

SEPARATION AND IDENTIFICATION OF BIO-ACTIVE COMPOUNDS FROM *PERGULARIA DAEMIA*

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Abstract: Medicinal plants play an important role in traditional medicine. One such ethnomedicinal plant is *Pergularia daemia*. It is a hispid perennial twinning herb distributed in the roadsides of tropical and sub tropical regions. The whole plant possess more medicinal values and traditionally used in the treatment of various ailments. This study aims to determine the qualitative and quantitative phytochemical screening of methanolic extract of leaves of *Pergularia daemia* and also to determine the components using UV spectrometry and FTIR analysis. The qualitative analysis of the leaves showed the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, phenols, tannins, aminoacids, cardiac glycosides, carbohydrates and proteins. The quantification of the compounds like alkaloids, flavonoids and phenols were done. FTIR analysis shows the presence of three major peaks and UV Spectrometry shows the presence of two major peaks and exhibited the presence of two major components in the methanolic extract of the leaves. The result confirms that the leaves of *Pergularia daemia* possess significant phytochemicals and acts as the source of many pharmacological studies and a curative for various ailments.

Key words: FTIR analysis, Medicinal plant, *Pergularia daemia*, Phytochemical screening, UV spectrometry.

I. INTRODUCTION:

Medicinal plants play an important role in traditional medicine as they are readily available and cheaper than modern medicine (Ramachandran, *et. al.*, 2013). The medicinal plants are useful for healing as well as for curing human diseases due to the presence of the phytoconstituents (Vijaya Packirisamy, *et. al.*, 2014). They play a significant role in maintaining our human health. According to World Health Organization, approximately 80% of the population currently uses herbal medicine (Kathishwaran, *et. al.*, 2010). The prime reason is that the other systems of medicine have number of side effects. Plant based system of medicine does not possess serious problems. One such traditionally used plant is *Pergularia daemia*.

Pergularia daemia is a slender, hispid, fetid smelling, twinning, perennial lactiferous herb (Doss and Anand, 2013) found in tropical and sub tropical regions. It belongs to Asclepiadaceae family which include more than 2000 species (Kathishwaran, *et. al.*, 2010). It is popularly known as “Veliparuthi” in Tamil and “Hariknot” in English. This plant has been used in the traditional medicine for wide range of ailments. The whole plant is used as anti-helminthic, anti-pyretic, laxative, expectorant and infantile diarrhoea. Each part of the plant possesses various therapeutic properties. Shoots of the plant are used commonly to treat whooping cough (Kokwaro, *et. al.*, 1981). Aerial parts of this plant have hepatoprotective (Sureshkumar and Mishra, 2006) and anti diabetic properties (Wahi, *et. al.*, 2002). The leaf latex is used as pain killer and relief for toothache (Hebbar *et. al.*, 2010). The dried leaves are used in treating bronchitis, asthma, rheumatic fever, amenorrhoea and dysmenorrhoea (Dutta and Gosh, 1947).

The aim of this study is to identify the phytochemicals of leaves of *Pergularia daemia* in methanolic extract both qualitatively and quantitatively and to separate the components by using FTIR analysis and UV-Visible spectrophotometry.

II. MATERIALS AND METHODS:

2.1 Collection of plant sample

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

2.2 Preparation 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 methanol for 3 to 5 days. After 5 days, the extract was 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 by the method described by (Kokate, *et. al.*, 1986 and Harbourne, *et. al.*, 1980).

2.3.1 Test for alkaloids (Mayer's test)

To the 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 (Liebermann Burchard test)

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

2.3.6 Test for phenols (Lead Acetate test)

To the 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 the 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 the 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 the 1ml of extract, add 5ml of distilled water and evaporate it to dryness. Then to the Sample add 2ml of glacial acetic acid containing trace amount of ferric chloride solution. Then add 1ml of concentrated sulphuric acid to 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, add 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 quarter 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 (Harbourne, *et. al.*, 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 (Harbourne, *et. al.*, 1980).

2.4.3 Determination of total phenols

Few grams of sample were boiled with 50 ml of ether for the extraction of phenols for 15 minutes. 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 (Harbourne, *et. al.*, 1980).

