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Phytochemical Investigation of Methanolic Extract of the Leaves of *Pergularia daemia*

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Abstract: The present study deals with the phytochemical examination of therapeutic importance of *Pergularia daemia*, an important medicinal plant. Medicinal plants are as source of great economic value in the Indian subcontinent and continue to provide valuable therapeutic agents. This study involves the preliminary phytochemical screening, the separation and identification of compounds were present in crude extract of *P. daemia* leaves by TLC, HPLC and HPTLC. Further, FTIR analysis of the crude extract has been studied. Qualitative analysis of the methanolic extract prepared from *P. daemia* leaves revealed the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrates and proteins. A preliminary survey of the scan of the methanolic extract of *P. daemia* evidenced the presence of multiple components in the extract. The results obtained after qualitative analysis confirmed by spectral analysis. It shows the presence of two major peaks observed in the HPTLC, HPLC and IR spectrum and exhibited the presence of two principle components in the methanolic extract of the leaves. The results suggested that the phytochemical properties of the leaves for curing various ailments. The plant *P. daemia* is an important source of various types of compounds with diverse chemical structures as well as pharmacological activities.

Key words: Phytochemical screening, *Pergularia daemia*, methanolic extract, medicinal plant

INTRODUCTION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in medicinal plant leaves and each works differently. Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO) more than 80% of the world's population, mostly in developing countries depend on traditional plant based medicines for their primary healthcare needs (WHO IUCN/WWF, 1993). India has about 45,000 plant species and among them many have been claimed to possess medicinal properties. The need for scientific validation of these useful medicinal plants is very essential. Many of these medicinal plants possess a number of properties such as antidiabetic, antioxidant, anticancer and anti-inflammatory etc. Many plant based chemopreventive agents are recognized as valuable and cost effective approach to control oral cancer incidence. Although, modern synthetic drugs are mostly used in developed countries, the use of herbal drugs in the Western world is well accepted and a continuously high demand for plant material and extracted natural

products can be observed. The importance of ethno pharmacological investigations in the discovery of new therapeutic agents from plants has been discussed extensively (Meyer *et al.*, 1997).

Pergularia daemia-asclepiadaceae (Khare, 2007) known as Pergularia in English, Veliparuthi in Tamil, Uttaravaruni in Sanskrit and Utranajutuka in Hindi belongs to the *Pergularia* species is a perennial twinin herb and is widely distributed in the tropical and subtropical regions of Asia and South Africa and have multiple applications in different folk medicines. Traditionally the plant is useful as anthelmintic, laxative, anti-pyretic and expectorant and is also used in infantile diarrhoea. This drug was also strongly recommended for malarial intermittent fevers (Kirtikar and Basu, 1983). The latex or a decoction of the roots is used in many countries as a medicine to treat several illnesses, such as venereal diseases, arthritis, muscular pains, asthma, rheumatism, snake-bites. The latex may also be used as a fish poison (Patrick *et al.*, 1992). Latex of this climber used for relief from toothache (Hebbar *et al.*, 2010). The Indian *Ayurvedic* system, aerial parts of this plant reported the various pharmacological activities like antifertility (Golam Sadik *et al.*, 2001), wound healing (Kumar *et al.*, 2006), antidiabetic (Wahi *et al.*, 2002), hepatoprotective (Sureshkumar and Mishra, 2006),

cardiovascular effect (Dhar *et al.*, 1973), antibacterial activity (Senthilkumar *et al.*, 2005). The plant is useful in the diseases of vatha, convulsion, asthma, poisoning; the root is useful in mental disorder, anaemia, leprosy and piles (Yoganarasimhan, 2000). The Stem bark also be used as remedy for cold (Dokosi, 1998). Leaf paste mixed with castor oil is applied to joints in inflammation, liver complaints, spleen enlargement; leaves have hypoglycemic activity (Pandey, 2001). The juice of the leaves is given in asthma and applied to rheumatic swellings in combination with lime or ginger; it is also used in the preparation of medicinal oil given in rheumatism, amenorrhoea and dysmenorrhoea. The roots of *P. daemia* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing various liver disorders and oxidative stress related degenerative diseases. The plant extract is also used for uterine and menstrual troubles and to facilitate parturition. However, very less research has been done on this plant leaf and there is a wide scope for investigation. In keeping this view in mind, the present investigation has been carried out to shed light on the preliminary phytochemical screening of *P. daemia* leaves.

