Extraction of *Barleria prionitis* (root) and evaluation of tyrosinase inhibition activity

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Abstract

The present study evaluated the tyrosinase inhibition activity and antioxidant activity for Vitiligo (pale white patches) disease by using Barleria prionitis (root) extracts with solvent extracts of ethyl acetate. The tyrosinase inhibition activity of the extracts was determined by inhibition concentration (IC50 values). Results showed that ethyl acetate had the highest tyrosinase inhibition activity by decreasing the percentage of inhibition concentration. The extracts' antioxidant activities were assessed by scavenging 1,1-diphenyl-2- picrylhydrazyl radicals (DPPH test).

The results showed that ethyl acetate root extract of Barleria prionitis has a higher antioxidant activity which proved that it strongly helps to tyrosinase inhibition activity. The findings indicate that extract from ethyl acetate has the strongest tyrosinase inhibitory activity which helps to increase melanin pigmentation in Vitiligo disease affected patients.

Keywords: *Barleria prionitis*, Tyrosinase inhibition activity, Vitiligo disease, Antioxidant activity, DPPH.

Introduction

Barleria prionitis, commonly referred to as a porcupine flower and semmulli in Tamil, belonging to the Acanthaceae family of the genus Barleria and is well recognized for its use in ethnomedicine. A variety of traditional qualities are provided by the prionitis species of the Genus Barleria. This herb is found in hotter areas of India and is widely cultivated in nurseries as an ornamental plant [5,12]. It is widely grown in Indian States and Asian countries. The following are the taxonomy and nomenclature:

Kingdom: Plantae
Order: Scrophulariales
Family: Acanthaceae
Genus: *Barleria*
Species: *B.prionitis*

Vitiligo disease: Vitiligo is an autoimmune illness that develops white patches on the skin due to a loss of pigmentation. When the immune system targets the body's own tissues and organs, an autoimmune illness occurs[6,10,15]. Melanocytes, which are skin cells, produce melanin, which gives skin its color[1,13,17]. Vitiligo is a skin disease caused by a deficiency of melanin pigmentation. Melanocytes in the skin are unable to produce melanin in the presence of vitiligo disease. Vitiligo diseases can affect any part of the skin including the face, neck, hands and skin folds. It can also harm your hair and the inside of your mouth. Vitiligo is most common in people in their twenties but it can appear at any age. The number and shape of spots differ from every individual[4,12,16,18].

Figure 1: ***Barleria prioni***

Vitiligo is a disorder that causes the skin to lose its natural coloration[2]. This condition is known as an autoimmune disease which is characterized by the presence of depigmented macules and patches of different shapes on the skin. These changes occur due to the destruction or dysfunction of melanocytes, the cells responsible for producing melanin[3]. Multiple regions of the body such as the skin, hair and mucous membranes, undergo the formation of discolored white marks. If the affected area of the skin measures less than 1 centimeter in width, it is referred to as a macule, whereas if it exceeds this measurement, it is termed as a patch[14]. Patients with vitiligo often face social exclusion and discrimination in many societies, leading to both psychological and physical consequences[9].

Types of vitiligo disease: The most common type of vitiligo causes an area of skin discoloration to appear in various parts of the body. It spreads very quick and faster than other forms[7,8]. This type affects one side of the body or parts such as hands or face. A depigmented lesion of the skin, mucosa and hair that has been acquired over time. Melanocytes are lost due to autoimmune processes. Mucosal affects mucous membranes of the mouth and genital area. Focal is a unusual type where the area of skin discoloration develops in small area and does not spread. Universal a rare type of vitiligo disease. There is no pigment in more than 80% of the skin.

Material and Methods

Sample collection: *Barleria prionitis* root was collected from Karumalai koodal, Mettur district, Tamilnadu, India. The root was shade dried for 15 days (38±1°C), it does not decompose phytochemical compounds. For further use, the

powdered root was stored in an airtight container after being ground using a mortar and pestel.

Solvent extraction: About 60g of powdered *Barleria prionitis* root was weighed accurately. Put 30g in Soxhlet apparatus. Extraction was carried out with ethyl acetate (70-75ºC) solvent.

Phytochemical analysis: A standard chemical procedure was used to analyze the phytochemical properties of the root extracts.

FT-IR Spectrum: FT-IR spectra were obtained from KBr discs with a variety of solvent extracts using FT-IR-7600 (a single-beam spectrometer).

Preparation of Phosphate Buffer Solution: Take 35 ml of solution B (6.805 grams of potassium dihydrogen ortho phosphate are dissolved in 50 ml of double distilled water maintain the pH at 4.5) in reagent bottle and add 35 ml of solution A (8.709 g of potassium phosphate is dissolved in 50 ml of double distilled water and maintain the pH 9.2. 1 ml of phosphate buffer solution added to 95ml of double distilled water obtaining a concentration 0.05M.

