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ANTIOXIDANT PROPERTIES OF *BARLERIA PRIONITIS* AERIAL PARTS

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ABSTRACT

Using in vitro tests to measure total phenolic content (TPC) and free radical scavenging activities, this study explores the antioxidant potential of Barleria prionitis. After the plant material was gathered, verified, and defatted using petroleum ether, it was extracted with 50% ethanol. β-carotene bleaching, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging tests were used to evaluate antioxidant activity, while the Folin-Ciocalteu technique was used to measure TPC. It was found that the leaves had the highest total phenolic content (67.48 mg/g GAE) and the best antioxidant activity in all tests. They also had the lowest IC_{50} values for DPPH (336.15 µg/ml) and hydroxyl radical scavenging (568.65 µg/ml), and the highest reducing power (0.79 ASE/ml). The leaves' remarkable antioxidant qualities were further validated by the β-carotene bleaching experiment, which showed an activity level of 79.20%, far higher than the activity levels of the blossoms and stems. Based on these results, B. prionitis leaves might be a good choice for pharmaceutical and nutraceutical treatments for diseases associated with oxidative stress since they contain powerful bioactive chemicals that have antioxidant properties.

Keywords: *Barleria prionitis, total phenolic content etc.*

INTRODUCTION

Chemical reactions like oxidation can harm cells by releasing free radicals. Antioxidants are substances that prevent this damage. Many biological and environmental activities, such as metabolic reactions, pollution, radiation, and stress, produce free radicals, which are unstable molecules. According to Halliwell and Gutteridge (2015), oxidative stress, caused by these ROS, has been associated with long-term health problems such cancer, diabetes, heart disease, and neurological disorders. The human body has antioxidant systems that rely on both enzymatic and non-enzymatic mechanisms to combat oxidative stress. Catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD), and vitamins C and E are examples of non-enzymatic antioxidants; carotenoids, flavonoids, and polyphenols are examples of enzymatic antioxidants (Lobo et al., 2010). More and more people are worried about the negative effects of synthetic antioxidants, hence there has been a shift in focus towards natural antioxidants derived from plants.

Antioxidant-Rich Medicinal Plants

Bioactive chemicals with strong antioxidant capabilities are abundant in medicinal plants. The antioxidant and stress-relieving properties of these plants are due to their polyphenol, flavonoid, tannin, alkaloid, and terpenoids content (Cai et al., 2004). Traditional medicinal plants have been the subject of several antioxidant research, with an emphasis on their possible use in the prevention and management of disorders associated with oxidative stress (Wink, 2015). The safety, effectiveness, and bioavailability of antioxidants produced from

plants are driving up their demand. Research on the antioxidant properties of several medicinal plants has shown their usefulness in promoting health and warding off illness. Barleria prionitis stands out among these plants as a promising contender thanks to its varied phytochemical profile and array of therapeutic uses.

A Brief Introduction to Barleria Prions

The Acanthaceae family includes the perennial plant or dwarf shrub known as barleria prionitis. Its tropical and subtropical range includes much of Southeast Asia, Africa, and India. Inflammation, respiratory problems, fever, wounds, and microbial infections are just some of the many conditions that this plant has long been used to treat in traditional medicine (Patel et al., 2012). Various bioactive chemicals, including phenolics, alkaloids, terpenoids, flavonoids, and sterols, are found in the various sections of the Barleria prionitis plant, including the leaves, stems, flowers, and roots (Rastogi & Mehrotra, 1993). The therapeutic value of B. prionitis is enhanced by its phytochemicals, which have antioxidant, anti-inflammatory, antibacterial, and hepatoprotective effects (Panchal et al., 2014).

Barleria prionitis and Its Antioxidant Characteristics

The antioxidant properties of Barleria prionitis, including its capacity to decrease oxidative stress and scavenge free radicals, have been the subject of several investigations. The plant's high flavonoid and phenolic content is responsible for its antioxidant capacity, which helps to neutralise ROS and protect cells from harm (Kumar & Gupta, 2015). The capacity of phenolic acids, tannins, and flavonoids to transfer hydrogen atoms or electrons makes them famous for their role in stabilising free radicals and breaking oxidative chain reactions. B. prionitis is an attractive therapeutic option for oxidative stress-related disorders due to the presence of these bioactive chemicals, which boost its pharmacological potential. The extensive antioxidant activity of B. prionitis extracts has been proven in many in vitro tests, including DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and β-carotene bleaching. The plant has a long history of usage in herbal medicine, and recent research suggests that its antioxidant qualities are a key component in the anti-inflammatory, hepatoprotective, and antibacterial benefits it has. B. prionitis has great promise for use in pharmaceuticals and nutraceuticals, especially with the increasing need for natural antioxidants as a replacement for synthetic ones.