III. Fourier Transform Infrared Spectrometer

ATR model FTIR spectrometer was used for the analysis of methanolic extract of *Pergularia daemia*. The spectrum was focused in the mid region. The spectrum was recorded using Attenuated Total Reflectance (ATR) beach measurement (Kathishwaran, *et. al.*, 2010).

IV. UV-VISIBLE Spectrometry:

The powdered leaves were dissolved in methanol and scanned in entire UV visible range (200-800 nm).

V. 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 qualitative analysis shows the presence of alkaloids, steroids, terpenoids, carbohydrates, reducing sugars, phenols, tannins, saponins, proteins and amino acids. Phytochemicals such as saponins, terpenoids, and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). *Pergularia daemia* has 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 FTIR analysis shows the presence of three major peaks with three principle components in methanolic extract of leaves Fig 1. The UV spectrum shows the presence of two major peaks in Fig 2. The other peaks were seemed to be retention peaks. This extract 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
Alkaloid	+
Steroids	+
Flavonoids	-
Terpenoids	+
Saponins	+
Phenols	+
Tannins	+
Cardiac glycosides	+
Aminoacids	+
Proteins	+
Carbohydrates	+

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

Tests	Methanol
Alkaloid	8.56 ± 0.08
Flavonoid	2.03 ± 0.02
Phenols	16.53 ± 0.35

Fig. 1. The results of FTIR analysis of the leaves of *Pergularia daemia*.

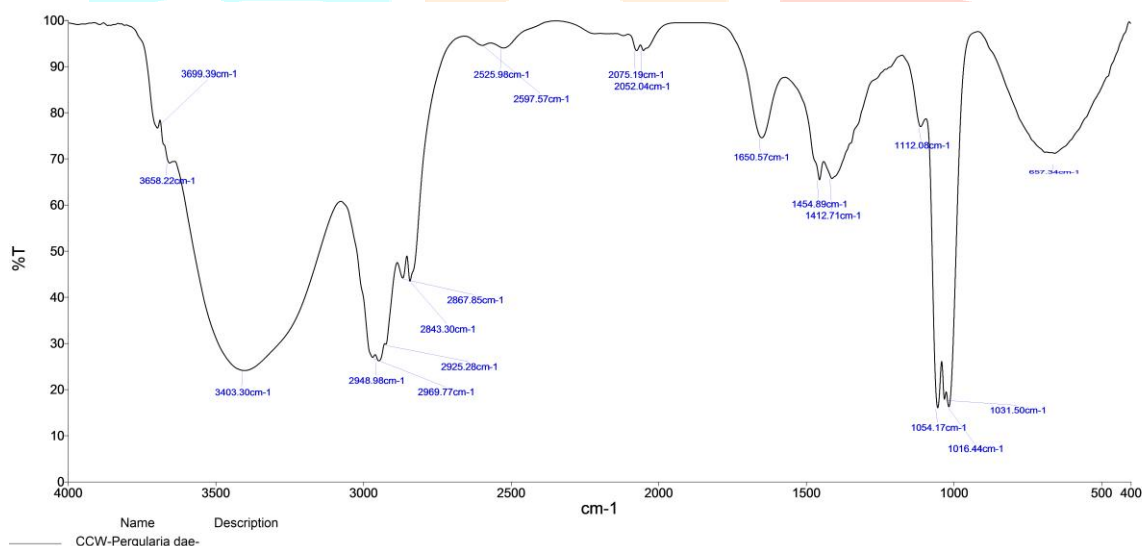
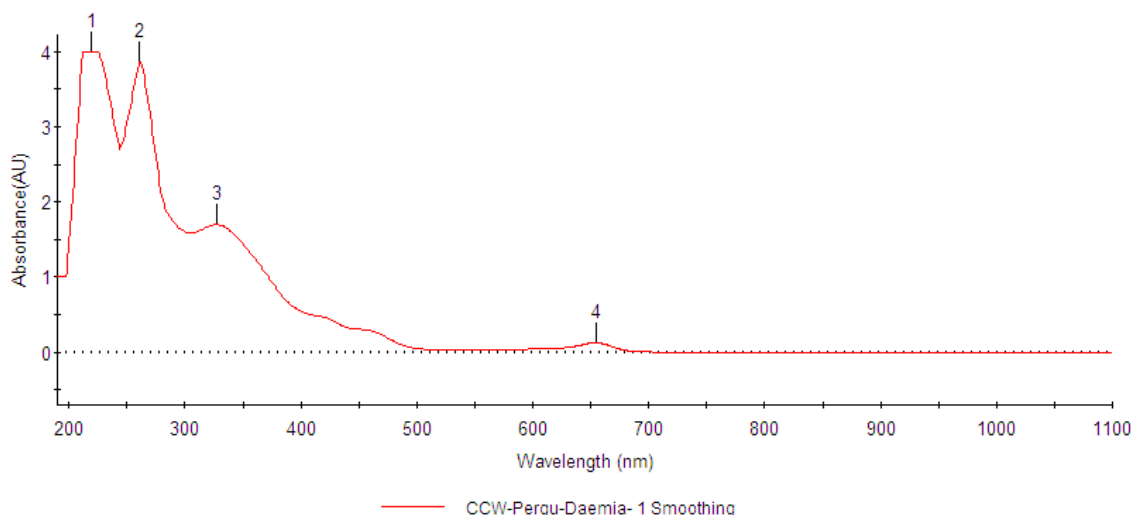


