PHYTOCHEMICAL INVESTIGATION ON THE ROOTS OF *PERGULARIA DAEMIA* AND HPLC ANALYSIS

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***Abstract*:** Herbal medicines act as a treasure and thus play an important role in primary health care in many developing countries. One such herbal medicine is *Pergularia daemia*. The roots of *Pergularia daemia* are found to have more medicinal properties. The present study deals with the qualitative and quantitative analysis of the roots of *Pergularia daemia* in different solvents. The qualitative analysis of the roots showed the presence of alkaloids, steroids, saponins, phenols, tannins, aminoacids, cardiac glycosides, carbohydrates and proteins. The quantification of the compounds like alkaloids, flavanoids and phenols were done. This study deals with the separation of compounds present in crude methanolic extract of *Pergularia daemia* leaves by High Performance Liquid Chromatography. The results suggested that the roots of *Pergularia daemia* has significant phytocomponents and can be used as a curative for many medical ailments. HPLC shows the presence of five major peaks and exhibited the presence of five major components in the methanolic extract of the leaves.

***Keywords*-** Herbal medicine, *Pergularia daemia*, Phytochemical, Medicinal properties, HPLC

I. INTRODUCTION

Plant medicines are used worldwide in the traditional treatment for many diseases (Vyas *et al*., 2011). The medicinal plants are useful for healing as well as for curing human diseases due to the presence of the phyto constituents (Vijaya Packirisamy, Vijayalakshmi and Krishna Moo, 2014). The prime reason for using plant medicine is that they are effective and they possess fewer side effects. One of the most commonly used ethanomedicinal plant with numerous properties is *Pergularia daemia.*

The plant *Pergularia daemia* belonging to Asclepiadaceae family is a pungent smelling perennial twinning herb. It is commonly known as "Veliparuthi" in Tamil. It has multiple applications since ancient times. The whole plant has various properties like antifertility (Golam *et al,* 2011), wound healing (Kumar, 2006), antidiabetic (Wahi *et al*, 2002) and cardiovascular effect (Sureshkumar and Mishra, 2007).The roots of *Pergularia daemia* possess more medicinal properties. Fresh roots of plant are used as an abortifacient (Kokwaro, 1981) and used to treat gonorrhoea (Samuelsson and 1991). The decoction of the roots is used as medicine to treat several illnesses such as veneral diseases, arthiritis, muscular pains, asthma, constipation, rheumatism and snake bites. The roots are also useful in treating leprosy, mental disorders, anaemia and piles (Yoganarasim, 2000). It is also used in the treatment of whooping cough and for bronchitis since ancient times (Sridevi, 2014). The present study aims at the comparative analysis of qualitative and quantitative phytocomponents of the roots of *Pergularia daemia* in different solvents and to separate the bioactive components using high performance liquid chromatography technique.

II. MATERIALS AND METHODS

2.1 Collection of plant sample

Fresh roots were collected from Trichy district, Tamil Nadu, India.

2.2 Preparation of the extract

The roots 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.

2.3 Qualitative analysis of phytochemicals

Preliminary phytochemical screening was carried out (Harborne, 1980) and (Karthiswaran, 2010).