The amount of phenolic compounds and the amount of flavonoids

Among the many types of plant antioxidants, phenolic chemicals stand out. According to Rice-Evans et al. (1997), these substances shield cells from oxidative damage by reducing agents, hydrogen donors, and singlet oxygen quenchers. B. prionitis has a high total phenolic content (TPC), which means it has a lot of antioxidant power (Chauhan et al., 2013). The impact of flavonoids, another important class of natural antioxidants, in scavenging free radicals is substantial. According to Kumar et al. (2014), B. prionitis has a high total flavonoid content (TFC), which makes it an effective antioxidant by increasing its reducing power and scavenging activities.

Activity Engaging in Free Radical Scavenging

Barleria prionitis has a considerable antioxidant capacity, as demonstrated by several in vitro tests that examined its ability to scavenge free radicals. To determine if plant extracts may donate hydrogen atoms and neutralise DPPH free radicals, one of the most used tests is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Research has shown that both the water and methanolic B. prionitis extracts are powerful DPPH scavengers, on par with well-known antioxidants such as quercetin and ascorbic acid (Sharma et al., 2016). Similarly, B. prionitis's high phenolic and flavonoid content contributes to its outstanding ABTS radical scavenging action, as assessed by the ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) test (Verma & Singh, 2017). The FRAP test, which is another popular approach, assesses the reducing capacity of plant extracts by measuring the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{3+}). Evidence of strong reducing activity in the aerial portions of B. prionitis supports their antioxidant potential (Gupta & Mehta, 2018). As an additional line of defence against oxidative damage, B. prionitis has hydroxyl radical scavenging activity. Lipid peroxidation and DNA damage are caused by severe oxidative stress, which is exacerbated by hydroxyl radicals and other highly reactive oxygen species. According to Choudhary et al. (2015), the therapeutic potential of B. prionitis extracts is further supported by the fact that they can efficiently scavenge hydroxyl radicals, protecting biomolecules from oxidative degradation. In light of these results, B. prionitis may have promising pharmacological and nutraceutical uses as a natural antioxidant.

Possible Uses of Antioxidants in the Treatment of Barleria Prionitis

Barleria prionitis has tremendous promise for use in several industries, including healthcare, nutrition, cosmetics, and pharmaceuticals, because to its high antioxidant characteristics. The pharmaceutical industry has the potential to use the plant's abundant antioxidant components to create herbal remedies and natural antioxidant supplements for the treatment of illnesses associated with oxidative stress, including diabetes, cardiovascular disease, and neurological disorders. It shows promise as a therapeutic intervention due to its capacity to neutralise free radicals and decrease oxidative damage. Natural preservatives derived from plants are extensively used in the food business to keep perishable goods from going bad due to oxidation. When it comes to preserving food, natural solutions like B. prionitis are often preferred than synthetic ones because of the health dangers associated with the former (Shahidi & Zhong, 2015). Additionally, plant antioxidants have become more popular in the cosmetics business due to their anti-aging and protective properties. Skincare products that include antioxidants produced by Bacillus prionitis can protect skin from free radicals, ultraviolet light, and other environmental toxins, delaying the onset of wrinkles and other indications of ageing while simultaneously hydrating and strengthening the skin. Reactive oxygen species (ROS) are neutralised, collagen breakdown is prevented, and skin suppleness is maintained by its bioactive components. With its wide range of uses and impressive effectiveness, B. prionitis is becoming an important asset for several businesses, contributing to better health, longer life expectancy, and environmentally friendly products.

REVIEW OF LITERATURE

Jain et al. (2017) examined the free radical scavenging capabilities of several medicinal plants, including Barleria prionitis, in a comparative research. Researchers evaluated the plant's antioxidant capacity using a battery of in vitro tests, including DPPH and ABTS. When compared to other aerial components of B. prionitis, the results showed that the leaves

had the maximum antioxidant activity. This work adds to the growing body of data that this plant might be a powerful antioxidant, lending credence to its use in nutraceuticals and alternative medicine.

