International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Qualitative And Quantitative Analysis of a Common Herb: *Pergularia daemia*

Poornima Rajan[1], Dr. Horne Iona Averal[2]

[1]Research scholar, Department of Zoology, Holy Cross College, Trichy- 2, Tamil Nadu, India.

[2]Associate Professor and Head, Department of Zoology, Holy Cross College, Trichy- 2, Tamil Nadu, India.

Abstract: ***Pergularia daemia is a widely distributed plant in the tropical and sub tropical area. Traditionally the plant has been used for various ailments like anti- pyretic, expectorant, infantile diarrhoea and for various menstrual abnormalities. In the present study the quantitative and qualitative analysis of the plant has been done. The qualitative analysis of the methanolic leaf extract of Pergularia daemia showed the presence of various compounds like terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides and coumarins. The quantification of the compounds like alkaloid, flavonoids, total phenols and tannins has also been done. From the results it is evident that the plant Pergularia daemia is found to have significant amount of phytocomponents.***

Keywords: *Pergularia daemia*, qualitative analysis, traditional medicine and veliparuthi

1. Introduction

Herbs can be defined as a plant, plant part or extract which are used for flavor, fragrance or medicinal purposes. Herbal treatment for treating diseases is the most ancient form of treatment. It includes Ayurveda, Siddha, Homeopathy etc., which has been using plant and plant formulations to treat various ailments. According to World Health Organization, more than 80% of people in poor and underdeveloped countries depend on plant based medicines for their primary health care needs [1]. India is a home of 45, 000 plant species. Among these, many of the plants have medicinal properties and their scientific validation is very much essential. They are possessed to have various properties like anti- oxidant, anti- cancer and anti- inflammatory etc. Plant based products have been used extensively because of its efficacy, safety, nearly no side effects, easily available and low cost. Traditional medicines have preventive, curative and rehabilitative role in combating a disease. For these reasons now the pharmaceutical companies started to enter the medicinal plant research. One such plant is *Pergularia daemia*. It is a hispid perennial herb which grows along the road sides of India and other tropical and sub- tropical regions. It is popularly known as 'Veliparuthi' in Tamil. This plant has been used in the traditional medicine for a wide range of aliments. The entire plant is used as anti-helmenthic, anti- pyretic, laxative, expectorant and infantile diarrhea. Each part of the plant has various therapeutic values. The root of the plant is effecting in treating the disorders like asthma, mental disorder, anemia, leprosy and piles [2]. The dried leaves are used in treating bronchitis, asthma, rheumatic fever, amenorrhea and dysmenorrhea and wounds [3]-[8]. Roots and shoots are prescribed for Whooping cough [9]. All these medicinal properties of *Pergularia daemia* is may be due to the presence of various phytochemicals. Hence this study aims to determine the phytochemicals so that they can be used further for the designing of the drugs in future. The methanol extract of the leaves of *Pergularia daemia* was subjected for qualitative and quantitative analysis for the determination of phyto-components.

2. Materials and Methods

2.1 Collection of the Plant

The fresh leaves were collected from Golden Rock, Trichy, Tamil Nadu, India.

2.2 Preparation of the Extract

The leaves of *Pergularia daemia* were washed nicely in the tap water and rinsed in distilled water. The leaves were then air- dried at a room temperature of about 37^0 C for two weeks. After which the leaves are pulverized using a sterile electric blender to obtain a powder form. Then they were sieved under the 40 mesh size to get a uniform powder. 100g of air- dried powder was extracted with methanol at a temperature of about 40^0-60^0C in a soxhlet extractor for 18-20 hours and the solution was evaporated to dryness under reduced pressure and controlled temperature by using the rotary evaporator. The extract was filtered by using No. 1 Whatman filter paper and stored in a refrigerator at 4^0C in an air tight container for further phytochemical analysis.

3. Preliminary Phytochemical Screening

Generally medicinal values of the plants are dictated by their phytochemical & other chemical constituents. Number of phytochemical studies has demonstrated the presence of several classes of chemical compounds. *Pergularia daemia* plant was subjected to phytochemical tests to identify the nature of chemical constituents. This phytochemical analysis indicates the presence (or) absence of secondary metabolites like alkaloids, flavanoids, etc using standard procedures described by (Kokate, 1986 and Harborne, 1998). The methanol extract of the plant was tested for various bioactive compounds.