2.3.1 Test 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.

2.3.2 Test 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.

2.3.3 Test 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.

2.3.4 Test for flavonoids (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 flavonoids.

2.3.5 Test 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.

2.3.6 Test 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.

2.3.7 Test 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.

2.3.8 Test 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.

2.3.9 Test 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.

2.3.10 Test 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.

2.3.11 Test 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.

2.3.12 Test 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.

2.3.13 Test 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.

2.4 Quantitative estimation of phytochemicals

2.4.1 Alkaloid 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 (Harborne, 1980).

2.4.2 Flavonoid 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 (Harborne, 1980).

2.4.3 Determination 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 (Harborne, 1980).

2.5 High 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 (Karthiswaran, 2010).

III. RESULTS AND DISCUSSION

The results of qualitative analysis of roots of *Pergularia daemia* are summarized in the Table 1. The results of quantitative analysis of the roots are summarized in Table 2. The methanolic extract of roots showed the presence of high number of phytocomponents when compared with ethanol, petroleum ether, and chloroform and aqueous. The methanolic extracts revealed the presence of alkaloids, steroids, phenols, tannins, cardiac glycosides, aminoacids,and proteins. Phytochemicals such as alkaloids has hypoglycemic activities (Cherian, 1995). The roots have high amount of tannins and they play a major role in the treatment of intestinal disorders like diarrhoea and dysentery (Akinpelu and Onakoya, 2006). The result of HPLC shows the presence of five major peaks with five principle components in methanolic extract of roots Fig 1. The other retention peaks were seems to be less important. It helps to undertake further studies on isolation and identification of specific phytocomponents for pharmacological studies.

Table 1. Qualitative analysis of the roots 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 II. Quantitative analysis of the roots of *Pergularia daemia*

Tests	Methanol	Ethanol	Petroleum ether	Chloroform	Aqueous
Alkaloid	5.56 ± 4.08	4.31 ± 0.12	2.65 ± 1.97	0.32 ± 1.0	4.95 ± 2.23
Flavanoid	0.13 ± 0.02	0.09 ± 0.02	0.05 ± 0.04	0.04± 0.01	0.12 ± 0.07
Phenols	14.53 ± 1.35	10.25 ± 1.3	2.09 ± 0.09	1.89 ± 1.02	2.12 0.32

IV. CONCLUSION

The qualitative and quantitative analysis shows that the roots of *Pergularia daemia* contain important phytoconstituents such as alkaloids, steroids, cardiac glycosides, phenols, tannins and proteins. The methanolic extracts are rich in phytoconstituents when compared with other extracts. The HPLC shows the five major peaks with the retention of thirty four minor peaks. Thus, the current investigation reveals the presence of various medicinally valued bioactive components of roots of *Pergularia daemia* which has many curative properties. Further researches are going on to discover its biological activity and enhance the pharmacological activities of it in the field of medicine.

REFERENCES

[1] Akinpelu, A. D. Onakoya, ZTM. 2006. Antimicrobial activities of medicinal plants used in folkore remedies in South Western. *African journal of biotechnology*, *5*, 1078-1081.

[2] Cherian, S. Augusti. K. T. 1995. Insulin sparing action of leucopelargonidin derivative isolated from *Ficus bengalesis* linn. Indian journal of experimental biology. 33, 608-611.

[3] Golam, S. Gafur, M. A. Shah, M. A. B. Khurshid, A.H.M. Biswas, M. H.U. Hassan, P. 2001. Antifertility activity of *Pergularia daemia*. Journal of Medical Sciences, 1, 22-24.

[4] Harborne, J. B. 1980. Phytochemical methods, Chapman and Hall limited, London. 49 – 189.

[5] Karthiswaran, K. Mirunalini, S. Dhamodharan, G. Krishnaveni, M. Arulmozhi, V. 2010. Journal of biological sciences, *10(3):* 242-246.

[6] Kokwaro, J. O. 1981. Korean Journal Pharmacognosy, 12(3) 149-52.

[7] Kokate, C. K. 1986. Practical pharmacognosy. Vallabh Prakashan, New Delhi, 1st ed.15-30.

[8] Kumar, B. Yadav, D. K.. Govindarajan, R. Pushpangadan, P. 2006. Wound healing activity of *Pergularia daemia* (Forsk.) Chiov, Pharmacognosy and Ethnopharmacology, 1, 12-14.

[9] Samuelsson, G. Farah, M. H. Claeson, P. Hagos, M. Thulin, M. Hedberg, O. Warfa, A. M. Hassan, A. O. Elmi, A. H. Abdurahman, A. D. Elmi, A. S. Abdi, Y. A. Alin, M. H. 1991. Journal of Ethnopharmacology, 35(1), 25-63.

