must be primed 50 mM of phosphate buffer (pH 7.6). UV irradiation for 15 minutes started the reaction. The absorbance at 590 nm was immediately determined. Positive indicators, including vitamin C were used. Utilizing the formula, the share of inhibition was determined.

$$\% \text{ of inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

$ABTS^{\bullet+}$ radical cation scavenging activity

Using activity tec 11, 7 mM potassium persulfate and 2.45 mM ABTS+ stock produced antioxidant capacity. The combination was then left in the dark and at a specific temp for twelve to sixteen hours prior to use. To attain an 0.700.02 at 734 nm, dilute using 5 mM phosphate-buffered saline. For two days, this solution remained relatively stable. Different concentrations of (5–30 g/mL) one milliliter of diluted ABTS+ solution. After 5 minutes, 734 nm was established. Vitamin C was employed because it referenced the standard. The samples was determined as

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$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Phosphomolybdenum reduction activity

By using this method, B. prionitis methanol leaf extract's ability for reduction was evaluated[12]. The leaves extract were diluted with 1 mL of a sodium phosphate (28 mM), sulphuric acid, ammonium molybdate (4 mM) at concentrations ranging from 50 to 300 g/mL (600 mM). 90 minutes were spent incubating in a water bath at 95°C. At 695 nm, coloured complex's absorbance was quantified. A such reference was ascorbic acid. The following formula was used to computed:

$$\% \text{ of reduction} = \frac{\text{Sample} - \text{Control}}{\text{Control}} \times 100$$

Ferric (Fe^{3+}) reducing power activity

By using potassium ferricyanide method[13], the decrease of methanol leaf extract of B. prionitis was ascertained. One mL of potassium ferricyanide [K3Fe (CN)6] at 1%, one mL of phosphate buffer (0.2 M, pH 6.6), one mL of plant extract at various concentrations (50–300 g/mL) were combined. After that, the mixtures were incubated for 20 minutes at 50°C. Each mixture received one mL of 10% (w/v) trichloroacetic acid. Then, 0.1% (w/v) $FeCl_3$ was added to the 1 mL mixture, determined at 700 nm. Accepted references were Vitamin C.

$$\% \text{ of reduction} = \frac{\text{Sample} - \text{Control}}{\text{Control}} \times 100$$

Antihemolytic

Preparation of human red blood cells (HRBC)

Healthy female volunteers' blood was drawn into EDTA vials, which were then gently swirled, spun at 1500 g for five minutes, and the blood and buffy coat were extracted. Resultant erythrocytes were collected with centrifugation 1500 g , 5 minutes after being washed three times with 10 mL of PBS, which is 0.2 M phosphate buffer containing 150 mM NaCl. Each time the buffy coat was centrifuged, it was carefully removed before the 10% v/v erythrocyte suspension stock was made in PBS, refrigerated at 4°C, and used within 6 hours.

Heat induced hemolysis

In a nutshell, 2 mL of phosphate buffer saline were combined with 0.2 mL of blood cell suspension and varied concentrations of methanol leaves derived from *B. prionitis* (pH 7.4). The mixture was maintained in an incubator with water at 54°C for 20 minutes. Following the centrifuges at 2500 rpm, a UV/Vis spectrometer was then used to detect the supernatant's absorbance at 540 nm. For the experiment, phosphate buffer was utilised as a control14. The following formula was used to compute the percentage of inhibition:

$$\% \text{ of hemolysis inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

Antibacterial

For antibacterial action, bacteria such *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* species like *Escherichia coli*, *Shigella flexneri*, and *Proteus vulgaris* were utilised.

Agar well Diffusion method

Method[15] according to the standards, nutrient broth agar medium was made. The substances were added to a 500 ml volumetric flask with 200 milliliters of water, whirled, autoclaved for 30 minutes. Its most current medium was prepared prior to being poured into sterile Petri plates. 15 minutes were given for the medium to set up. The inoculums were dispensed using sterile cotton swabs that had previously been submerged in the tube holding the inoculums and dispersed equally on to agar that has hardened in the Agar plate.