Enzyme solution: 0.6g of tyrosinase enzyme was added to 3ml of phosphate of buffer solution.

L-DOPA: 250mg of L-DOP11A was added to 25 ml of phosphate buffer solution to obtain a concentration of 0.05M. The yellow colour is determined by measuring absorbance with spectrophotometer at 475 nm. The yellow colour indicates the increase of melanin pigmentation for Vitiligo disease.

Antioxidant activity: In the presence of a substrate (antioxidant) that can donate a hydrogen atom, DPPH is reduced to its reduced form with the loss of violet color by using ethyl acetate extraction of *Barleria prionitis* (root) to estimate the anti-oxidant activity.

Preparation of DPPH assay: 19.7 mg of DPPH (1,1diphenyl-picrylhydrazyl) was dissolved with 50ml of methanol to obtain a concentration of 1 M.

The yellow colour is determined by measuring absorbance with spectrophotometer at 517 nm. The yellow colour indicates that better antioxidant activity.

Results and Discussion

Phytochemical analysis: Outcomes of the phytochemical tests of the various extracts from the root of *Barleria prionitis* are listed in table 3.

Table 1
Chart on Tyrosinase inhibition activity

	B	C	T1	T2	T3	T4	T5
Buffer	900µL	800µL	880µL	860µL	840µL	820µL	800µL
DMSO	100µL	100µL	-	-	-	-	-
Compound in DMSO	-	-	20µL	40µL	60µL	80µL	100µL
Enzyme	-	100 U	100 U	100 U	100 U	100 U	100 U
30 min incubation at 37ºC							
L-DOPA (0.05M)	500µL	500µL	500µL	500µL	500µL	500µL	500µL

Table 2
Chart on antioxidant activity (DPPH radical scavenging activity)

	C	T1	T2	T3	T4	T5
Sample (Ethyl acetate / Ascorbic acid)(µL)	-	20	40	60	80	100
Methanol (ml)	2	1.98	1.96	1.94	1.92	1.90
DPPH(1,1diph enyl-2- picrylhydrazyl)(ml)	0.5	0.5	0.5	0.5	0.5	0.5

Table 3
Phytochemical analysis of root extracts of *Barleria prionitis*

Test for	Sample (Ethyl acetate)
Alkaloids	+
Polyphenols	+
Carbohydrates	+
Proteins	+
Amino acids	+
Flavanoids	+

+ indicates presence

FT-IR spectrum of *Barleria prionitis* (root): As new compounds are identified in plants, spectroscopy can contribute to the elucidation of their structure. An ethyl acetate extract of *Barleria prionitis* roots was analyzed using FT-IR spectroscopy. Figure 5 illustrates the FT-IR spectrum of *Barleria prionitis* roots. As confirmed by FTIR spectra, the extracts contained aromatic compounds, alcohols, ketones, amines, ethers and phenolic compounds.

In this study, FT-IR spectra were recorded in the wavelength range of 400 cm^{-1} to 4000 cm^{-1}. In the spectrum, the peaks are located at 3406 cm^{-1}, which indicates that the phenol group has undergone an O-H stretching. Alkene C-H stretching bonds are represented by the peaks at 2922 cm^{-1} and 2852 cm^{-1}. Furthermore, the peaks found at 1638 cm^{-1} and 1638 cm^{-1} are indicative of C=C aromatic conjugates. There is a peak at 1450 cm^{-1} that corresponds to a carbonyl group. As indicated by the peaks observed at 1062 cm^{-1}, these represent vibrations caused by stretching of Si-O.

Liquid chromatography Mass spectrometry (LC-MS): The LC-MS method, which is a chemical analysis method, can be used to separate, identify and quantify both unknown and known compounds, as well as to provide structural and chemical information about different plant extracts. In trace analysis of multi component containing substances, it analyzes small molecules with greater sensitivity and selectivity.