MATERIALS AND METHODS

Collection of plant samples: The fully mature *Pergularia daemia* leaves were collected in August-September 2009 from Thirumayam village in Pudukkottai District of Tamil Nadu, India and authenticated by the Botanist, Department of Botany, Annamali University.

Processing of plant samples: The leaves of *P. daemia* are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are dried in an oven at a temperature of 35-40°C for 3 days. The dried leaves of each plant are pulverized, using a sterile electric blender, to obtain a powdered form. The powdered form of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of methanolic leaves extract of *Pergularia daemia*: The methanolic extract of *P. daemia* was prepared by soaking 100 g of dried powdered samples in 250 mL of methanol for 12 h. The extract is filtered by using Whatman filter paper. The filtrate was used for phytochemical screening.

Preliminary phytochemicals screening: Phytochemical tests were studied or analysed on the methanolic extract of the powdered form of the leaves sample using standard

qualitative methods as described by Edeoga *et al.* (2005) and Harborne (1998).

Qualitative analysis on phytochemical constituents

Test for flavonoids: A few drops of 1% NH₃ solution is added to the methanolic extract of plant leaves in a test tube. A yellow coloration is observed if flavonoid compounds are present.

Test for tannins: The 0.5 g of powdered sample of plant leaves is boiled in 20 mL of distilled water in a test tube and then filtered. The filtration method used here is the normal method, which includes a conical flask and filter paper. The 0.1% FeCl₃ is added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Test for carbohydrates: The 0.5 mL of powdered sample of extract, 5 mL of Benedict's reagent was added and boiled for 5 min. Formation of bluish green colour showed the presence of carbohydrate solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

Test for alkaloids: Five milliliter of the extract was added to 2 mL of HCl. To this acidic medium, 1 mL of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Test for proteins: To a small amount of methanolic leaves extract, 5-6 drops of Million's reagent was added. A white precipitate which turns red on heating was formed and it indicates the presence of proteins.

Test for steroids: One milliliter of the extracts was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for terpenoids: Five milliliter of methanolic extract is mixed with 2 mL of CHCl₃ in a test tube. Three milliliter of concentrated H₂SO₄ is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Thin layer chromatography: Two microliter of sample was applied on precoated silica gel GF254 aluminium plates (MERCK, 8×7 cm size). Develop the plate in Methanol: Chloroform (3:7) mobile phase. A spot in HPTLC was

performed using a CAMAG. It is scanned in 2 Densitometer and linomatt IV applicator.

High performance thin layer chromatography (HPTLC):

The methanolic extract of *P. daemia* leaves were carried out in HPTLC (CAMAG TLC SCANNER II). The HPTLC was performed using a CAMAG HPTLC spectrophotometer provided with a scanner II densitometry a Linomat applicator.

High performance liquid chromatography (HPLC):

High Performance Liquid Chromatographic system equipped with LC10AT pump, Spectrasystem UV3000 HR detector in combination with Camag software. Active compounds were purified using HPLC. A Varian 5000 Liquid Chromatography with a C18 column (Phenomenex, 250×4.60 mm) was used with a Spectrasystem UV3000 HR detector (detection at 254 nm). The sample was analyzed by HPLC (Schimadzu, Japan) model used. The mobile phase components were filtered through 0.2 μ membrane filter before use.