Gas chromatographyMass Spectrometry (GCMS)

The material introduced with HP-5 column.Circumstances were employed: Temperatures categorised between 50 and 250°C at 10°C/min injection mode. The following MS settings were analysed: a 50–600 mass , a temperature of 250°C and 70 eV.[16].

Identification of components

The characterization of the spectral data has done. A comparison was made between the mass spectra of the unknown and known compounds recorded in the collection.

RESULTS AND DISCUSSION

Phytochemical analysis

These were found in the phytochemical examination of *B. prionitis* leaves extracted in methanol (table1)

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Table 1. Phytochemical Test of methanol leaves of *B. prionitis.*

S. No	Phytochemicals	Tests	Results
1	Alkaloids	Hager's reagent	+
2.	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+
3.	Steroids	Acetic anhydride + Con. H_2SO_4	+
4.	Phenols	$FeCl_3$ solution	+
5.	Flavonoids	NaOH solution	+
6.	Tannins	Lead acetate solution	+
7.	Glycosides	Sodium nitroprusside solution + Con. H_2SO_4	+
8.	Saponins	Foam test	+

Estimation of total phenols and flavonoids

Due to the presence of several antioxidants in plants, they have evolved efficient defensive damaging effects of visible, ultraviolet, and radiation. Numerous biomolecules includes vitamin E, acids, acetophenones, phenylpropanoids, coumarins, isocoumarins, benzophenones, stilbenes, quinones, betacities, phenolic acids, flavonoids, biflavonoids, benzophenones, xanthones, stilbene. Inhibiting enzymes that produce free radicals, binding metal ions, are just a few of the mechanisms by which these biomolecules demonstrated their activity. "Herbal phenols" refers to a more variety of plant compounds, including functional derivatives, that share the common feature. They can be used in the preservation of fats and oils due to their oxidation susceptibility. Phenolic substances also boost the activity of antioxidant enzymes, which has an indirect impact on the level of dangerous oxygen radicals inside the live cell. Radical events like DNA oxidation and superoxide generation can function as prooxidants in large doses, along with the heterocycle's oxidation number, when classifying flavonoids. Flavonoids can be divided into a total of around 12 subgroups 18. Flavanones and catechins, which they act on in the blood vessels, boost the capillaries' resistance. According to the flavonoids' structural makeup, they have an anti-inflammatory function. Diabetes, hypertension, and atherosclerosis can all be treated with flavonoids, which also remove degenerative alterations on capillaries. It has been discovered that flavonoids inhibit enzymatic systems and enzymes while stimulating bile production. A significant percentage of flavonoids have antibacterial, antiviral, and some cytotoxic action. Cytotoxic flavonoids all share the trisubstituted ring A and methylation at position C4 as structural characteristics. Because of their polyphenolic makeup, they can scavenge harmful free radicals like hydroxyl and superoxide. The overall flavonoid content of the extract was 14.410.63g/mg due to the phenol content, which was 122.70.76g/mg of GAE. In light of their phenol and flavonoid contents, these findings offer useful information on the antioxidant potential of *B. prionitis* leaves extract in methanol.

Table 2. Estimation of total phenols and flavonoids

S. No	Phytochemicals	Amount (µg/mg)
1.	Phenols	122.7±0.76
2.	Flavonoids	14.41±0.63

DPPH radical scavenging activity

The much more extensively used antioxidant assay to gauge an antioxidant's effectiveness is the DPPH free radical test. 64.981.15% at a concentration of 300 g/mL. (Table 3, Fig 2). By converting the methanol extract of *B.prionitis*' leaves demonstrated. Standard ascorbic acid's IC50 value of 11.98 g/mL, a comparison point which had an IC50 of 221.680 g/mL.