3.1 Test for Terpenoids (Salkowski test)

2 ml of chloroform and 2 ml of concentrated sulphuric acid were added to 2 ml of the methnol extract. A reddish

coloration of the interface indicates the presence of terpenoids.

3.2 Test for Flavonoids

A few drops of 10% lead acetate solution was added to 1 ml of the extract. A yellow coloration indicates the presence of flavonoids.

3.3 Test for Saponins (Foam test)

5 ml of extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously after which it was observed for the formation of an emulsion.

3.4 Test for Tannins (Braymer's test)

2 ml of the extract was added to 2 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Green precipitate was regarded as positive, for the presence of tannins.

3.5 Test for Alkaloids (Hager's test)

Few drops of Hager's reagent was added to 2 ml of the extract and shaken gently to extract the alkaloidal base. Yellow precipitate was regarded as positive for the presence of alkaloids.

3.6 Test for Steroids (Salkowski test)

To 2 ml of extract, 2 ml of chloroform and few drops of concentrated sulphuric acid were added. Reddish brown ring was regarded as positive for the presence of steroids.

3.7 Test for Glycosides (Liebermann's test)

To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic acid were added. Violet to blue and blue to green color was regarded as positive for the presence of glycosides.

3.8 Test for Phlobatannins (Precipitate test)

To 2 ml of extract, 2 ml of 1% hydrochloric acid was added and boiled. Red precipitate was regarded as positive for the presence of phlobatannins.

3.9 Test for Proteins (Xanthoproteic test)

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added and boiled. White precipitate was regarded as positive for the presence of proteins.

3.10 Test for Coumarins

To 2 ml of extract, 3 ml of 10 % sodium hydroxide was added. Yellow color was regarded as positive for the presence of coumarins.

4. Quantification of the compounds

4.1 Determination of Total Alkaloid Content

The alkaloid content was determined gravimetrically (Harborne, 1973). 5 g of plant sample was weighed using a weighing balance and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 hours before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added in drops in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried in an oven at 60°C for 30 min, transferred into desiccators to cool and then re-weighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by difference in weight of the filter paper. The experiment was repeated thrice and the reading of the average was recorded.

Determination of Total Alkaloid content
Total Alkaloid content= Final weight of sample/initial weight of extract *100

4.2 Determination of Total Flavonoid Content

The total flavonoid content (TFC) of the extract was determined using the Aluminium chloride Assay through colorimetry(Kalita P *et al.*, 2013). An aliquot (0.5 ml) of extract was mixed with 2ml of distilled water followed by the addition of 0.15 ml of sodium nitrite ($NaNO_2$, 5% w/v) and allowed to stand for 5 min. Later, 0.15 ml of aluminium chloride (10% $AlCl_3$) was added and incubated for 5 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and the volume was made up to 5ml with distilled water. After 15 min of incubation, absorbance was measured at 510 nm. Distilled water was used as blank. The TFC was expressed in mg of quercetin equivalents (QE) per gram of extract. All the determinations were carried out six times.

Determination of Total Flavonoid content
TFC= C*V/M
Where
TFC = Total Flavonoid content, mg/ g of sample extract in QE (quercetin equivalent)
C = the concentration of quercetin established from the calibration curve (mg/Ml)
V = the volume of extract (ml)
M = the weight of sample extract (g).

4.3 Determination of Total Phenols

The method described by Wolfe *et al.*, 2003 with little modification by using Folin- Ciocalteau reagent was used to determine total phenols content in the extract. A volume of 0.5 ml of the extract (1 mg/ml) was mixed with 2.5 ml of 10% Folin- Ciocalteau and 2 ml of sodium carbonate (75% w/v). The resulting mixture was vortexed for 15 seconds and incubated for 30 minutes for color development. The absorbance of total phenols was measured at 765 nm using

UV-VIS double beam (Mapada UV-1800, China) spectrophotometer. The experiment was conducted in triplicates.

Determination of Total Flavonoid content
TPC= C*V/M
Where
TPC = Total Phenolic content, mg/ g of sample extract in CE (catechol equivalent)
C = the concentration of catechol established from the calibration curve (mg/Ml)
V = the volume of extract (ml)
M = the weight of sample extract (g).