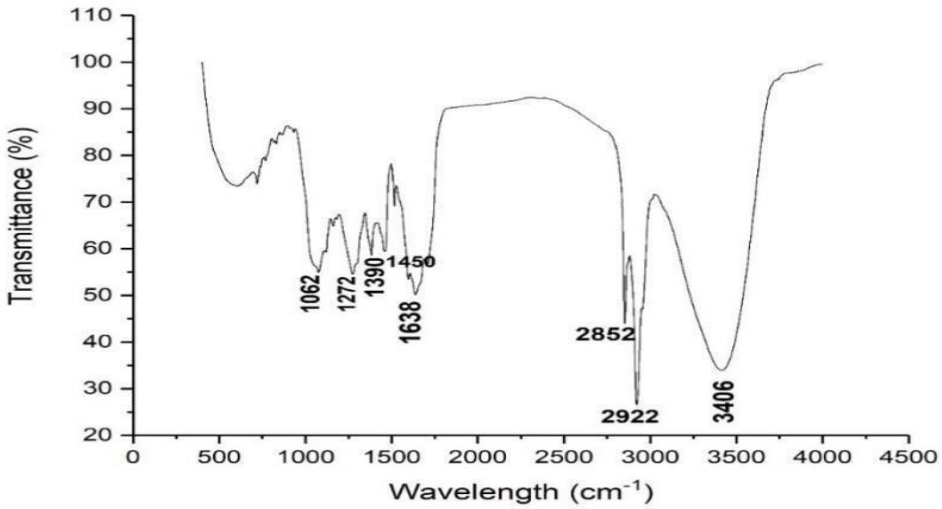

Figure 2: Infrared absorption spectra of ethyl acetate extract of *Barleria prionitis*(root)

Results of LC-MS studies of *Barleria prionitis* (root)
LC-MS studies of ethyl acetate extract of *Barleria prionitis*

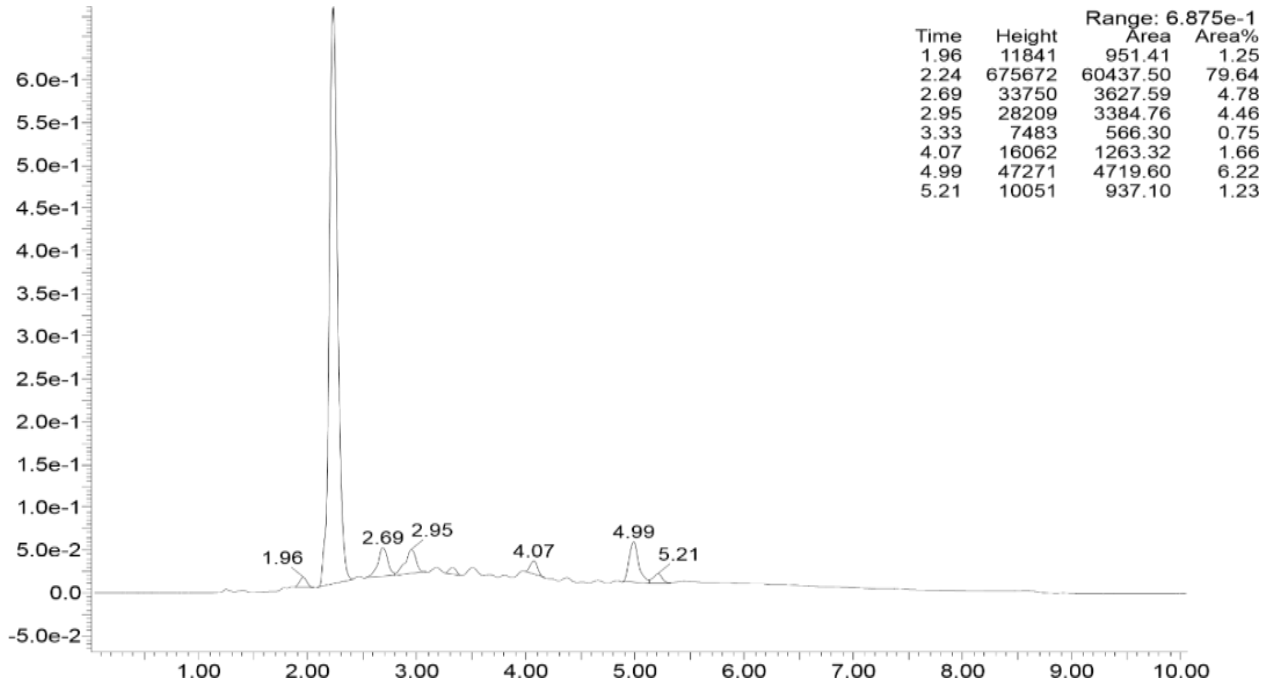

Figure 3: Result of LC at retention time and range of compounds present in Ethyl acetate extract of *Barleria prionitis* (root)