Table 3. DPPH radical scavenging activity

S. No.	Concentration (µg/mL)	% of inhibition
		DPPH radical
1	50	16.49±0.74
2	100	23.56±0.64
3	150	37.07±0.20
4	200	45.11±0.12
5	250	63.29±0.75
6	300	64.98±1.15

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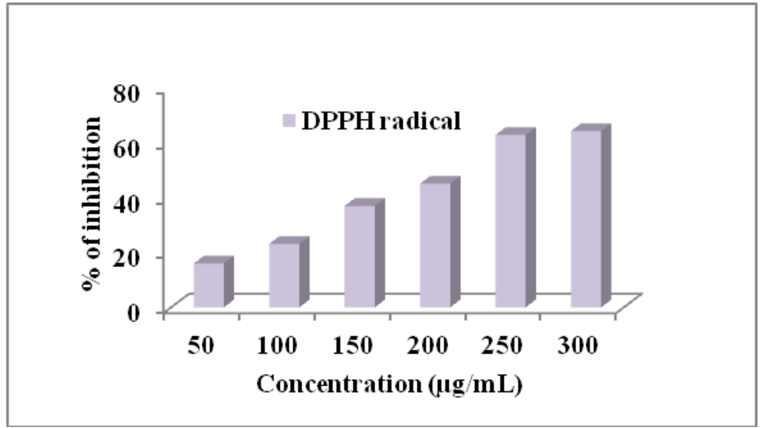

Figure 2. Concentration of DPPH radical of *B. Prionitis.*

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

As it generates several additional free radicals and oxidising agents, extremely damaging to its effects can be amplified. Because they remove superoxide anions from the environment, flavonoids are potent antioxidants. In this procedure, transforms the yellow dye (NBT2+) into the 590 nm-diameter blue formazan. The reduction in absorbance caused by antioxidants suggests that superoxide anion in the reaction mixture 20 has been consumed. The IC50 was determined as 86.88 g/mL concentration, at 300 g/mL concentration (Table 4, Figure 3).

Table 4 Superoxide ($O_2^{\cdot-}$) activity

S. No.	Concentration (µg/mL)	% of inhibition
		Superoxide radical
1	20	3.77±0.71
2	40	19.11±0.16
3	60	21.55±0.63
4	80	39.77±0.55
5	100	57.55±0.48
6	120	63.33±0.32

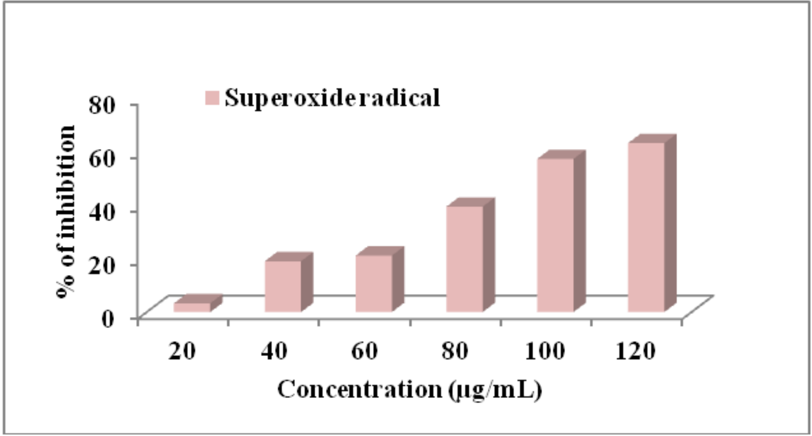

Figure 3. Concentration of Superoxide ($O_2^{\cdot-}$) radical of *B. prionitis.*

ABTS$^{\cdot+}$ radical cation scavenging activity

A chromophore, *B. prionitis* leaves extract's ABTS•+ radical cation was decreased by methanol, and the amount was measured[21]. Peaked at 81.08% at 30 g/mL (Table 5, Fig. 4), and the IC50 was 4.28 g/mL (Figure 2) The standard ascorbic acid was used as a comparison (IC50 = 4.21 g/mL).