Sharma and Verma (2016) investigated the connection between the antioxidant activity of various plant extracts and phenolic components. Results demonstrated that when polyphenol and flavonoid contents in Barleria prionitis increased, so did its antioxidant capacity. The study highlighted the substantial correlation between the phenolic content and the reducing capacity of plant extracts as evaluated by the FRAP test. Furthermore, the study hinted that temperature and soil composition are two environmental elements that can affect medicinal plants' antioxidant capabilities.

Kumar et al. (2015) investigated the potential antioxidant effects of Barleria species, particularly their ability to reduce and scavenge radicals. B. prionitis's high phenolic and flavonoid content gave it excellent antioxidant activity, according to the study's use of the DPPH and FRAP tests. In order to prevent cellular damage caused by oxidative stress, the results showed that the plant's aerial components, particularly the leaves, were good at scavenging free radicals. These results provided credence to the long-standing belief in B. prionitis's ability to ward against degenerative illnesses in Ayurvedic practice.

Patel and Rao (2014) examined the relationship between the antioxidant activity of several medicinal herbs and their total phenolic content (TPC). Because phenolic chemicals are so important in free radical scavenging, their results showed that plants with more TPC had more antioxidant capacity. This study verified, using the Folin-Ciocalteu technique, that the leaves and flowers of Barleria prionitis had a high concentration of polyphenols. This study provided more evidence that foods and medicines made from phenolic-rich plants might have antioxidant properties.

Siddiqui et al. (2012) put natural antioxidants in the spotlight as a means of warding off diseases caused by oxidative stress. According to their research on medicinal plants, the main antioxidants that neutralise free radicals are phenolic and flavonoid components. The phenolics and flavonoids present in barleria prionitis give it its reducing power and radical scavenging action; these features make it a popular medicinal herb. Research like this might lead to the incorporation of medicinal plants like B. prionitis into new drug formulations by shedding light on their antioxidant profiles.

OBJECTIVES OF THE STUDY

Following are the main Objective of this study: -

1. To explore the applications of *Barleria prionitis* antioxidants in health and industry.
2. To assess the phenolic and flavonoid content of *Barleria prionitis* and its antioxidant potential.

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HYPOTHESIS

Following are the main Hypothesis of this study: -

H₁: There is a significant application of *Barleria prionitis* antioxidants in health and industry.

H₂: There is a significant contribution of total phenolic and flavonoid content to the antioxidant potential of *Barleria prionitis*.

RESEARCH METHODOLOGY

The antioxidant properties of Barleria prionitis were examined in this study using in vitro assays. Plant material was sourced from NBRI, Lucknow, verified, and then extracted with 50% ethanol after defatting with petroleum ether. The resulting extract was then filtered, concentrated, and lyophilised. The Folin-Ciocalteu method was used to measure total phenolic content (TPC), while β-carotene bleaching, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging assays were employed to assess antioxidant activity. Absorbance measurements at specific wavelengths quantified antioxidant potential, offering insights into the bioactive properties of B. prionitis.

RESULTS

HYPOTHESIS TESTING:

Hypothesis	Statistical Test	Significance Level (α)	Decision Criteria	Result
H_1: There is a significant application of *Barleria prionitis* antioxidants in health and industry.	Chi-square test / ANOVA / Regression Analysis	0.05 (5%)	$p < 0.05 \rightarrow$ Reject H_0 (Significant) $p > 0.05 \rightarrow$ Fail to reject H_0 (Not Significant)	To be determined
H_2: There is a significant contribution of total phenolic and flavonoid content to the antioxidant potential of *Barleria prionitis*.	Pearson's Correlation / Regression Analysis	0.05 (5%)	$p < 0.05 \rightarrow$ Reject H_0 (Significant) $p > 0.05 \rightarrow$ Fail to reject H_0 (Not Significant)	To be determined

Barleria prionitis antioxidants' statistical importance in health, industry, and antioxidant potential is determined using the hypothesis testing framework. The potential medicinal, gastronomic, and cosmetic uses of antioxidants derived from Barleria prionitis will be evaluated using a Chi-square test, analysis of variance, or regression analysis. The antioxidant activity of a substance may be assessed by using regression or Pearson's

correlation analysis on the total phenolic and flavonoid content. A significant link will be confirmed if the p-values are less than 0.05, which will lead to the rejection of the null hypothesis (H_0). In any case, if the p-values are more than 0.05, it will not be possible to reject the null hypothesis, meaning that there is no statistically significant impact.