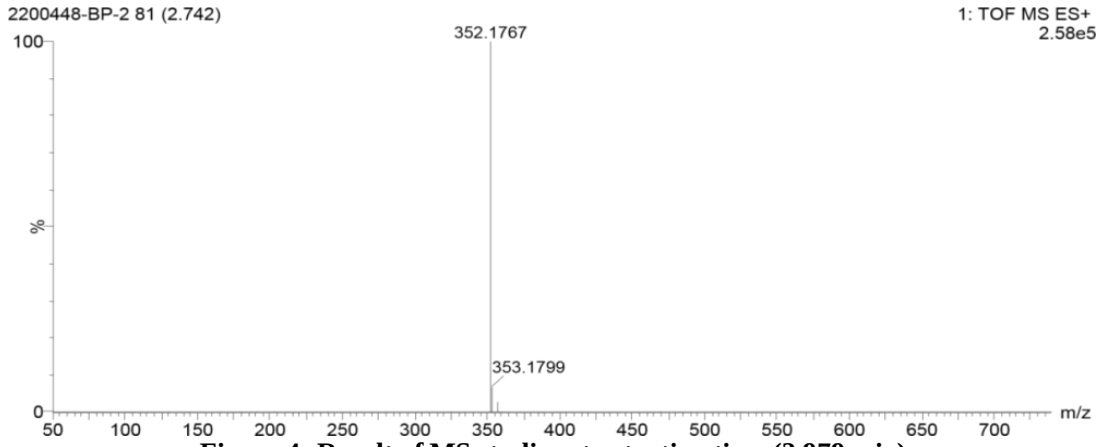

Figure 4: Result of MS studies at retention time (2.979 min)

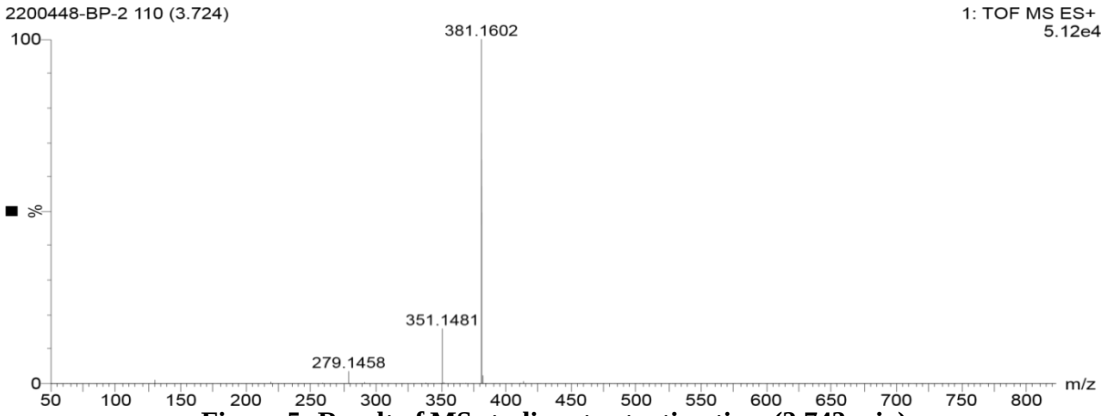

Figure 5: Result of MS studies at retention time (2.742 min)

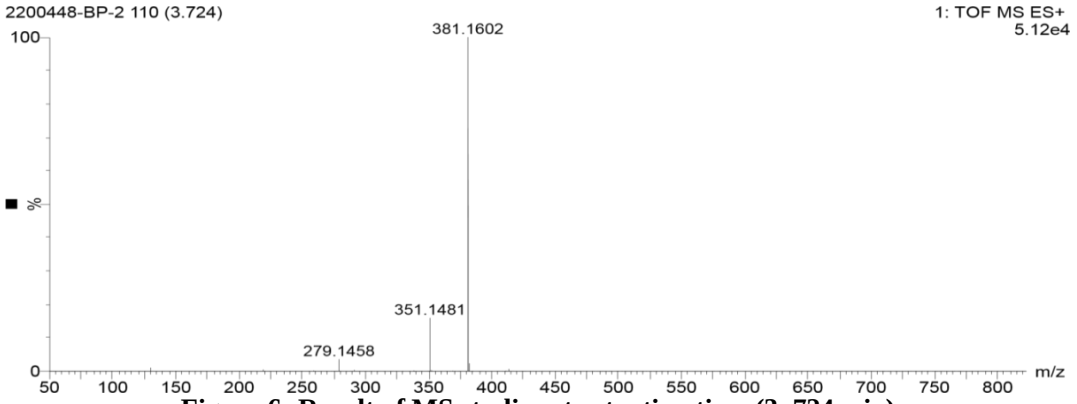

Figure 6: Result of MS studies at retention time (3. 724 min)