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Table 5. ABTS·+ radical cation activity

S. No.	Concentration (µg/mL)	% of inhibition
		ABTS·+ inhibition
1	5	14.83±0.93
2	10	36.48±0.87
3	15	61.48±0.81
4	20	67.56±0.73
5	25	75.00±0.53
6	30	81.08±0.60

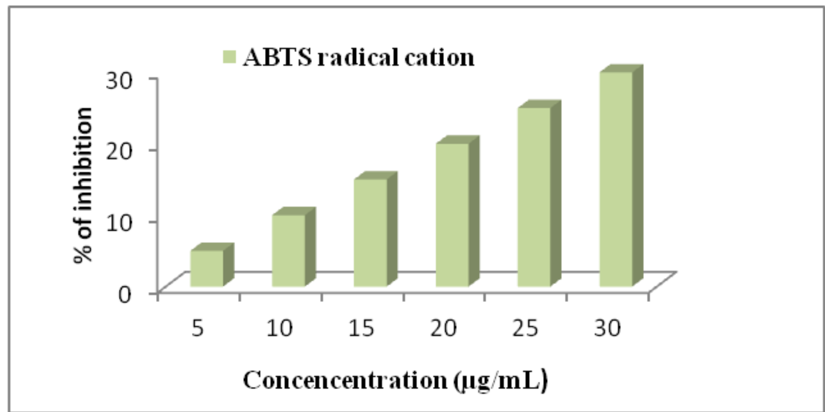

Figure 4. Concentration of ABTS·+ radical cation of *B. Prionitis.*

Phosphomolybdenum reduction activity

This method [22], was used to evaluate the reduction of radicals. 300 g/mL, the highest phosphomolybdenum reduction was 81.790.94%, and the RC50 was 44.31 g/mL. The Concentration (RC50 = 6.34 g/mL) was used as a comparison. A quantitative technique called the PM test is used to examine the rate of the reduction reaction between the molybdenum ligands and the antioxidant and oxidant.

Table 6. Phosphomolybdenum reduction activity.

S. No.	Concentration (µg/mL)	% of reduction
		Phosphomolybdenum reduction
1	50	81.41±0.93
2	100	71.71±0.87
3	150	65.05±0.81
4	200	62.30±0.73
5	250	71.57±0.53
6	300	81.79±0.60

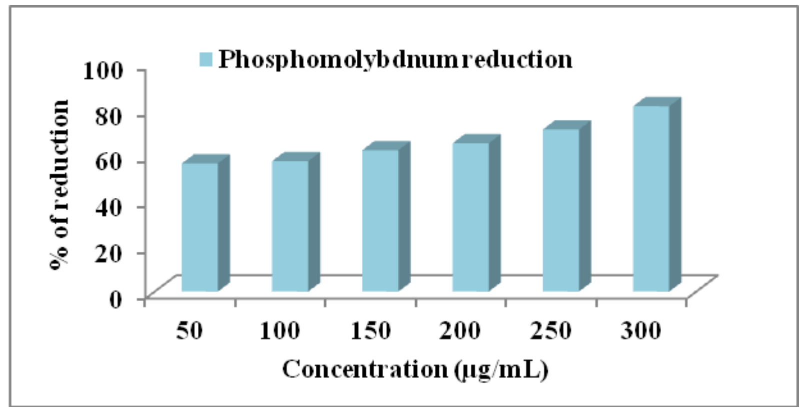

Figure 5. Concentration of Phosphomolybdenum reduction of *B .prionitis.*

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Ferric (Fe^{3+}) reducing activity

By $Fe3^+$ to $Fe2^+$ through the extract's ability to donate electrons and the subsequent production of ferro-ferric complex, *B.prionitis* leaf extract reducing power was demonstrated. The potential to reduce grows as the extract's concentration rises 23. At a concentration of 300 g/mL, the highest Fe3+ reduction was 83.610.820% (Table 7, Fig. 6), and the RC50 was 151.88 g/mL. The standard ascorbic acid (RC50 = 7.72 g/mL) used as comparison. Increased reaction mixture in this experiment also denotes higher reduction potential. Utilizing Fe3+ to Fe2+ reduction test, the extract's reducing ability was determined.

