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Chromatographic Characterizations of Bioactive Compounds in Barleria Prionitis

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Abstract
The number of people suffering from renal disorders is increasing manifold every year. It is cause of concern all over the world. This research was planned as herbal medicines are safe and their potential for human consumption is quite high. Study of plant *Barleria prionitis* followed by soxhlet extraction method using the different solvents like ethanol, methanol, and chloroform. The qualitative chemical investigation was carried out with the crude extract of leaves of *B. prionitis* and the phytoconstituents were identified by performing various chemical tests, which demonstrated the presence of various phytoconstituents. Representative Silica TLC separations and Column chromatography of *B. prionitis* extracts was carried out using different solvent systems. The different fractions of phytoconstituents are further characterized by HPTLC. Separation of extract in chloroform, Rf value is 0.744, resulted in bands. Similarly in methanol extract max Rf value is 0.8 and also seen in ethanol extract max Rf value of 0.849. Specific compounds identified in plant samples by HPTLC include tannins, alkaloids, and flavonoids. For instance, peaks at Rf values of 0.01 (Tannin 1) and 0.39 (Gallic acid) suggest the presence of tannins. Alkaloids are indicated by peaks at Rf values of 0.40 (Chelidonine), 0. 0.53 (Alkaloid 1), 0.69 or 0.70 (Alkaloid 2) indicates the presence of alkaloids The peaks at R_f values 0.33 (quercetin-3-O-rutinoside), 0.39 (Flavonoid 2), 0.64 (quercetin-3-O-glucopyranoside) 0.73 (quercetin-3-O-rhamnoside) and 0.90 (flavanol-o- glycoside) indicates the presence of flavonoids.

Keywords: Phytoconstituents, HPTLC, soxhlet extraction, tannins, alkaloids

Introduction

Plants are essential to human survival as a source of basic materials for things like medicine, food, and fuel. The World Health Organization estimates that more than 80% of the global population uses complementary and alternative medicines. Several researchers have focused on the potential of traditional plant-derived medications for treating microbial infections, as they have been utilized in most regions of the globe. Substances found in plants have gained popularity in recent years as researchers discovered new application of these plants. Focusing on the medicinal importance on ethno medicinal utilization of plants has shown that 74% of pharmacologically active phytochemical components; were discovered recently. Now, it is believed that only 14%-28% of higher plant species are exploited for medicinal reasons. Over the last several decades, herbal medicines have benefited from a renewed focus on research and marketing related to pharmaceuticals derived from plants. Owing to the widespread use of commercial antimicrobial medications for the treatment of infectious illnesses, multiple drug resistance has emerged as a serious problem today. The juice extracted from B. prionitis leaves is used to treat stomach diseases, urinary infections, ulcers, and fevers in India as traditional medicine. When youngsters have catarrhal infectants or a fever, their parents give them a mixture of the leaf juice and honey. If someone is having a toothache, chewing on some leaves will be helpful. Leaves have been used to cure piles and soothe discomfort in certain indigenous tribes. A topical application of the leaf juice may help with both acne and cuts on the foot. If one suffers from whooping cough, the dried stem bark is used as an expectorant and diaphoretic. Additionally, inflammations and gastrointestinal ailments may be treated using the plant's aerial parts. Boils and glandular swellings may be reduced by applying the root paste topically. The blossoms are used internally to cure a variety of medical conditions, including headaches, abscesses, swelling, bleeding from the urinary tract (haemoptysis), problems with the seminal fluid (urinary tract discharge), and obesity. Leg

stiffness, scrotal enlargement, and sciatica are also treated using the entire plant. The whole plant, and especially the roots, have medicinal use. It is also used to treat various chronic conditions including dropsy, jaundice, and hepatic blockage. In cases of bronchial asthma, the whole-plant ash is mixed with honey and administered. To prevent premature greying of the hair, joint pain, and gout, using the oily, unrefined extract of this plant has been observed to show positive results.

