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Phytochemical Studies on the Leaves of *Pergularia Daemia* Collected from Villupuram District, Tamil Nadu, India

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Abstract*: Pergularia daemia is a fetid smelling, perennial twinning herb, widely distributed in the roadsides of tropical and sub tropical areas. It is used as an important medicinal plant since ancient times. The present study deals with the qualitative and quantitative analysis of the leaves of Pergularia daemia in different solvents and also this study deals with the separation of compounds present in crude methanolic extract of Pergularia daemia leaves by High Performance Liquid Chromatography. The qualitative analysis of the leaves showed the presence of alkaloids, steroids, terpenoids, flavanoids, saponins, phenols, tannins, aminoacids, cardiac glycosides, carbohydrates and proteins. The quantification of the compounds like alkaloids, flavanoids and phenols were done. HPLC shows the presence of two major peaks and exhibited the presence of two major components in the methanolic extract of the leaves. The results suggested that Pergularia daemia has significant phytocomponents and can be used as a source for many pharmacological studies and a curative for various ailments.*

Keywords: *ancient times, HPLC, medicinal plant, Pergularia daemia, phytochemical screening.*

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I. INTRODUCTION

Medicinal plants are a pride to our nature. It is estimated that there are more than 45,000 species of medicinal plants present in our country. They are possessed to have various properties like antioxidant, anti inflammatory, anti cancer etc. Of these only 60% of plants are officially used by practitioners and 40% of plants are used traditionally [1]. One such traditionally used ethnomedicinal plant is *Pergularia daemia* which is used to treat a number of ailments. It is a latex plant, pungent smelling and perennial twinning herb belonging to the family Asclepiadaceae [2]. Seeds of this plant have velvety hairs and are dispersed through wind with cottony appearance. Hence, these plants are found mostly along the roadsides forming hedges of tropical and subtropical regions [3]. It is commonly known as "Veliparuthi" in Tamil and "Hariknot" in English. In Tamil, the term "Veli" denotes "a guardian" or "a protector". It is interesting to note that the Siddha medicine had named only two medicinal plants which act as a shield for humans. One is Veliparuthi (*Pergularia daemia*) and the other is kodiveli (*Plumbago zeylanica*), as they both have multiple valuable properties. *Pergularia daemia* can be used as an antihelminthic, laxative, antipyretic and anti inflammatory [4]. Aerial parts of this plant have hepatoprotective [5] and anti diabetic properties [6]. The leaf latex is used as pain killer and as a relief for toothache [7]. The dried leaves are used in treating bronchitis, asthma, rheumatic fever, amenorrhea and dysmenorrhea [8].

The present study aims at comparative analysis of qualitative and quantitative phytocomponents present in the leaves of *Pergularia daemia* in different solvents and to separate the bioactive components using high performance liquid chromatography technique.

II. MATERIALS AND METHODS

2.1Collection of plant sample

The fresh leaves were collected from Tirukoilur, Villupuram district, Tamilnadu, India.

2.2Preparation of the extract

The leaves of *Pergularia daemia* were washed thoroughly in tap water to remove dust particles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder. The dried powdered sample was soaked in different solvents like methanol, ethanol, chloroform and petroleum ether for 3 to 5 days. Aqueous extract of the leaves were also prepared by soaking the dried powder in distilled water. After 5 days, the extracts were filtered using No.1 Whatman filter paper and stored in air tight container for further analysis.

2.3Qualitative analysis of phytochemicals

Preliminary phytochemical screening was carried out [9] and [10].

2.3.1Test for alkaloids (Mayer's test)

To 1ml of extract, 1 ml of Mayer's reagent (Potassium iodide solution) was added. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

2.3.2Test for steroids (Libermann Burchard test)

To 1ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

2.3.3Test for terpenoids (Salkowski test)

To 1 ml of extract, 2ml of chloroform and few drops of sulphuric acid were added. Formation of reddish brown ring indicates the presence of terpenoids.

2.3.4Test for flavanoids (Alkaline reagent test)

To 1 ml of extract, few drops of dilute ammonium solution and few drops of concentrated hydrochloric acid were added. A yellow colouration indicates the presence of flavanoids.

2.3.5Test for saponins (Froth test)

To 1 ml of extract, 5 ml of distilled water was added and shaked vigorously. Formation of froth indicates the presence of saponins.

2.3.6Test for phenols (Lead Acetate test)

To 1ml of extract, 1 ml of lead acetate solution was added. Formation of precipitate indicates the presence of phenols.