4.4 Determination of Total Tannins

One-fifth gram (0.20 g) of the sample was added to 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80˚C for 1 h to ensure a uniform mixing. The extract was filtered into a 100 ml volumetric flask, followed by adding 20 ml of distilled water, 2.5 ml of Folin- Denis reagent and 10 ml of 17% sodium carbonate was also added and thoroughly mixed together. The mixture was made up to 100 ml with distilled water, then mixed and allowed to stand for 20 minutes. The absorbance of the sample was measured after bluish-green color development at 760 nm using the UV-VIS double beam (Mapada UV-1800, China) spectrophotometer (Method of A.O.A.C, 1975).

Determination of Total Flavonoid content
TT= C*V/M
Where
TT = Total Tannins ml/g of sample extract in CE (Catechol Equivalent)
C = the concentration of catechol established from the calibration curve (mg/Ml)
V =the volume of extract (ml)
M = the weight of sample extract (g).

5. Results

On the basis of phytochemical screening of methanol extract of the leaves of *Pergularia daemia*, it is apparent that it has various phytochemical components. There is presence of terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides and coumarins but there is absence of phlobatannins and proteins (Table. 1). The extract has been further subjected to quantification of major phytocompounds like alkaloids, flavonoids, phenols and tannins. The total alkaloid content is found to be 0.04514 g alkaloids/g extract . The amount of flavonoids present in the sample is determined by Quercetin Standard graph (Figure. 1) where the absorbance of the extract at 510nm is found to be 0.42 and by using the formula, the amount of flavonoids is found to be 123. 529 mg/g extract. The phenolic content of the leaf extract is found by using Standard curve (Figure. 2) where the absorbance of the sample (1 mg/ ml and 0.5ml) at 765 nm is 0.22. The total phenolic content is expressed in catechol equivalent and found to be 15 mg/ g CE. The total tannins present in the leaf extract is found using a Standard curve (Figure. 3) where the absorbance of the sample (0.2 g or 200 mg) at 760 nm is 0.17 by using the formula, the total tannin content of the leaf extract is found to be 3.956 mg/ g CE. The total tannins are also expressed in Catechol Equivalent.

Table 1: Phytochemical components present in the methanol extract of the leaves of *Pergularia daemia*

S. No	Phytoconstituents	Test performed	Presence/ Absence
1	Terpenoids	Salkowski test	Present
2	Flavonoids	-	Present
3	Saponins	Foam Test	Present
4	Tannins	Braymer's Test	Present
5	Alkaloids	Hager's Test	Present
6	Steroids	Salkowski Test	Present
7	Glycosides	Liebermann's Test	Present
8	Phlobatannins	Precipitate Test	Absent
9	Proteins	Xanthoproteic Test	Absent
10	Coumarins	-	Present