In recent years, phytochemical study of plant products has emerged as a unique field within organic chemistry and plant biochemistry. It is concerned with the wide range of organic compounds that plants store, and it contributes to our understanding of their chemical composition, distribution, and biological functions.

Leaves, stems, roots, and bark, all are rich in secondary metabolites, are used to create aqueous and organic extracts in this technique. Secondary metabolites, such as alkaloids, terpenes, and flavonoids, are then measured in the plant extracts. For every category of substances, there are established assays that may be found in the scientific literature. Next, the combination is often analysed for its component count and makeup using a basic separation method such as Thin-Layer Chromatography (TLC).

Materials and Methodology

The leaves were first washed, then dried and at the end, they were pulverized. Further they were extracted by using Soxhlet extraction method. This Soxhlet method was operated for 8-10 days. There were 3 solutions in this Soxhlet extraction using viz; ethanol, methanol and chloroform solvents entirely and completely done.

Once the chromatogram has been developed, the solute spots must be made visible in order to determine their R_f values, the most routinely used method of detection is examination of the plate under an ultraviolet (UV) light. this is done to detect fluorescence using light sources that have their maximum emission lines at 254 nm or 366 nm. The more specific methods of detection involve spraying the plates with reagents designed to react with specific functional groups to produce visible derivatives. The solvent system was poured into a rectangular chromatographic glass chamber to a depth of 0.5 cm. The chamber was lined with a piece of filter paper to ensure adequate saturation. The extract was transferred to a silica gel G plate with the help of a capillary tube. The distance between the two locations was kept at about 2.0 cm. The applied spots were dried at room temperature and the plate was gently placed in a glass chamber. The angle of the plate with the vertical was kept at approximately 15°. The chromatogram was developed until the solvent front moved approximately 10.0 cm. The plate was removed and the solvent front was marked. The plate was dried at room temperature and inspected with UV light or sprayed with a specific detection reagent. Coloured spots were marked and the Rf value of each isolated component was calculated.

Detection of Reagents used (Wagner and Blodt, 1996).

1. UV 254 nm and 366 nm. Pen
2. 2% ferric chloride solution in methanol.

Throughout the TLC studies it was observed that the derivatization of plates with different detection reagents did not give a good resolution and the number of spots was also lower when observed under ultraviolet light, therefore, detection with light was selected. The mobile phase consists of the substance to be separated (either a single solvent or a combination of solvents) and the stationary phase consists of constituents that can absorb the solvent. Different solvents ranging in polarity in increasing order were used in the extraction process to generate the plant extracts. Using the distillation method and the proper solvents, the extracts were reduced to crude extracts. In addition, the polarity of the phytocompounds may be inferred with their Rf values using different solvent systems. The TLC plates were carefully dried, and UV light at 254 nm (lower wave length) and 366 nm (longer wave length) were used to identify spots (higher wave length).

"Rf = Distance travelled by solute/Distance travelled by solvent."

Column chromatography of Barleriaprionitis of different extract done by trying different solvent systems viz. [n-Butanol:acetic acid: HCl(6:3:1)]for alkaloids, Acetic acid: HCl: Water (10:3:30) for Tannins, Ethyl acetate: Acetic acid: formic acid: water (100:11:11:27)] for flavonoids and it was noticed that different fractions of phytoconstituents were obtained which were sent for further characterizations. The dried plant extract (10 g) was dissolved in 100 ml of HPTLC grade methanol and filtered. This solution was used as a test solution for the HPTLC study.