Fourier Transform Infrared Spectrophotometer (FTIR):

ATR model FTIR Spectrophotometer (Bruker Co., Germany) was used for the analysis of the methanolic crude extract of *P. daemia* sampler. The spectrum was focus in the mid IR region of 400-4000 cm⁻¹ by the KBr pellet technique. The spectrum was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

RESULTS AND DISCUSSION

Qualitative analysis carried out for methanolic extract of the leaves of *P. daemia* showed the presence of five major groups of phytochemical constituents is summarized in Table 1. Phytochemical screening of the plant leaves revealed the presence of flavonoids, steroids, carbohydrates, alkaloids, tannins and terpenoids were present in *P. daemia* extract. Proteins are absent in *P. daemia* leaf extract. Flavonoids are mainly found in the leaves. From the amount of precipitate formed and degree of colour change, it was deduced that the leaf extract of *P. daemia* yielded the lowest concentration of tannins. A preliminary survey of the scan of the methanolic extract of *Pergularia daemia* evidenced the presence of multiple components in the extract.

Preliminary investigations used TLC to separate the extracts, with a mixture of toluene:ethanol (96:4) as the developing solvent. The results obtained after qualitative analysis confirmed by spectral analysis using HPLC, IR and HPTLC showed the presence of two major peaks

Table 1: The analysis of phytochemicals in the methanol leaves extract of *P. daemia*

Phytochemicals	Inference
Flavonoids	+
Tannins	+
Carbohydrates	+
Alkaloids	+
Proteins	-
Steroids	+
Terpenoids	+

+: Presence; -: Absence

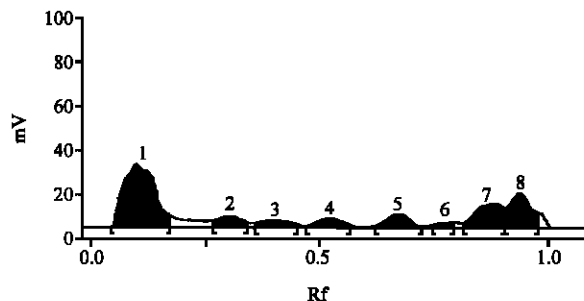


Fig. 1: HPTLC chromatogram of methanolic leaves extract of *Pergularia daemia*

observed in the HPLC spectrum and exhibited the presence of two principle components in the methanolic extract of the leaves. IR spectral analysis was performed for both the major bands (band 1 and 2) that obtained after TLC analysis. Figure 1 and 2 show the analysis of methanolic leaves extract of *P. daemia* by HPTLC and HPLC. The results of the IR spectrum revealed that band 1 possess compounds of carboxylic acid and alkenes band 2 possesses compounds that are having a aromatic groups, when analysed by FTIR were found to have two major components (Fig. 3). The other retention peaks were subsequent results seems to be ambiguous. It helps to undertake further studies on the isolation and identification of specific phytoconstituents, which may be responsible for the many pharmacological actions.

Phytochemicals such as saponins, terpenoids, flavonoids and alkaloids have anti-inflammatory effects. Flavonoids, tannins and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). From clinical studies, it is shown that terpenoids strengthen the skin, increase the concentration of antioxidants in wounds and restore inflamed tissues by increasing blood supply. The terpenoids have also shown to decrease blood sugar levels in animal studies (Luo *et al.*, 1999). Phytochemical screening of methanol extracts of *P. daemia* leaves used in this study revealed that the crude extracts contained alkaloids, carbohydrates, flavonoids, tannins and terpenoids. *Pergularia daemia* crude extract showed no indication of the presence of steroids. The presence of flavonoids in the leaves enables to be used as an