Fig 2. The results of UV-Visible spectrometry of the leaves of *Pergularia daemia*.

VI. CONCLUSION

The qualitative and quantitative analysis shows that the leaves of *Pergularia daemia* contain important phytoconstituents such as alkaloids, steroids, terpenoids, flavonoids, phenols, tannins and proteins. 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. The work is in progress to brighten its the pharmacological profile of it in the field of medicine.

REFERENCES

- [1] Akinpelu AD, 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 KT (1995). Insulin sparing action of leucopelargonidin derivative isolated from *Ficus bengalensis* linn, *Indian journal of experimental biology*, (33): 608-611.
- [3] Doss A and. Anand S. P (2013), "Antihyperglycemic activity of methanol and aqueous extracts of *Pergularia daemia* Linn", *African Journal of Biotechnology*, 13(1): 170-174.
- [4] Dutta A, Ghosh S (1947). *Daemia extensa*, *Indian journal of pharmacy*, (9), 58-60.
- [5] Harborne, JB (1980): *Phytochemical methods*. Chapman and Hall limited, London. 49 – 189.
- [6] Hebbar SS, Harsha VH, Shripathi V, Hedge GR (2010). Ethnomedicine of dharwad district in Karnataka, India plants use in oral health care. *Journal of Ethnopharmacology*. 94, 261-266.
- [7] Karthiswaran K, Mirunalini S, Dhamodharan G, Krishnaveni M, Arulmozhi V (2010). *Journal of biological sciences*
- [8] Karthiswaran K and Mirunalini S (2010). "Therapeutic Potential of *Pergularia daemia* (Forsk.): The Ayurvedic Wonder", *International Journal of Pharmacology*, 6:836-843. 10(3): 242-246.
- [9] Kokate CK (1986): *Practical pharmacognosy*, Vallabh Prakashan, New Delhi, 1st ed., 15-30.
- [10] Kokwaro J O (1981), "A review of research on plants for fertility regulation in Africa. Proc who symposium on plant-derived products for fertility regulation", Seoul, Korea February, 8.
- [11] Ramachandran J, Sankaranarayanan S (2013), "Antifungal activity and the mode of action of Alkaloid Extract from the Leaves of *Adhatoda Vasica*", *International journal of Ethnomedicine and Pharmacological research*, 1: 80- 87.
- [12] Sureshkumar SV, Mishra SH (2006). Hepatoprotective effect of extracts of *Pergularia daemia* Forsk. *Journal of Ethnopharmacology*. 107: 164-168.
- [13] Vijaya Packirisamy, Vijayalakshmi Krishna Moorthy (2014), Antibacterial and Phytochemical Evaluation of *Pergularia daemia* from Nagapattinam region, *International Journal of Scientific and Research Publications*, 4.
- [14] Wahi AK, Ravi J, Hemalatha S, Singh PN (2002). Antidiabetic activity of *Daemia extensa*., *Journal of National Remediation*, 2(1): 80-83.