Get the Extract Ready

The leaves of Barleria prionitis were cleaned with distilled water after being gathered, then air-dried in a controlled environment and ground into a powder. Defatting the powdered plant material was the initial step in processing it. Petroleum ether was used to remove any lipids. The leftover marc was subsequently extracted three days later using 50% ethanol. After filtering, the extract was concentrated with a rotary evaporator (Buchi, USA) and then dried at reduced pressure and low temperature with a lyophilizer (Labconco, USA) to yield a solid residue. Table 1 displays the % yield of various plant sections.

Table 1: The Yield Percentage of Various Bacillus prionitis Components

S. No.	Sample	Solvent Used for Extraction	% Yield (Extract)
1	Leaf	ethanol: water (1:1)	24.10
2	Flower	ethanol: water (1:1)	17.50
3	Scenery	ethanol: water (1:1)	20.90

Total Phenolic Content (TPC) Estimation

The Folin-Ciocalteu colorimetric technique, which uses gallic acid as a standard, was used to quantify the total phenolic content (TPC). Dry weight equivalent gallic acid (GAE) in milligrammes per kilogramme was the unit of measurement. Stirred at room temperature, 25 milligrammes of plant extract was dissolved in 10 millilitres of a mixture of 50% MeOH:H_2O (1:1). Next, 1 millilitre of the extract solution was combined with 1 millilitre of Folin's Reagent (1N) and 2 millilitres of 20% Na_2CO_3. After 30 minutes of incubation at room temperature, the mixture was vortexed using a cyclomixer. After adding distilled water to get the total amount to 25 mL, the absorbance was measured at 725 nm.

The β-Carotene Bleaching Assay is used to measure antioxidant activity (AOA)

The antioxidant capacity of the extracts was assessed in this experiment using a modified technique. The first step was to dissolve 2.0 mg of β-carotene in 20 mL of chloroform. After that, 20 µL of linoleic acid and 200 µL of Tween 40 were combined with a 3 mL portion of this solution. A stable emulsion was formed by adding 100 mL of oxygenated water after evaporation under decreased pressure. Afterwards, 3

millilitres of the emulsion was mixed with 40 microlitres of the sample and left to incubate at 50°C for an hour. After 60 minutes and at the beginning (0 minute), the absorbance was recorded at 470 nm.

Reducing Power Estimation (RP)

Using quercetin as a reference standard, the ferric reducing-antioxidant power (FRAP) test was used to measure the reducing power of the extracts. To make a final volume of 1 mL, various portions of the extract were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide. After 20 minutes of incubation at 50°C, 2.5 mL of 10% w/v trichloroacetic acid was added to stop the reaction. Half a millilitre of 0.1% FeClმ was added after 2.5 millilitres of the reaction mixture had been diluted with distilled water of the same amount. At 700 nm, the absorbance was measured after 10 minutes. One millimole equals one ascorbic acid equivalent (ASE), which is the standard unit of measurement for reducing power (RP).

Anti-Free Radical Activity as Measured by DPPH Test

Extracts from the leaves, flowers, and stems of B. prionitis were tested for their ability to scavenge DPPH radicals using a modified technique. Using potassium phosphate buffer (10 mM, pH 7.4), various portions of the extract were combined with 2.9 mL of a recently made 2.8 mM DPPH solution, resulting in a final volume of 1 mL. The next step was to incubate the mixture for 1 hour at 37°C. Following the incubation period, 1 millilitre each of thiobarbituric acid (TBA) at 1% and 2.8% were added to the mixture. After being heated for 15 minutes in a water bath that was boiling, the reaction mixture was cooled, and the absorbance was measured at 532 nm.

The Phenolic and Antioxidant Profile of Bacillus prionitis Components

Folin-Ciocalteu reagent, a combination of phosphotungstic and phosphomolybdic acids, was used to oxidise phenol hydroxyl groups in a basic solution in order to determine total phenolic content (TPC). Table 2 summarises the results for total phenolic content (TPC), antioxidant activity (AOA), reducing power, and free radical scavenging activity (such as DPPH and hydroxyl radicals).

Without antioxidants, β-carotene quickly loses its colour in the β-carotene bleaching experiment because of combined oxidation with linoleic acid. The TPC and AOA values were greatest in B. prionitis leaves.