Observations and Results

Representative silica TLC separations of Barleriaprionitis extracts, containing alkaloids**.** Test solvent mixture used for alkaloids was [n-Butanol: acetic acid: HCl(6:3:1)]. Separation of extract in chloroform, Rf value is 0.744, resulted in bands. Similarly in methanol extract max Rf value is 0.8 and also seen in ethanol extract max Rf value of 0.849, The chromatogram of were developed first in three extracts, and then allowed to dry and separated in(Acetic acid: HCl: Water (10:3:30)for Tannins. Similar experiment was repeated for flavonoids with solvent mixture like Ethyl acetate: Acetic acid: formic acid: water (100:11:11:27)**.** These results demonstrate the Rf values of different extracts in

Table 1: Table for TLC separation

Tests	Solvent front	Detecting agent	Chloroform	Methanol	Ethanol
Test for alkaloids [n-Butanol: aceticacid: HCl(6:3:1)]	10.5cm	U.V and visible light	0.319	0.8	0.471
			0.531	0.6	0.849
			0.744	-	-
Test for Tannins [(Acetic acid: HCl: Water (10:3:30)]	10.5cm	U.V and visible light	0.833	0.753	Absent
Test for flavonoids [Ethyl acetate: Acetic acid: formic acid: water (100:11:11:27)]	10.5cm	U.V and visible light	Absent	0.461	0.825

Table 2: Table for Column chromatographic separation

Solvent system	Plant extract (solvent used)	Fraction obtained	Weight of fraction in gm.	Colour of fraction	Fraction used
Test for alkaloids [n-Butanol:acetic acid: HCl(6:3:1)]	Ethanol	SB AE2	0.24	Light yellow	SB AE2
	Methanol	SB AM1	0.26	Light yellow	SB AM1
	Chloroform	SB AC3	0.47	Light yellow	**SB AC3**
Test for Tannins [(Acetic acid: HCl: Water (10:3:30)]	Ethanol	-	-	-	-
	Methanol	SB TM2	0.23	Green	**SB TM2**
	Chloroform	SB TC1	0.25	Green	SB TC1
Test for flavonoids [Ethyl acetate: Acetic acid: formic acid: water (100:11:11:27)]	Ethanol	SB FE1	1.25	Colourless	**SB FE1**
	Methanol	SB FM2	0.48	Colourless	SB FM2
	Chloroform		-	-	-

In Table 3 we have found two compounds from fraction SB TM2 with Rf value is 0.391 that indicates the presence of galic acid and another is 0.01. Table 3 shows the Rf value of alkaloids from fraction SB AC3such as R_f values 0.40 (Chelidonine) 0.53 (Alkaloid 1), 0.69 or 0.70 (Alkaloid 2). Table 5 from fraction SB FE1 shows peaks at R_f values 0.33 (quercetin-3-O-rutinoside), 0.39 (Flavonoid 2), 0.64 (quercetin-3-O-glucopyranoside) 0.73 (quercetin-3-O-rhamnoside) and 0.90 (flavanol-o- glycoside) indicates the presence of flavonoids.

Table 3: Table for Tannins High Performance Thin Layer Chromatographic separation

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	-0.01	0.1	0.01	109.1	14.39	0.05	11.2	1387.6	109.1
2	0.35	20.1	0.39	68.4	9.02	0.42	19.3	2041.0	68.4
3	0.59	26.0	0.62	35.0	4.61	0.63	32.0	854.2	35.0
4	0.66	33.1	0.71	44.5	5.87	0.71	44.5	1320.7	44.5
5	0.77	60.8	0.90	501.2	66.10	0.95	7.6	22912.8	501.2

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	0.00	1.0	0.01	110.6	14.14	0.06	13.2	1476.5	5.20
2	0.36	26.7	0.39	74.1	9.47	0.43	22.0	2207.1	7.77
3	0.59	28.0	0.64	39.2	5.02	0.65	34.1	1385.6	4.88
4	0.68	37.3	0.73	48.0	6.13	0.73	45.8	1709.0	6.02
5	0.79	62.0	0.90	510.2	65.24	0.96	4.2	21614.4	76.13

The peaks at R_f values of 0.01 (Tannin 1), 0.39 (Gallic acid) indicates the presence of Tannin in sample.