Table 7. Fe^{3+} reducing power activity

S. No.	Concentration (µg/mL)	% of reduction
		Fe^{3+} reduction
1	50	33.17±0.44
2	100	48.00±0.54
3	150	49.38±0.59
4	200	52.33±0.32
5	250	69.30±0.67
6	300	83.61±0.82

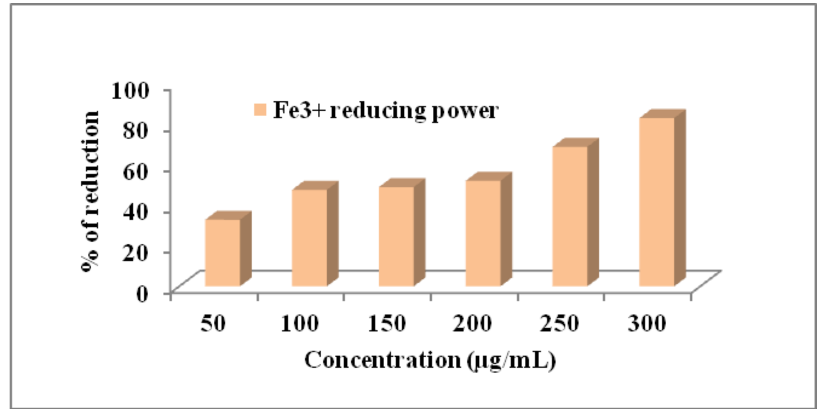

Figure 6. Concentration of Fe^{3+} reducing power of *B. prionitis.*

Antihemolytic

Erythrocytes, numerous and have a wide range of biological and physical properties, have been extensively used in drug delivery because they are abundant in the body. The erythrocytes are the primary target of the polyunsaturated fatty acids (PUFA), which are redox active oxygen transport molecules and powerful activated oxygen species promoters, and haemoglobin molecules. Hemolysis may be caused by oxidative damage to the lipids and proteins of the erythrocyte membrane, which may also occur as a result of hemoglobinopathies, oxidative medications, an excess of transition metals, different radiation, and deficits in erythrocyte antioxidant coordination[25]. When red blood cells were exposed to any toxicant, such as hydrogen peroxide, the amount of hemolysis seemed to be considerably more overwhelming. This test evaluated whether B. prionitis shielded against oxidative damage. This experiment attempted to determine whether B. prionitis prevented or did not prevent oxidative damage to the erythrocyte membrane.120 g/mL, methanol leaves of B. prionitis showed a percentage inhibition of hemolysis of 65.230.55%, and the IC50 was 91.98 g/mL. Figure 7 in Table 8 Using a spectrophotometer set at 560 nm, the amount of haemoglobin in the suspension was calculated.

Table 8. Antihemolytic activity

S. No.	Concentration (µg/mL)	% of inhibition
		Antihemolytic activity
1	20	49.0±0.55
2	40	54.04±0.34
3	60	57.05±0.50
4	80	58.96±0.84
5	100	60.32±0.77
6	120	65.23±0.55