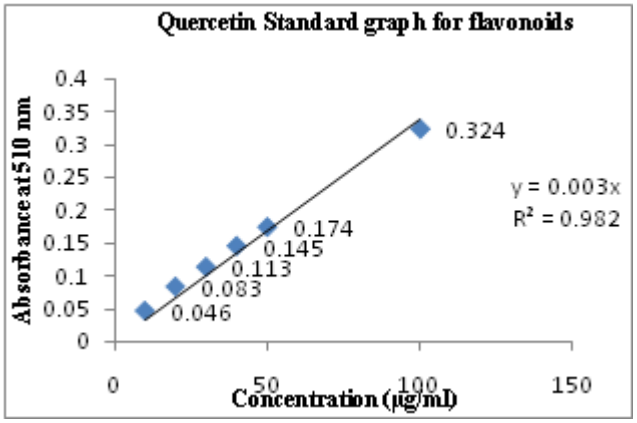

Figure 1: Determination of Total Flavonoids

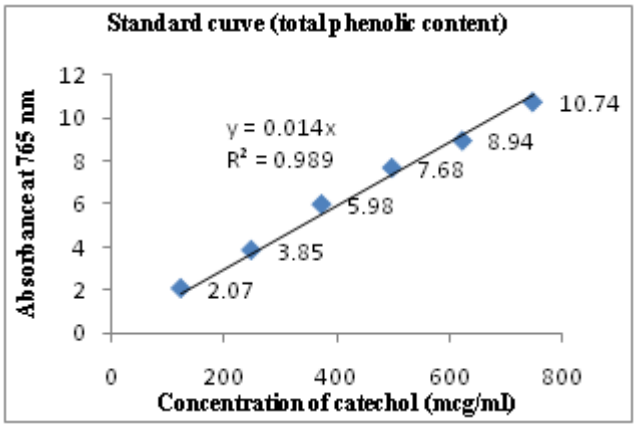

Figure 2: Determination of Total Phenols

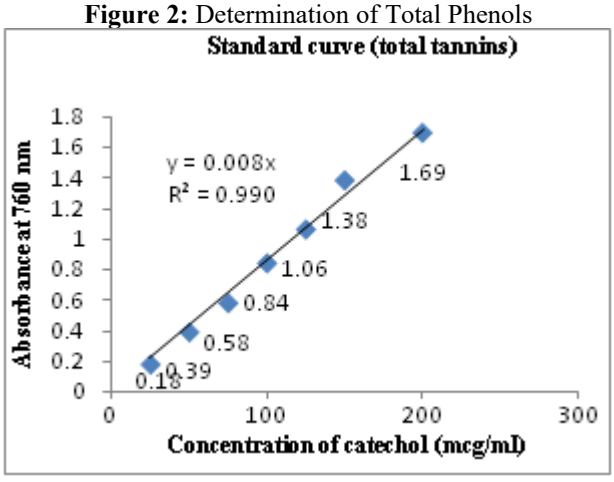

Figure 3: Determination of Total Tannins

6. Discussion

As there are various undesirable effects of synthetic drugs, people are demanding natural drugs for safety in recent years. Therefore scientists are in search for alternative medicine to synthetic drugs. Some chronic diseases require long term therapy in that case synthetic drugs may produce side effects. Phytochemicals such as saponins, terpenoids flavanoids and alkaloids have hypoglycemic activities [16]. According to clinical studies, terpenoids have the ability to strengthen the skin, increase the concentration of antioxidants in the wounds and it also augments the blood supply in the inflamed tissue for fast recovery. The terpenoids also have the capacity to decrease the blood sugar level in animals [17]. *Pergularia daemia* has tannins and tannins play a major role in the treatment of intestinal disorders such as diarrhoea and dysentery [18]. The flavonoids present in the leaves facilitate us for using as an anti-oxidant. Antioxidants are able to neutralize highly unstable and extremely active molecules which are called as free radicals which affects the cells of the human body each day [19]. According to a study done at Children's Hospital and Research Center Oak Land Collaboration with Scientists at Heirich University in Germany the flavonoids like epicatechin, quercetin and luteolin was able to inhibit the development of fluid that causes diarrhoea by targeting the intestinal fibrosis transmembrane conductance regulates c1-transport inhibiting CAMP stimulated C1 secretion in the intestine [20]. Bhaskar and Balakrishnan (2009) carried out an in vitro screening of antioxidant activity on *Pergularia daemia* root extract. In their preliminary phytochemical test both aqueous & ethanolic extract indicated the presence of alkaloid, glycosides, steroid, flavanoid, saponin, terpenoid, tannin& phenolic compound .The result obtained from their study shows that *Pergularia daemia* exhibited antioxidant activity which may be due to the presence of polyphenolic & other phytochemical constituents and may be used in preventing oxidant stress related degenerated diseases which is similar to our study.

7. Conclusion

The qualitative and quantitative analysis of the methanolic leaf extract of *Pergularia daemia* reveals the presence of medicinally valued bio active components like terpenoids, flavonoids, tannins, alkaloids, steroids, glycosides and coumarins. The presence of flavonoids enables the plant as a potent anti- allergic, anti- inflammatory, antioxidant, anti-cancer activity and also it also play a role in preventive cardiovascular diseases. As the medicinal importance of similar components in other plant extracts are previously established, there is no doubt that these components in *Pergularia daemia* will also have wonderful medicinal and disease curing ability. The research is in progress to discover its biological activity and enhance the pharmacological profile of it in the area of traditional medicine.