Table 4: Table for Alkaloids High Performance Thin Layer Chromatographic separation

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	.0.02	1.7	0.01	560.8	47.69	0.08	14.4	11601.0	31.99
2	0.50	5.0	0.53	17.4	1.48	0.54	10.7	275.1	0.76
3	0.55	16.5	0.58	26.3	2.24	0.59	24.0	556.3	1.53
4	0.65	25.8	0.69	41.8	3.56	0.70	24.1	1179.5	3.25
5	0.80	60.8	0.91	529.6	45.03	0.95	6.8	22655.0	62.47

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	-0.02	2.3	0.01	569.2	45.52	0.08	12.9	12993.0	32.50
2	0.40	9.6	0.40	11.8	0.94	0.41	0.1	737	0.18
3	0.51	9.3	0.53	26.0	2.08	0.55	19.8	453.6	1.13
4	0.58	23.3	0.62	38.7	3.09	0.63	32.5	1173.8	2.94
5	0.66	34.2	0.70	46.1	3.69	0.71	43.0	1463.0	3.66
6	0.74	47.8	0.78	62.3	4.98	0.79	60.4	1908.4	4.77
7	0.79	60.5	0.91	496.4	39.70	0.96	6.3	21909.7	54.81

The peaks at R_f values 0.40 (Chelidonine) 0.53 (Alkaloid 1), 0.69 or 0.70 (Alkaloid 2) indicates the presence of alkaloids

Table 5: Table for Flavonoids High Performance Thin Layer Chromatographic separation

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	0.00	1.0	0.01	110.6	14.14	0.06	13.2	1476.5	5.20
2	0.36	26.7	0.39	74.1	9.47	0.43	22.0	2207.1	7.77
3	0.59	28.0	0.64	39.2	5.02	0.65	34.1	1385.6	4.88
4	0.68	37.3	0.73	48.0	6.13	0.75	45.8	1709.0	6.02
5	0.79	62.0	0.90	510.2	65.24	0.96	42	21614.4	76.13

The peaks at R_f values 0.33 (quercetin-3-O-rutinoside), 0.39 (Flavonoid 2), 0.64 (quercetin-3-O-glucopyranoside) 0.73 (quercetin-3-O-rhamnoside) and 0.90 (flavanol-o- glycoside) indicates the presence of flavonoids.

Peak	Start RF	Start Height	Max RF	Max Height	Max %	End RF	End Height	Area	Area %
1	-0.00	0.5	0.01	199.4	22.46	0.01	92.6	1162.1	6.42
2	0.01	95.3	0.02	199.9	22.52	0.04	7.6	1418.4	7.83
3	0.29	19.3	0.33	30.5	3.44	0.36	18.9	1097.6	6.06
4	0.81	47.4	0.90	457.9	51.58	0.95	5.8	14428.9	79.69

Conclusion

India is often regarded as the world's premier botanical garden due to its vast array of plant species, many of which have medicinal value. The World Health Organization estimates that over 80% of the global population relies on complementary and alternative medicines, with plant-derived treatments playing a significant role. Numerous studies have explored the therapeutic potential of traditional plant-based remedies for various ailments, as they remain widely preferred across many regions. Specific compounds identified in plant samples include tannins, alkaloids, and flavonoids. For instance, peaks at Rf values of 0.01 (Tannin 1) and 0.39 (Gallic acid) suggest the presence of tannins. Alkaloids are indicated by peaks at Rf values of 0.40 (Chelidonine), 0. 0.53 (Alkaloid 1), 0.69 or 0.70 (Alkaloid 2) indicates the presence of alkaloids the peaks at R_f values 0.33 (quercetin-3-O-rutinoside), 0.39 (Flavonoid 2), 0.64 (quercetin-3-O-glucopyranoside) 0.73 (quercetin-3-O-rhamnoside) and 0.90 (flavanol-o- glycoside) indicates the presence of flavonoids.

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