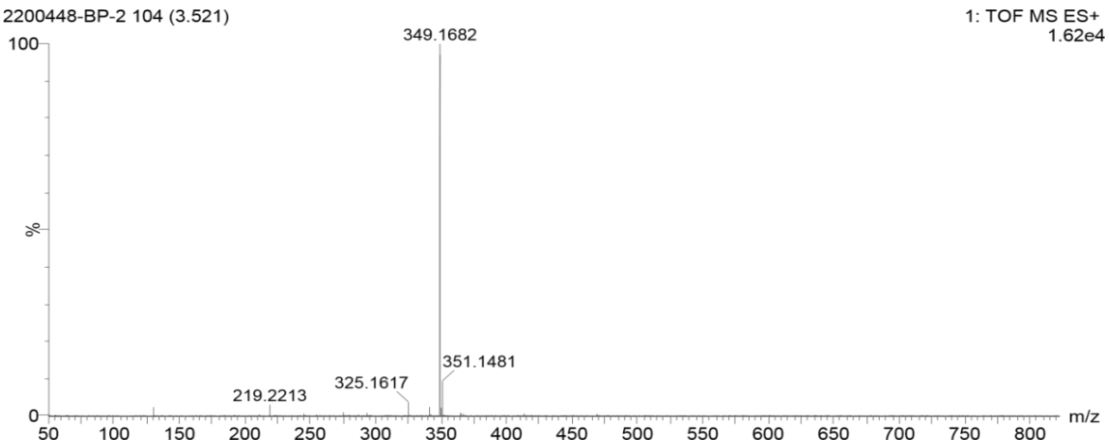

Figure 7: Result of MS studies at retention time (3.521 min)

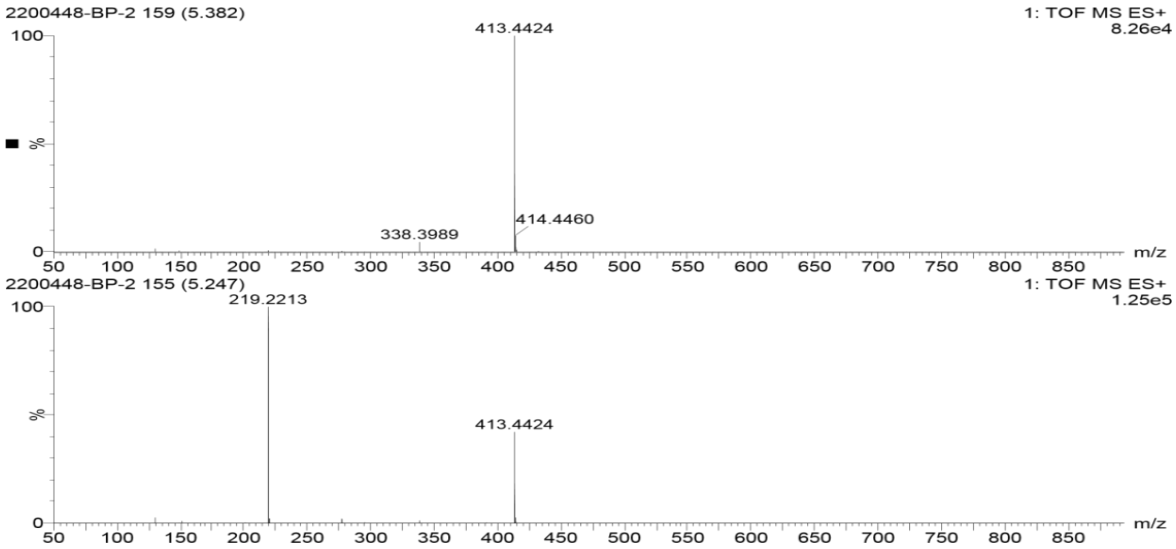

Figure 8: Result of MS studies at retention time (5.382 and 5.247min)

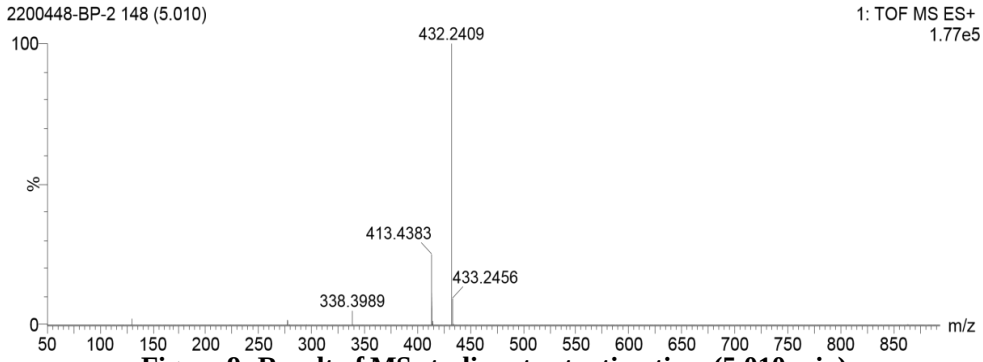

Figure 9: Result of MS studies at retention time (5.010 min)