2.3.7Test for tannins (Lead acetate test)

To 1ml of extract, 1ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

2.3.8Test for tannins (Ferric chloride test)

To 1ml of extract, 1ml of ferric chloride solution was added. Formation of blue, black or brownish green colour indicates the presence of tannins.

2.3.9Test for cardiac glycosides (Keller killiani test)

To 1ml of extrct, 5ml of distilled water was added and evaporated to dryness. Then to the Sample 2ml of glacial acetic acid containing trace amount of ferric chloride solution was added. Then 1ml of concentrated sulphuric acid was added along the sides of the tube. Formation of brown ring underlayed with blue colour indicates presence of cardiac glycosides

2.3.10Test for aminoacids (Ninhydrin test)

To the 1ml of sample, 3 to 4 drops of Ninhydrin solution was added and boiled in water bath for 10 minutes. Formation of purple or blue colour indicates the presence of amino acids.

2.3.11Test for proteins (Biuret test)

To the 1ml of extract, 1ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

2.3.12Test for carbohydrates (Barfoed test)

To the 2ml of extract, 1ml of Barfoed's reagent was added and boiled in water bath for few minutes. Formation of reddish brown precipitate indicates the presence of carbohydrates.

2.3.13Test for reducing sugars (Fehling's test)

To the 1ml of extract, equal quantities of Fehling solution A and B were added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

2.4Quantitative estimation of phytochemicals

2.4.1Alkaloid determination

5 gm of sample was added to 200 ml of 10% acetic acid in ethanol in a beaker. The beaker was tightly covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quater of the original volume. The entire solution was precipitated by the drop wise addition of concentrated ammonium hydroxide solution. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is alkaloid, which was dried and weighed [10].

2.4.2Flavanoid determination

10 gm of sample was added to 100 ml of 80% aqueous methanol in a beaker. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was then evaporated to dryness and weighed [10].

2.4.3Determination of total phenols

Few grams of sample were boiled with 50 ml of ether for 15 minutes for the extraction of phenols. To the 5ml of extract, 10 ml of distilled water, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were left for 30 minutes. This was measured at 505 nm [10].

2.4.4High performance liquid chromatography

High performance liquid chromatographic system is equipped with LC10AT pump, spectrasystem UV3000 HR detector in combination with camag softrware. The detection takes place at 254 nm. The mobile phase components were filtered through 0.2μ membrane filter before use [11].

III. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the leaves of *Pergularia daemia* is summarized in the Table 1. The quantification of important phytocompounds of this plant is summarized in Table 2. The methanolic extract of leaves showed the presence of high number of phytocomponents when compared with ethanol, petroleum ether, chloroform and aqueous. The methanolic extracts revealed the presence of alkaloids, steroids, terpenoids, saponins, phenols, tannins, cardiac glycosides, aminoacids, proteins, carbohydrates and reducing sugars. Phytochemicals such as saponins, terpenoids, flavanoids and alkaloids have hypoglycemic activities [12]. Pergularia daemia has high amount of tannins and they play a major role in the treatment of intestinal disorders like diarrhoea and dysentery [13]. The leaves also have flavanoids which play a major role as antioxidant [14]. The result of HPLC shows the presence of two major peaks with two principle components in methanolic extract of leaves Fig 1. The other retention peaks were seems to be ambiguous. It helps to undertake further studies on isolation and identification of specific phytocomponents for pharmacological studies.

Table 1: Qualitative phytochemical analysis of the leaves of *Pergularia daemia*.

Tests	**Methanol**	**Ethanol**	**Petroleum Ether**	**Chloroform**	**Aqueous**
Alkaloid	+	+	-	-	+
Steroids	+	+	+	+	+
Flavanoids	-	+	+	-	+
Terpenoids	+	+	+	-	+
Saponins	+	+	+	+	+
Phenols	+	+	-	+	-
Tannins	+	+	+	-	+
Cardiac Glycosides	+	+	+	+	+
Aminoacids	+	-	-	-	-
Proteins	+	-	+	-	-
Carbohydrates	+	-	-	-	-
Reducing Sugars	+	+	+	+	+

Table 2: Quantitative phytochemical analysis of the leaves of *Pergularia daemia*

