Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(6): 2106-2111
Received: 01-09-2019
Accepted: 05-10-2019

**Akhil GH**
M.V.Sc Scholar, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy, Kerala, India

**Bibu JK**
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

**Sujarani S**
Assistant Professor, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy, Kerala, India

**Shynu M**
Assistant Professor, Dept. of Veterinary Biochemistry, College of Veterinary & Animal Sciences, Mannuthy, Kerala, India

**Usha PTA**
Professor and Head, Dept. of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy, Kerala, India

**Corresponding Author:**
**Bibu JK**
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

# Phytochemical analysis and *in vitro* antioxidant activity of methanol extract of leaves of *Pergularia daemia*

**Akhil GH, Bibu JK, Sujarani S, Shynu M and Usha PTA**

**Abstract**
The phytochemicals present in the plants are proved to have a good antioxidant potential which is beneficial in maintaining normal physiological homeostasis in a biological system. Deficiency of these compounds leads to oxidative stress resulting in inflammation, genotoxicity and cancer. The present study was undertaken to evaluate the phytochemicals and *in vitro* antioxidant activity of methanol extract of leaves of *Pergularia daemia.* Qualitative phytochemical screening showed the presence of steroids, glycosides, tannins, diterpenes, triterpenes, flavonoids and saponins. The extract had produced a significant antioxidant activity in a concentration-dependent manner in DPPH and superoxide radical scavenging assays. The maximum concentration used for the study which was 320 µg/mL, produced maximum inhibition of DPPH and superoxide radical of 73.19±1.34 and 68.69±0.56 per cent respectively. The $IC_{50}$ concentration of the extract was found to be 100.67±7.9 and 39.63±1.41 µg/mL for DPPH and superoxide radical scavenging assay respectively. Thus, the study proved a potent antioxidant activity of methanol extract of leaves of *Pergularia daemia* in scavenging free radicals.

**Keywords:** *Pergularia daemia,* DPPH, superoxide, qualitative phytochemical screening, flavonoids

## 1. Introduction
Majority of the physiological effects produced by plants in the body are due to the presence of phytochemicals in them. Phytochemicals present in the plants play a major role in maintaining and improving the health status of living beings. Different phytochemicals have different added advantages in biochemical reactions that occur in the body.

Antioxidants are the agents that protect biological systems against oxidative stress. The reactive oxygen species (ROS) are the most harmful free radicals generated during normal aerobic cellular respiration by macrophages, polymorphonulcear leukocytes and peroxisomes. The exogenous causes include pollutants, pesticides, organic solvents and tobacco smoke. The most commonly occurring ROS species consists of superoxide anion, $H_2O_2$, reactive hydroxyl (-OH) radicals, peroxyl (ROO-) radicals, peroxynitrite anion (ONOO-) and nitric oxide. The free electron present on the outer shell of these species is highly reactive and unstable. They cause destruction of major macromolecular components of cell like nucleic acid, proteins and lipids. In some pathological conditions like inflammation, genotoxicity and cancer, there is overproduction of ROS [1, 2, 3].

Antioxidants donate an electron to a free radical without making themselves unstable thereby maintaining the balance between ROS and antioxidants. Many of the plants contain phytochemicals that have potent natural antioxidant activity, few such phytochemical include flavonoids and polyphenolic compounds. These antioxidants prevent the damage produced by free radicals in biological system.

Flavonoids inhibits the action of nitric-oxide synthase in macrophages thereby reducing the production of nitric oxide and thus decreasing the nitric oxide mediated production of highly reactive peroxynitrite [4]. Additionally, flavonoids such as quercetin and silibin are known to inhibit xanthine-oxidase activity thereby decreasing the production of superoxide free radicals [5]. Quercetin, a well-known flavonoid directly inhibits lipid peroxidation that occurs due to the presence of ROS in the presence of iron [6].

*Pergularia daemia* (Asclepiadaceae), commonly known as Sagovani is usually found on roadside and compounds as a milky climber weed. This plant is commonly found in Karnataka, Tamil Nadu and Kerala and has been traditionally used as laxative, anthelminthic, antipyretic, analgesic, expectorant, antidiarrhoeal. Karthishwaran and Mirunalini (2010) [7] reviewed the therapeutic potential of *Pergularia daemia* as an ayurvedic wonder [7]. Jain *et al.*

(1998) [8]. Described the ethnopharmacological evaluation of ethanol extract of aerial parts of *P. daemia* [8]. Sutar and Pal (2015) [9]. Performed high performance thin layer chromatography (HPTLC) for the identification of flavonoids in petroleum ether and methanol extract of leaves of *P. daemia* [9]. Bhaskar and Balakrishnan (2009) [10]. Reviewed about different medicinal properties produced by *P. daemia* [10].

Hence the objective of the present study was to identify the phytochemicals present in the methanol extract of leaves of *Pergularia daemia* and its *in vitro* antioxidant activity using DPPH and superoxide radical scavenging assay.

## 2. Material and Methods

**2.1 Collection of plant samples:** The whole plant of *Pergularia daemia* was collected from the campus of University of Agricultural Sciences, Raichur, Karnataka in the month of February-March 2018. The plant material was authenticated by Raw Material Herbarium & Museum, NISCAIR-New Delhi (NISCAIR/RHMD/Consult/2018/3281-82) and voucher specimen was deposited in the Dept., of Veterinary Pharmacology & Toxicology.