[10] Sridevi, G. Prema Sembulingam, Sekar Suresh, K. Sembulingam. Phytochemical 2014. Analysis of *Pergularia Daemia* for Its Bioactive Components through Gas Chromatographic Mass Spectrometry (GCMS). IOSR Journal of Pharmacy, 4, 41-46, 2014.

[11] Sureshkumar, S. V. Mishra, S. H. 2006. Hepatoprotective effect of extracts of *Pergularia daemia* Forsk. *J. Ethanopharmacol.*, 107: 164-168.

[12] Vijaya Packirisamy and Vijayalakshmi Krishna Moorthy. 2014. Antibacterial and Phytochemical Evaluation of *Pergularia daemia* from Nagapattinam region. International Journal of Scientific and Research Publications, 4.

[13] Vyas, BA Vyas, R. B, Joshi, S. V. Santani, D. D. 2011. Journal of Young Pharmacist, *3(1)*, 36-40.

[14] Wahi, A. K. Ravi, J. Hemalatha, S. Singh, P. N. 2002. Anti diabetic activity of *Daemia extensa*, Journal of Natural Remedies, 2 (1), 80-83.

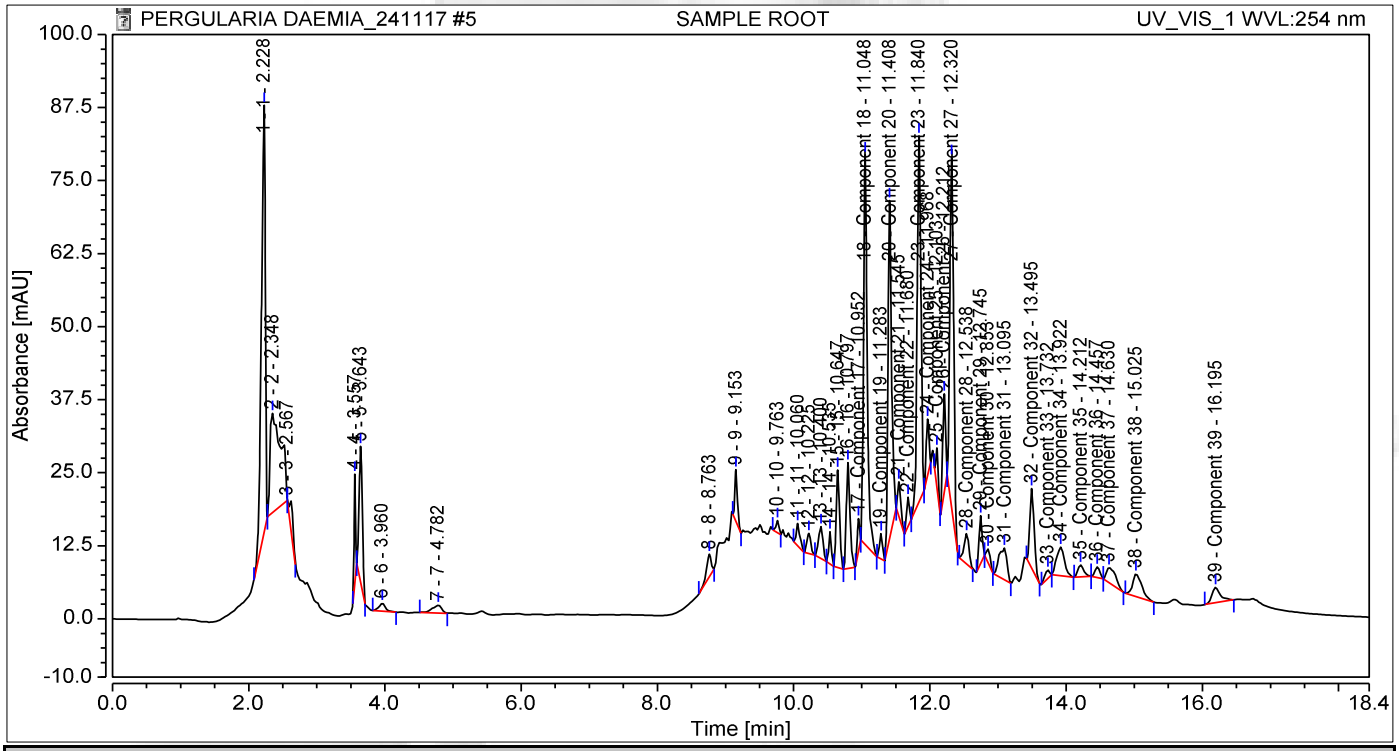
[15] Yoganarasimhan, S. N. 2000. Medicinal Plants of India.1. Bangalore: Interline Publishing Pvt. Ltd, 405.