Table 4
Result of LC-MS studies of ethyl acetate extract of *Barleria prionitis*(root)

S.N.	Area (%)	Time (sec)	Molecular Weight	Compound Name
1.	79.64	2.24	191.1	Syringic acid
2.	6.22	4.99	365.1	Diethylene triamine
3.	4.78	2.69	352.1	Nitrate
4.	4.46	2.95	366.1	Ampyridine
5.	1.66	4.07	365.1	Diethylene triamine
6.	1.25	1.96	191.1	Syringic acid
7.	1.23	5.21	219.2	Benzoin
8.	0.75	3.33	349.1	Amide

The results of LC-MS studies of ethyl acetate fraction in table 4 established that syringic acid was present highly at retention time (2.24sec).

Results for biological activity of tyrosinase inhibition activity: Among many different types of organisms, tyrosinase is a multi-copper enzyme that is crucial for melanogenesis and enzymatic browning. Thus, its inhibitors may be useful in the field of cosmetics, drugs and food as depigmenting agents or as anti-browning agents. The use of various screening techniques has resulted in the development of many natural, semi- synthetic and synthetic inhibitors for this purpose.

Results for biological activity for tyrosinase inhibition activity of ethyl acetate extract: Ethyl acetate is taken as a sample fraction with IC50 values of 97.5µM. Kojic acid is taken a standard with IC50 value of 40.16µM. Inhibition concentration (IC50) is the parameter used to demonstrate the activity of tyrosinase inhibition. In the ethyl acetate fraction, *Barleria prionitis* (root) exhibits very strong tyrosinase inhibition activity with an IC50 of 97.5 µM. These results showed that ethyl acetate has the best tyrosinase inhibition activity through decreasing IC50 values gradually. When compared sample fraction to standard, the ethyl acetate has a best tyrosinase inhibition activity. It helps to increase melanin pigmentation in white patches affected area for Vitiligo patients.

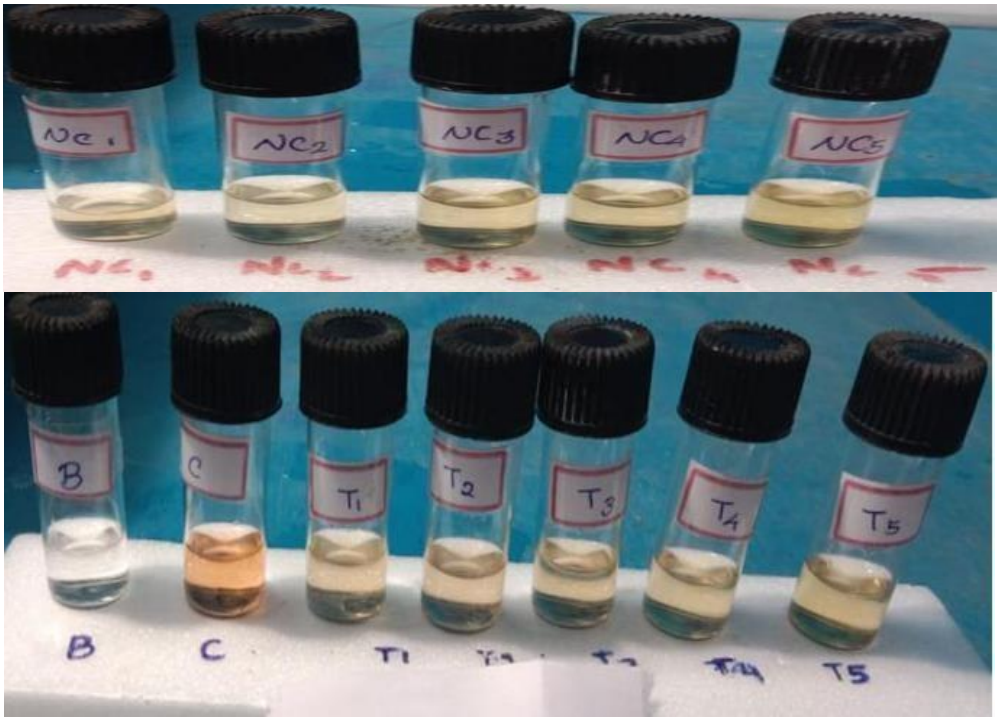

Figure 10: Tyrosinase inhibitory activity of the ethyl acetate extract *Barleria prionitis* (root)

Table 5
Tyrosinase inhibitory activity of the crude hexane, ethyl acetate and methanol extracts of *Barleria prionitis* (root)

Compound	% of inhibition					IC50(µM)
	Concentration (µg/mL)					
	50	100	150	200	250	
Ethylacetate	39.33	48.71	64.08	75.96	81.20	97.5
Kojic acid (Reference)	49.54	65.72	81.20	91.57	98.82	40.16