The reducing power test detects reductants by measuring the extracts' capacity to change ferric (Fe^{3+}) to ferrous (Fe^{2+}). Due to their low ASE/ml value, the results demonstrated that B. prionitis leaves have the greatest reducing power.

The amount of antioxidants in the reaction mixture has a direct correlation to their ability to scavenge DPPH radicals. The stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH) is transformed into α,α-diphenyl-β-picrylhydrazine by a reaction with antioxidants. The lower $IC_{)0}$ values in the leaves of B. prionitis compared to the flower and stem extracts showed that they had a far stronger capacity to scavenge DPPH and hydroxyl radicals.

Findings indicate that compared to B. prionitis stem and flower, leaves had the highest levels of phenolic content, antioxidant activity, reducing power, and free radical scavenging activity.

Table 2: The Potential of Bacillus prionitis as an Antioxidant and a Free Radical Scavenger

S. No.	Sample	β-Carotene Bleaching (AOA; %)	Reducing Power (ASE/ml)	DPPH (IC_{50} µg/ml)	OH (IC_{50} µg/ml)	TPC (mg/g GAE)
1	Leaves	79.2 ± 1.3	0.8 ± 0.1	336.2 ± 7.2	568.7 ± 6.1	67.5 ± 0.7
2	Flower	62.2 ± 2.6	1.4 ± 0.1	675.1 ± 8.3	809.2 ± 9.2	60.8 ± 0.5
3	Stem	48.3 ± 2.0	1.9 ± 0.1	1148.6 ± 10.8	862.5 ± 8.3	43.4 ± 0.2
4	BHT	51.3 ± 1.0	--	--	--	--
5	Quercetin	--	0.5 ± 0.0	0.02 ± 0.00	0.07 ± 0.01	--

A sample's IC_{50} value indicates the concentration needed to scavenge 50% of free radicals. The activity of scavenging hydroxyl radicals was measured using the deoxyribose technique. The reaction mixture included 50 µM of ascorbic acid, 20 µM of $FeCl_3$, 2 µM of EDTA, and 1.42 mM of H_2O_2.

DISCUSSION

The results of this study show that various portions of Barleria prionitis have antioxidant capacity, with the leaves showing the most bioactivity compared to the stems and flowers. The leaves had the highest total phenolic content (TPC), which is a key measure of

antioxidant capacity. This is in line with their excellent results in different in vitro antioxidant tests, such as β-carotene bleaching, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging operation. According to the β-carotene bleaching experiment, the leaf extract strongly protected against oxidative stress by avoiding oxidation-induced decolorisation, therefore inhibiting lipid peroxidation. The leaves' strong antioxidant capabilities were further validated by the reducing power experiment, which showed that they had the maximum capacity to reduce Fe^{3+} to Fe^{2+}. This suggests that there are more electron-donating bioactive chemicals in the leaves. The findings of the DPPH radical scavenging activity corroborated this fact as well; the leaves exhibited the strongest capacity to neutralise free radicals, as evidenced by their lowest IC_{50} values. Similarly, the leaf extracts showed the greatest protective impact against oxidative damage in terms of hydroxyl radical scavenging activity, further confirming their significance as an abundant source of antioxidants. All things considered, the results show that the leaves of B. prionitis have the most antioxidant qualities of the plants we evaluated. This makes them a great choice for pharmaceutical and nutraceutical applications that target diseases caused by oxidative stress. Potential therapeutic uses for B. prionitis leaf extracts in preventing and managing oxidative damage-linked diseases, such as metabolic syndromes, cardiovascular diseases, and neurodegenerative disorders, should be investigated due to their high phenolic content and strong free radical scavenging ability. Given the leaves' high bioactive potential, it's crucial to study their exact bioactive chemicals, how they work, and whether or not they have synergistic effects when combined with other natural antioxidants. This will help in selecting the most effective plant parts for medicinal uses.

CONCLUSION

Results show that phenolic content, antioxidant activity, reducing power, and free radical scavenging capability are all higher in Barleria prionitis leaves, not in the stem or bloom. In β-carotene bleaching, ferric reducing power, and DPPH radical scavenging experiments, the extracts' antioxidant potential will be shown. The research will prove that the leaves' high phenolic content is a major factor in their exceptional antioxidant activity. Based on these findings, the pharmaceutical, nutraceutical, and food sectors may find value in using B. prionitis leaves as natural antioxidants.

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