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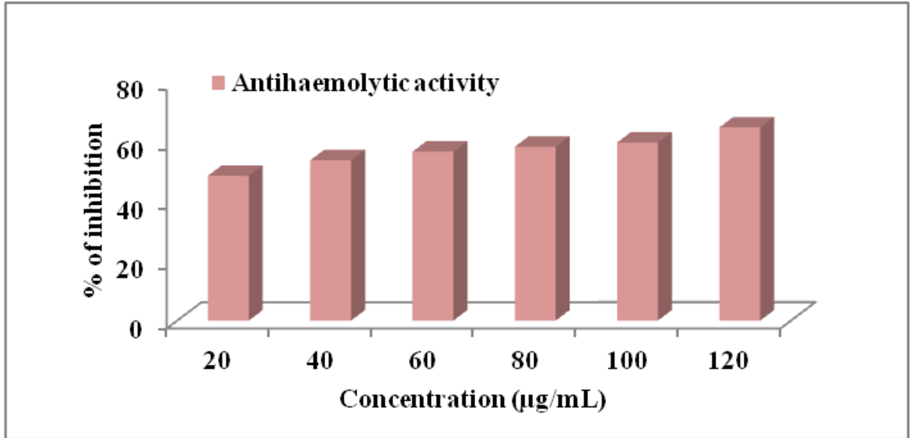

Figure 7. Antihemolytic activity of methanol leaves extract of *B. prionitis.*

Antibacterial activity

Bacteria including *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus cereus, Bacillus neoformans*, were tested for in vitro antibacterial activity using the methanol leaves extract of *B.prionitis* (*Escherichia coli*, *Shigella flexneri*, *proteus vulgaris*). At a dose of 500 g/mL, Staphylococcus aureus exhibited the greatest zone of inhibition, measuring 20 mm. May be present, which have antibacterial properties because they inhibit the growth of germs[26].

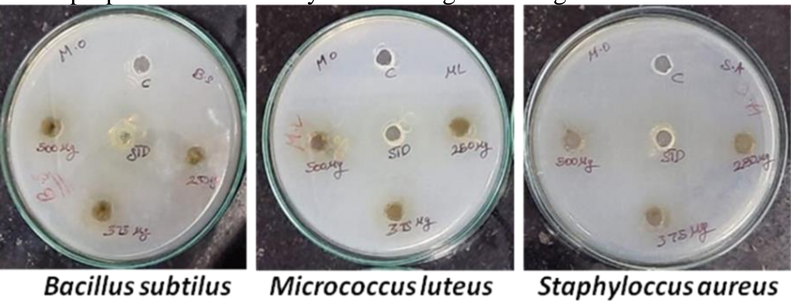

Figure 8. Antibacterial activity of *B. prionitis.*

Table 9. Antibacterial activity

Organisms	Zone of inhibition mm			Standard (Tetracycline)
	250 μg/mL	375 μg/mL	500 μg/mL	
Bacillus subtilis	19	12	15	35
Micrococcus luteus	16	14	15	18
Staphylococcus aureus	19	16	20	40
Escherichia coli	16	11	15	20
Shigella flexneri	19	12	16	25
Proteus vulgaris	18	15	15	21

GC-MS analysis

Antimicrobial compounds c-terpinen were eluted and recorded (Figure 9,Table 10).