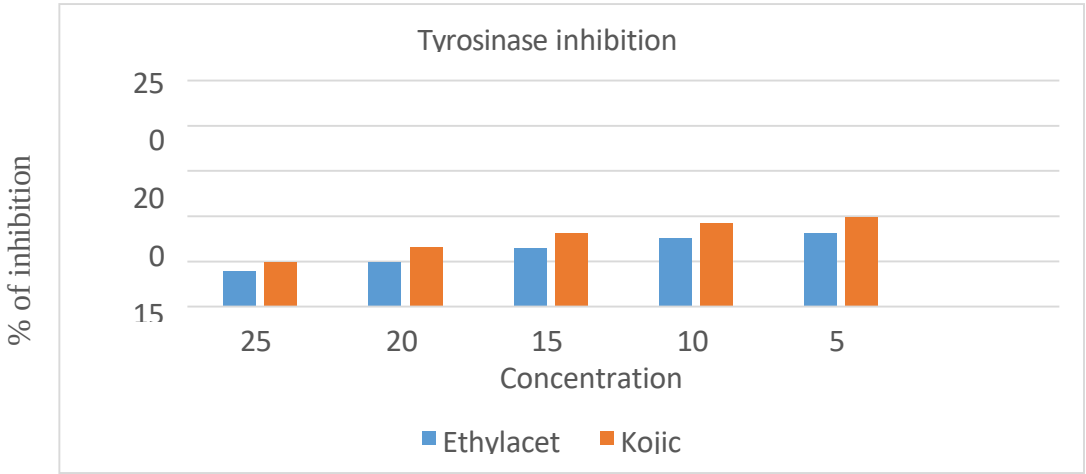

Figure 11: Graphical representation of tyrosinase inhibition activity of ethyl acetate extract of *Barleria prionitis* (root)

The IC50 value of ethyl acetate as a sample fraction is 50.48µM. The IC50 value of ascorbic acid is 33.1 µM which is used as a standard. Antioxidant activity is measured by the inhibition concentration (IC50), which is the concentration of antioxidants capable of reducing 50% of free radicals in the body. The highest antioxidant activity was found in the fractions of ethyl acetate based on IC50 values. Based on results from the antioxidant extraction test, it was found that *Barleria prionitis* root has a very strong antioxidant activity against ethyl acetate fractions, with IC50 value of 50.48 µM. When compared sample fraction to standard, the ethyl acetate has a best anti-oxidant activity.

There is a strong association between ethyl acetate fractions and antioxidant activity due to the presence of phenolic derivatives such as tannins and flavonoids. The hydroxyl

groups in flavonoid compounds can donate protons in the form of hydrogen ions to act as antioxidants. It is believed that this hydrogen ion will bind to free radical electrons within the nitrogen atoms of the DPPH, resulting in a reduction of the DPPH radical.

Ethyl acetate has best tyrosinase inhibition activity in this study. Following a few weeks, patients exhibited initial repigmentation on the side that underwent the extraction of *Barleria prionitis* treatment. Vitiligo, the condition characterized by the loss of pigment cells (melanocytes), cannot be halted by any medication. However, there are certain drugs that, when used alone, in combination, or in conjunction with light therapy, can aid in the restoration of some color. One such drug is *Barleria prionitis* extract, which possesses anti-flammatory properties. Additionally, the application of a corticosteroid cream to the affected skin can potentially bring back color, particularly if vitiligo is in its early stages. Although this type of cream is both effective and convenient to use, it is important to note that visible changes in skin color may take several months.