8. Acknowledgment

I would like to thank my Research Supervisor, Dr. Horne Iona Averal for her motivation and constant support. I thank the UGC for the funding the Major Project. I would also thank my family members for their encouragement, especially for my husband, Mr. Jayanthiran Sasidharan.

References

[1] WHO, IUCN, WWF, "Guidelines on the conservation of medicinal plants. Switzerland: IUCN Gland" 1993.
[2] S. N Yoganarasimhan, "Medicinal Plants of India. Vol. 1 Bangalore: Interline Publishing Pvt. Ltd," pp. 405, 2000.
[3] O.P Mittal, C Tammz, T Reichstein, "Glycosides and aglycons. CCXXVII. The glycosides of *Pergularia extensa,"* (45), pp. 907, 1962.
[4] V. Elango, L. Ambujavalli, E. Amala Basker, N. Sulochana, "Pharmacological and microbiological studies on *Pergularia extensa.* Fitoterapia," LVI (5), pp.300-302, 1985.
[5] H.K.N Kakrani, A.K Saluja, "Traditional treatment through herbs in Kutch district, Gujarat state, India. Part II. Analgesic, anti-inflammatory, anti-rheumatic, anti-arthritic plants. Fitoterapia; LXV," (5), pp. 427-430,1994.
[6] H De laszlo, P.S Henshaw, "Plant materials used by primitive peoples to affect fertility," Science CXIX (119), pp. 626-63, 11954.
[7] A. Dutta, S. Ghosh, "*Daemia extensa,"* Indian Journal of Pharmacy, (9), pp. 58- 60, 1947.
[8] P. Pushpangadan, C.K Atal, "Ethno-medico-botanical investigations in Kerala. Some primitive tribals of Western Ghats and their herbal medicine." Journal of Ethnopharmacology XI (1), pp. 59-77, 1984.
[9] J.O Kokwaro, "A review of research on plants for fertility regulation in Africa. Proc who symposium on plant-derived products for fertility regulation,"Seoul, Korea February, pp. 8, 1981
[10] C.K Kokate,"Practical Pharmacognosy", Vallabh Prakashan, New Delhi, 1st ed., pp.15-30, 1986.
[11] J.B. Harborne, "Methods of extraction and isolation. In: Phytochemical methods", Chapman and Hall, London, pp.60-66, 1998.
[12] J.B Harborne, "Phytochemical methods," London.Chapman and Hall, Ltd, pp. 49- 188, 1973.
[13] P. Kalita, B.K Tapan, T.K Pal, R. Kalita, "Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn.," Journal of Drug delivery and Therapeutics,. III (4), pp. 33-7. 2013.
[14] K. Wolfe, X. Wu, R.H Liu, "Antioxidant activity of apple peels," Journal of Agricultural and Food Chemistry, (51),pp. 609–614,2003.
[15] Association of Official Analytical Chemists Official methods of analysis of the Association of Official Analytical Chemists, Washington, D.C. (A.O.A.C), 1975.
[16] S. Cherian, and K. T. Augusti, "Insulin sparing action of leucopelargonidin derivative isolated from *Ficus bengalesis* Linn, Indian Journal of Experimental Biology, (33), pp. 608- 611, 1995.
[17] J. Luo, J. Cheung and E. Yevich, "Novel terpenoid – type quinines isolated from *Pycnanthu angolensis* of potential utility in the treatment of type-2 diabetes,

Journal of Pharmacology and Experimental Therapy, (288) pp. 529- 534, 1999.
[18] D. A. Akinpelu, Z.T.M. Onakoya, "Antimicrobial activities of medicinal plants used in folklore remedies in South Western. African Journal of Biotechnology, (5), pp. 1078- 1081, 2006.
[19] D. Stauth, "Studies force new view on biology of flavonoids," Oregon State University, USA, 2007.
[20] M.Schuier, H. Sies, B. Billek, H. Fischer, "Cocoa related flavonoids inhibits CFTR- mediated chloride transport across T84 human colon epithelia," Journal of Nutrition, (135), pp. 2320- 2325, 2005.
[21] H. V Bhaskar, N. Balakrishnan, "In vitro anti- oxidant property of laticiferous plant species from Western Ghats Tamil Nadu, India," International Journal of Health Research, (2), pp. 163-170, 2009.