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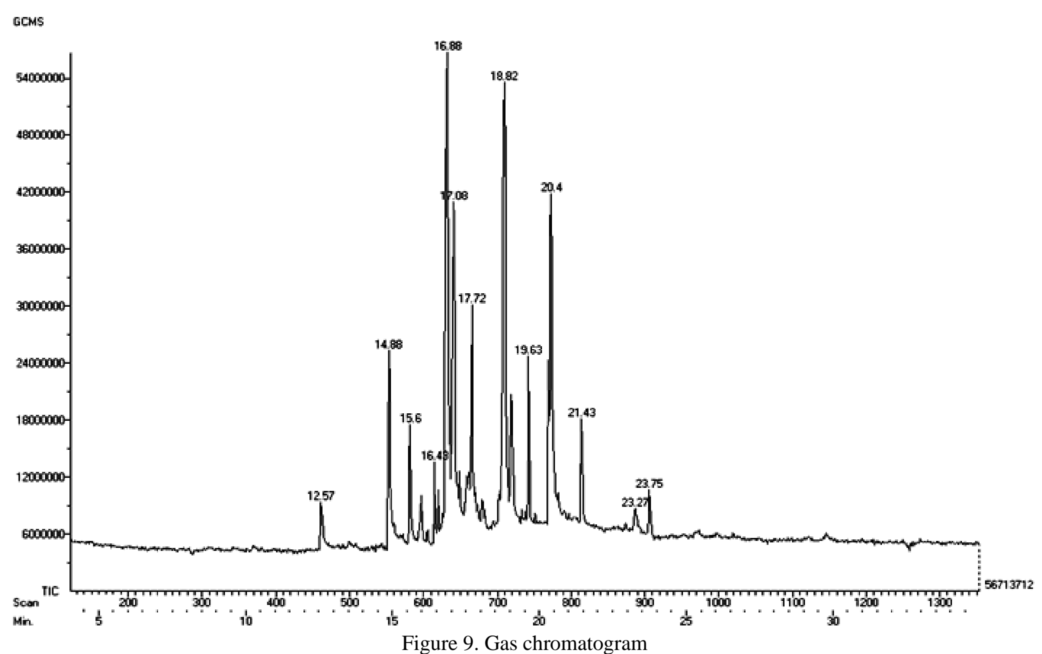

Figure 9. Gas chromatogram

Table 10. Compounds of GCMS analysis

Compound name	RT	Compound structure	Molecular weight g/mol	Molecular Formula
c-Terpinen	12.57		136.00	$C_{10}H_{18}O$
4-Hydroxy-3-methoxybenzyl alcohol	14.88		154.16	$C_8H_{10}O_3$
4-Tridecanone	15.6		198.34	$C_{13}H_{26}O$
Qunioline,2-(2,4-dimethylphenyl)	16.43		233.31	$C_{17}H_{15}N$
Flavone	16.88		222.24	$C_{15}H_{10}O_2$

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2H-1-Benzopyran-4,7-diol,3,4-dihydro-2-phenyl	17.08		242.27	$C_{15}H_{14}O_3$
6-Methoxyflavone	17.72		252.26	$C_{16}H_{12}O_3$
9-Octadecenoic acid[Z]-,methyl ester	18.82		296.5	$C_{19}H_{36}O_2$
1-Tricosene	19.63		322.6	$C_{23}H_{46}$
Methyl eicosa-5,8,11,14,17-pentaenoate	20.4		316.5	$C_{21}H_{32}O_2$
1-Tetradecene, 2-decyl	21.43		336.6	$C_{24}H_{48}$
Cyclobutane carboxylic acid, heptadecyl ester	23.27		338.56	$C_{23}H_{44}O_2$
Tricosane-2,4-dione	23.75		352.6	$C_{23}H_{44}O_2$ $C_{23}H_{44}O_2$ $C_{23}H_{44}O_2$

CONCLUSION

Antioxidants are substances that, while found in low concentrations, considerably slow down or stop an oxidizable molecule from oxidising. Antioxidants found in plants can be quite beneficial. According to the current study's findings, B. prionitis methanol leaves exhibit high antioxidant activities that lessen the negative impacts of free radicals. The findings of this investigation give reason for optimism of *B. prionitis* as an oxidative agent.

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CONFLICTS OF INTEREST

The author declares no conflict of interest

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REFERENCE

[1] Amo, S.O, Finnie, J.F, Staden, J.V. In vitro pharmacological evaluation of three *Barleria* species. J Ethnopharmacol. 2009; 121(2):274-277.

[2] Kaur, R, Kaur, G, Kapoor, A. Preliminary phytochemical screening and in vitro anthelmintic activity of whole plant extracts of *Barleria prionitis* Linn. against earthworms: Pheretima Posthuma. World J Pharm Pharm Science. 2015; 4(7):1340-1347.

[3] Khare, D, Tiwari, K.L. Effect of leaf exudates and leaf tissue sap of *Barleria prionitis L*, on spore germination of some spices of anamorphic fungi. Int J Res Eng ApplSci.2016; 6(4):1-7.