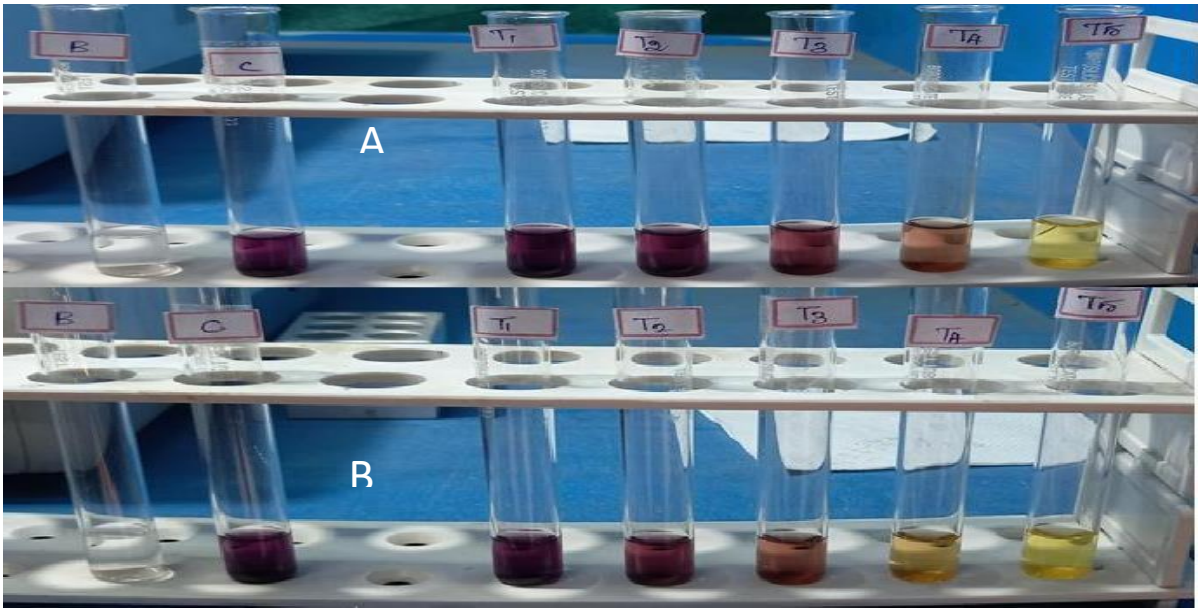

Figure 12: DPPH radical scavenging activities of various concentrations of *Barleria prionitis* root extract (A) Ethyl-acetate and (B) Ascorbic acid

Table 6
DPPH radical scavenging activity of the crude ethyl acetate extract of *Barleriaprionitis* (root

Compound	% of inhibition					IC50(µM)
	Concentration (µg/mL)					
	20	40	60	80	100	
Ethyl acetate	35.22	44.67	52.38	65.97	76.60	50.48
Ascorbic acid (Reference)	42.78	52.36	68.74	75.48	91.06	33.1

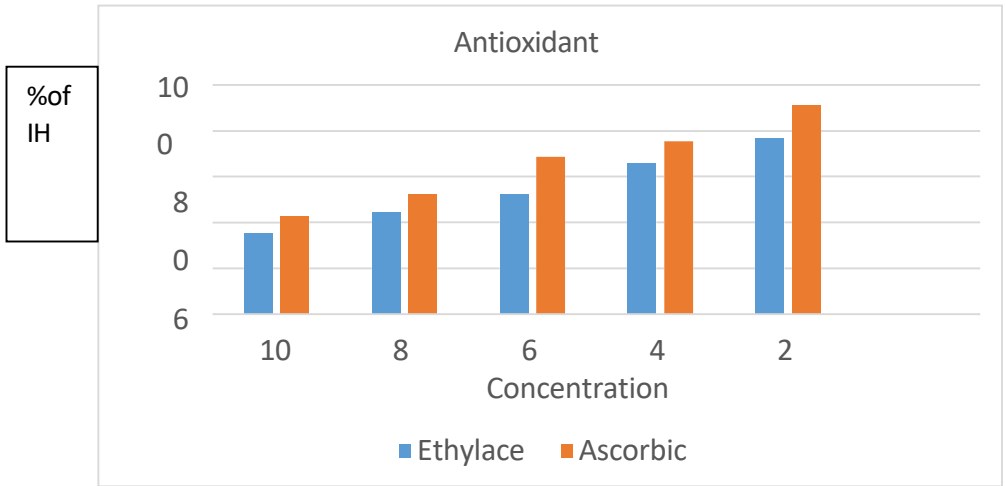

Figure 13: Graphical representation of antioxidant activity (DPPH Assay) in extract of *Barleria prionitis* (root)

Conclusion

The solvent ethyl acetate was used to prepare the crude extract from the root of *Barleria prionitis*. These root extracts of the crude were evaluated by phytochemical analysis which showed that certain amounts of compounds such as alkaloids, polyphenols, carbohydrates, proteins, amino acids and flavonoids were present. FT-IR studies were performed to identify the various functional groups present in all solvents extracts. LC-MS studies were performed to identify the actual molecular weight of the compounds by using ethyl acetate extracts. The results of present study have reported crude extract in ethyl acetate of *Barleria prionitis* root involving a tyrosinase inhibition activity and antioxidant activity.

The solvent extract of *Barleria prionitis* root in ethyl acetate showed the highest tyrosinase inhibition activity and antioxidant activity for Vitiligo disease, which helps to increase melanin pigment in Vitiligo disease-affected areas. Enhancing the process of melanogenesis can lead to an improvement in the production of melanin pigment which is often deficient in hypopigmentation conditions such as vitiligo. Additional research and clinical trials are required to confirm the effectiveness of *Barleria prionitis* extraction in the treatment of vitiligo.

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