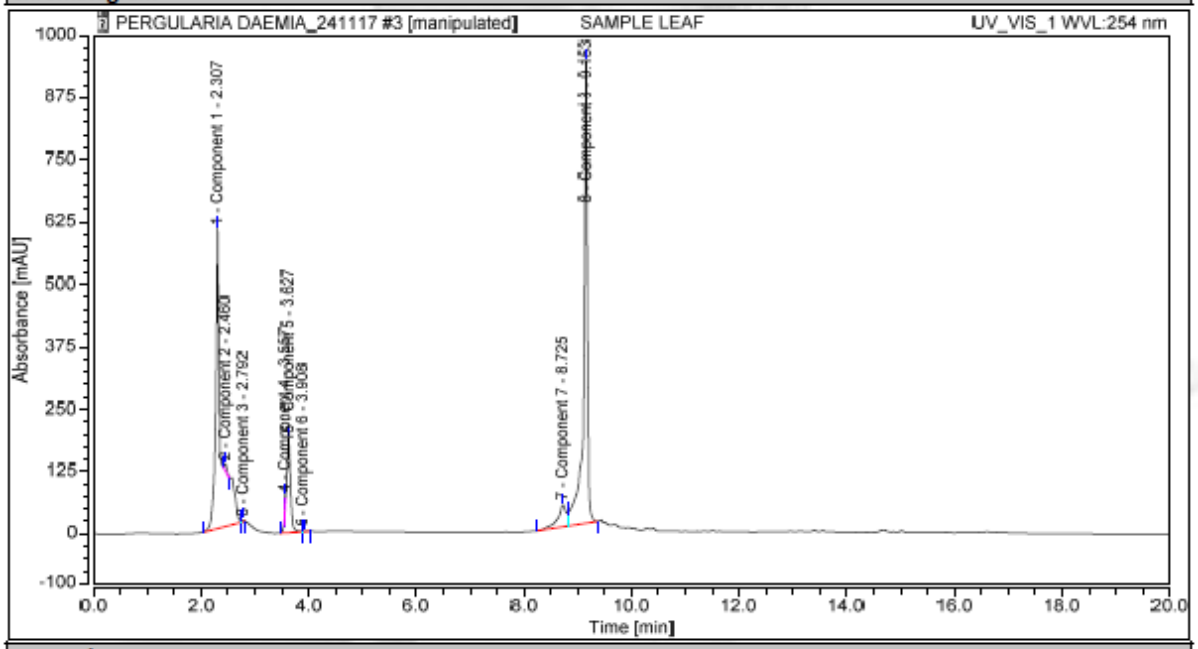
Tests	**Methanol**	**Ethanol**	**Petroleum Ether**	**Chloroform**	**Aqueous**
Alkaloid	8.56 ± 0.08	7.56 ± 1.20	1.25 ± 0.67	1.32 ± 1.0	7.45 ± 1.23
Flavanoid	2.03 ± 0.02	3.01 ± 1.0	3.85 ± 0.04	0.09 ± 0.01	4.15 ± 0.08
Phenols	16.53 ± 0.35	14.25 ± 2.3	5.09 ± 0.09	9.09 ± 2.12	6.72 ± 1.32

Chromatogram and Results

Injection Details

Injection Name:	**SAMPLE LEAF**	*Run Time (min):*	**20.00**
Vial Number:	**2**	*Injection Volume:*	**20.00**
Injection Type:	**Unknown**	*Channel:*	**UV_VIS_1**
Calibration Level:		*Wavelength:*	**254.0**
Instrument Method:	**PERGULARIA DAEMIA_P1**	*Bandwidth:*	**n.a.**
Processing Method:	**PERGULARIA DAEMIA_Q**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**24-Nov-17 15:42**	*Sample Weight:*	**1.0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	Component 1	2.307	71.099	601.828	41.74	33.13	n.a.
2	Component 2	2.460	0.791	16.094	0.46	0.89	n.a.
3	Component 3	2.792	0.285	3.909	0.17	0.22	n.a.
4	Component 4	3.557	0.510	30.835	0.30	1.70	n.a.
5	Component 5	3.627	16.625	190.867	9.76	10.51	n.a.
6	Component 6	3.908	0.113	1.274	0.07	0.07	n.a.
7	Component 7	8.725	7.750	44.026	4.55	2.42	n.a.
8	Component 8	9.153	73.177	927.585	42.96	51.07	n.a.
Total:			**170.350**	**1816.417**	**100.00**	**100.00**	

Fig 1: Chromatogram and Results

CONCLUSION

The qualitative and quantitative analysis shows that the leaves of *Pergularia daemia* contain important phytoconstituents such as alkaloids, steroids, terpenoids, flavanoids, phenols, tannins and proteins. The methanolic extracts are rich in phytoconstituents when compared with other extracts. Thus, the study reveals the presence of various medicinally valued bioactive components of *Pergularia daemia* which has many curing abilities. Further researches are going on to discover its biological activity and enhance the pharmacological activities of it in the area of medicine.

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