Chromatogram and Results

Injection Details

Injection Name:	**SAMPLE ROOT**	*Run Time (min):*	**18.45**
Vial Number:	**3**	*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 16:24**	*Sample Weight:*	**1.0000**

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	1	2.228	4.780	72.956	12.72	13.08	n.a.
2	2	2.348	3.020	16.985	8.04	3.05	n.a.
3	3	2.567	0.297	0.000	0.79	0.00	n.a.
4	4	3.557	0.409	18.602	1.09	3.34	n.a.
5	5	3.643	1.278	23.408	3.40	4.20	n.a.
6	6	3.960	0.152	1.374	0.41	0.25	n.a.
7	7	4.782	0.210	1.328	0.56	0.24	n.a.
8	8	8.763	0.297	3.802	0.79	0.68	n.a.
9	9	9.153	0.374	8.871	0.99	1.59	n.a.
10	10	9.763	0.110	2.106	0.29	0.38	n.a.
11	11	10.060	0.230	3.769	0.61	0.68	n.a.
12	12	10.225	0.233	3.459	0.62	0.62	n.a.
13	13	10.400	0.416	5.455	1.11	0.98	n.a.
14	14	10.535	0.245	5.502	0.65	0.99	n.a.
15	15	10.647	0.951	16.697	2.53	2.99	n.a.
16	16	10.797	1.113	18.084	2.96	3.24	n.a.
17	Component 17	10.952	0.216	5.587	0.58	1.00	n.a.
18	Component 18	11.048	4.185	66.656	11.13	11.95	n.a.
19	Component 19	11.283	0.227	4.344	0.60	0.78	n.a.
20	Component 20	11.408	3.497	57.842	9.30	10.37	n.a.
21	Component 21	11.545	0.321	5.969	0.85	1.07	n.a.

22	Component 22	11.680	0.251	5.014	0.67	0.90	n.a.
23	Component 23	11.840	3.980	62.660	10.59	11.24	n.a.
24	Component 24	11.968	0.459	9.611	1.22	1.72	n.a.
25	Component 25	12.103	0.286	6.803	0.76	1.22	n.a.
26	Component 26	12.212	0.825	16.784	2.19	3.01	n.a.
27	Component 27	12.320	3.902	59.988	10.38	10.76	n.a.
28	Component 28	12.538	0.439	5.214	1.17	0.93	n.a.
29	Component 29	12.745	0.444	8.163	1.18	1.46	n.a.
30	Component 30	12.853	0.151	2.285	0.40	0.41	n.a.
31	Component 31	13.095	0.716	5.413	1.90	0.97	n.a.
32	Component 32	13.495	1.017	13.689	2.71	2.45	n.a.
33	Component 33	13.732	0.120	1.423	0.32	0.26	n.a.
34	Component 34	13.922	0.670	4.899	1.78	0.88	n.a.
35	Component 35	14.212	0.210	2.029	0.56	0.36	n.a.
36	Component 36	14.457	0.152	1.823	0.40	0.33	n.a.
37	Component 37	14.630	0.417	2.553	1.11	0.46	n.a.
38	Component 38	15.025	0.606	3.881	1.61	0.70	n.a.
39	Component 39	16.195	0.383	2.608	1.02	0.47	n.a.
Total:			**37.589**	**557.637**	**100.00**	**100.00**	