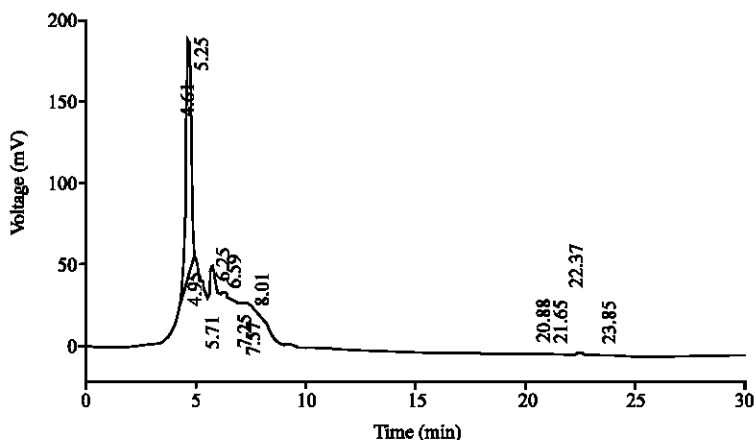


Fig. 2: HPLC chromatogram of methanolic leaves extract of *Pergularia daemia*

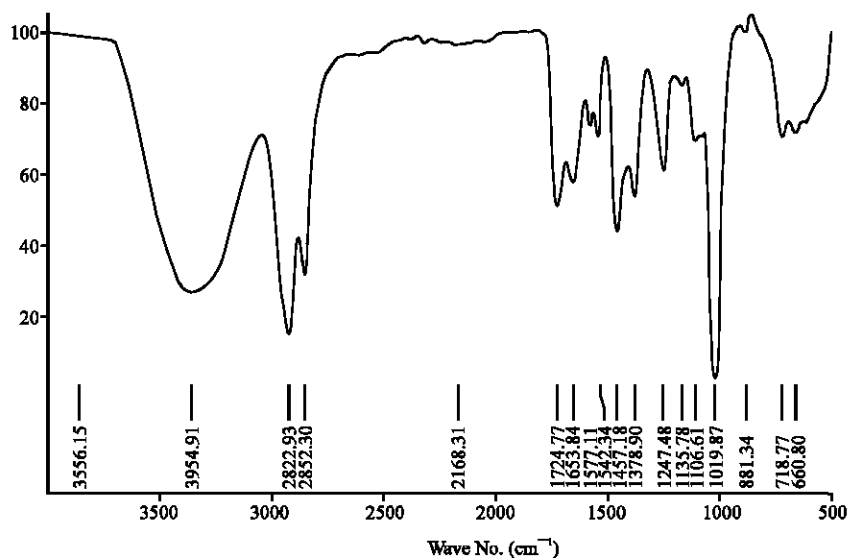


Fig. 3: FTIR Spectrum of methanolic leaves extract of *Pergularia daemia*

antioxidant. *Pergularia daemia* leaves has tannins as its main component used for treating intestinal disorders such as diarrhoea and dysentery (Akinpelu and Onakoya, 2006). The presence of flavonoids in the leaves of *P. daemia* enables them to be used as an antioxidant. Antioxidants neutralize highly unstable and extremely reactive molecules called free radicals, which attack the cells of human body every day (Stauth, 2007). The presence of flavonoids and tannins (Table 1) explains the reason why the leaves of *P. daemia* are used for the treatment of diarrhoea. The study done at Children's Hospital and Research Center Oakland, in collaboration with scientists at Heinrich Heine University in Germany, has shown that epicatechin, quercetin and luteolin which are type of flavonoids can inhibit the development of fluids that result in diarrhoea by targeting the intestinal

cystic fibrosis transmembrane conductance regulator Cl-transport inhibiting cAMP-stimulated Cl- secretion in the intestine (Schuier *et al.*, 2005).

The HPTLC analysis of *P. daemia* leaves extract shows two major peaks R_f 0.04 (44.34%) and 0.90 (16.04%) can also be further confirmed by HPLC which is also showing two major peaks R_t 4.607 (83.6826%) and 5.707 (9.4018%). The FTIR analysis confirmed the presence of carboxylic acid and Alkenes-CH₂, CH₃; Aromatic stretching which shows major peaks at 1019.87 and 2922.33 cm⁻¹.

CONCLUSION

The phytochemical screening on quantitative analysis shows that the leaves of the *P. daemia* are rich

in popular phytochemical constituents such as flavonoids, terpenoids, carbohydrates, steroids and alkaloids. Thus, the study has provided biochemical basis for ethno pharmacological uses of the plant in the treatment and prevention of various diseases and disorders. However, further studies are in progress in our laboratory to isolate the bioactive compounds which will show anticancer activity.

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