[4] Banerjee D, Maji, A.K, Mahapatra, S, Banerji P,*Barleria prionitis* Linn: A review of its traditional uses,phytochemistry, pharmacology, and toxicity.Res JPhytochem.2012; 6:31-41.

[5] Trease, G.E, and Evans, W. C. Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London.1983; 343-383.

[6] Raaman N, Phytochemical techniques.New India Publishing Agency,New Delhi.2006;306.

[7] Spanos G.A, Wrolstad, R.E. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural & Food Chemistry.1990;* 38: 1565-1571.

[8] Liu, X, Dong, M, Chen, X, Jiang, M, Lv, X, Yan, G. Antioxidant activity and phenolics of endophytic *Xylaria sp.* From *Ginkgo biloba. Food Chemistry.2007;* 105: 548-554.

[9] Blois M.S, Antioxidant determinations by the use of a stable free radical. Nature.1958; 29: 1199-1200.

[10] Ravishankara M.N, Shrivastava N, Padh H, RajaniM. Evaluation of antioxidant properties of root bark of Hemidesmus indicus. Phytomedicine. 2002; 9:153-160.

[11] Delgado-Andrade C, Rufian-Henares J.A, Morales, F.J. Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. J Agric Food Chem. 2005;53:7832-7836.

[12] Prieto, P, Pineda, M, and Anguilar, M. Spectrophotometric quantisation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem.1999; 269: 337-341.

[13] Yen G.C,Chen H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agri.Food Chem.1995; 43:27-32.

[14] Yang Z.G, Sun H.X, Fang W.H. Haemolytic activities and adjuvant effect of Astragalus Membranaceus Saponins (Ams) on the immune responses to ovalbumin in mice. Vaccine. 2005;23(44):5196–203.

[15] Kubo I, Muroi H, Himejima, M. Antimicrobial activity of green tea flavor components and their combination effects. Journal of Agric Food Chem.2002; 40: 245-248.

[16] Harini V, Vijayalakshmi M, Sivaraj C, Arumugam, P. Antioxidant and Anticancer Activities of Methanol Extract of Melochia corchorifolia L. Int. J. of Sci. and Res., 2017; 6(1): 1310-1316.

[17] Primiano T, Sutter R.T, Kensler W.T. Redox regulation of genes that protect against carcinogens. Comparative Biochemistry and Physiology. 1997;118(4):487-497.

[18] Middleton E.J. Biological properties of plant flavonoids: An overview. International Journal of Pharmacognosy. 1996;34(3):344-348.

[19] Kedare S.B, Singh R.P. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011; 48:412–22.

[20] Gülçin İ, Oktay M, Küfrevioğlu Ö.İ, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. Journal of Ethnopharmacology.2002; 79: 325-329.

[21] Hagerman A.E, Riedl K.M, Jones G.A , Sovik K.N, Ritchard N.T, Hartzfeld, P.W, Riechel T.L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem.1998; 46: 18871892.

[22] 22. Loo A.Y, Jain K, Darah, I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chem.2008; 107: 1151-1160.

[23] Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem.2007; 101: 1019

[24] Amarowicz R, Pegg R, Rahimi-Moghaddam P, Barl B , Weil, J. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 2004; 84: 551–562.

[25] Alam N , Ki N.Y, Lee J.S, Cho H.J , Lee T.S.In vitro Anti-hemolytic Effect , in vivo Anti-inflammatory and in vitro Antioxidant Activity of Anchusa azurea Mill. Anti Inflam Antiallergy Agents Med Chem, 2022

[26] Maher Obeidat, Mohamad Shatnawi, Mohammad Al-alawi, Enas Al-Zu`bi, Hanee Al-Dmoor, Maisa Al-Qudah, Jafar El-Qudah and Ismael Otri. Antimicrobial Activity of Crude Extracts of Some Plant Leaves. Research Journal of Microbiology.2012; 7: 59-67.

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