**2.2 Processing of plant samples:** The leaves of *Pergularia daemia* was separated from the whole plant and dried under shade at room temperature for a week. The dried leaves of the plant were pulverised with an electric pulveriser to obtain coarse powder. The powdered form was stored in an airtight container until further use.

**2.3 Preparation of methanol extract of leaves of *Pergularia daemia*:** Approximately, 100 g of powdered leaves sample was loaded into the thimble of Soxhlet apparatus in Whatman filter paper and extraction was performed at 67 °C. The methanol extract was concentrated using a rotary evaporator under reduced pressure (230mbars) and temperature (40 °C) for evaporating the solvent. The yield of the extract was calculated and the extract was kept under refrigeration in airtight glass container until further use.

**2.4 Qualitative phytochemical screening:** Phytochemical analysis of the methanol extract of leaves of *Pergularia daemia* was performed based on the method proposed by Harborne, (1991) [11].

### 2.4.1 Test for detection of steroids

**1. Salkowski's test:** Approximately fifty milligrams of the extract were dissolved in 3 mL of chloroform. Few drops of concentrated sulphuric acid were added and the solution was allowed to stand. Development of red colour indicated the presence of steroids.

**2. Liebermann burchardt test:** Approximately fifty milligrams of the extract were mixed with 3 mL of chloroform. To this, five drops of acetic anhydride and 1 mL concentrated sulphuric acid was added along the sides of the test tube. Development of a reddish ring at the junction of two layers confirmed the presence of steroids.

**2.4.2 Tests for detection of alkaloids:** One gram of the extract was mixed with 5 mL of ammonia and then extracted with an equal volume of chloroform. To this extract, 5 mL of dilute hydrochloric acid was added. The acid layer obtained was further tested with the following reagents for the presence of alkaloids.

**1. Dragendorff's test:** Eight drops of Dragendorff's reagent (Stock solution (1) one gram of bismuth subnitrate was dissolved in 2 mL of concentrated hydrochloric acid and 10 mL of water. Stock solution (2) six gram of potassium iodide was dissolved in 10 mL of water. Then both the stock solutions (1) and (2) were mixed together and it was mixed with 7 mL of concentrated hydrochloric acid and 15 mL of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 mL) was mixed with 1 mL of acid extract. Development of a reddish-brown precipitate indicated the presence of alkaloids.

**2. Mayer's test:** To 1 mL of the acid layer, eight drops of Mayer's reagent (1.4 g of mercuric chloride dissolved in 60 mL of water and poured into a solution of five gram of potassium iodide in 10 mL of water and then made up the volume to 100 mL with distilled water) were added. Development of a cream coloured precipitate indicated the presence of alkaloids.

**3. Wagner's test:** One millilitre of Wagner's reagent (two-gram iodine and six gram of potassium iodide dissolved in 200 mL of water) was added to 1 mL of the extract. Development of reddish-brown colour precipitate indicated the presence of alkaloids.

**4. Hager's test:** To 1 mL of the acid extract, eight drops of Hager's reagent (one gram of picric acid dissolved in 100 mL of water) were mixed. Development of yellow precipitate indicated the presence of alkaloids.

### 2.4.3 Tests for detection of glycosides

**1. Sodium hydroxide test:** Approximately 50 mg of the extract was mixed with 1 mL water and six drops of 10 per cent sodium hydroxide solution was added to it. Development of yellow colour indicated the presence of glycosides.

**2. Benedict's test:** Approximately 50 mg of the extract was mixed with 1 mL of water and then 5 mL of Benedict's reagent was added to it. Formation of brown or red precipitate indicated the presence of reducing sugars.

### 2.4.4 Test for detection of phenolic compounds

**Ferric chloride test:** Five milligrams of the extract were dissolved in 1 mL of water and five drops of 10 per cent ferric chloride were added to it. Formation of bluish black colour indicated the presence of phenols.

### 2.4.5 Tests for detection of tannins

**1. Ferric chloride test:** Treated two milligrams of the extract with 3 mL of one per cent ferric chloride solution. Development of a brownish green or a blue-black colouration indicated the presence of tannins.

**2. Gelatin test:** One gram of the extract was mixed with a few drops of one per cent solution of gelatin containing 10 per cent sodium chloride. Development of a white precipitate indicated the presence of tannins.

### 2.4.6 Tests for detection of flavonoids

**1. Ferric chloride test:** Treated 2 mL of the methanol extract (0.5-gram extract in 10 mL methanol) with four drops of neutral ferric chloride solution. Formation of green colour indicated the presence of flavonoids.

**2. Lead acetate test:** Treated 2 mL of the alcohol extract (0.5-gram extract in 10 mL methanol) with 3 mL of 10 per cent lead acetate solution. Development of yellow precipitate indicated the presence of flavonoids.

**2.4.7 Test for detection of diterpenes:** About five milligrams extract was mixed with 3 mL of five per cent copper acetate solution. Development of green colour indicated the presence of diterpenes.

**2.4.8 Tests for detection of triterpenes**

**1. Salkowski test**: Mixed 3 mL of chloroform to three milligrams of extract and it was shaken with 3 mL concentrated sulphuric acid. Development of yellow colour in the lower layer on standing indicated the presence of triterpenes.

**2. Liebermann burchardt test:** Three milligrams of the extract were mixed with 3 mL chloroform in a test tube. To this, five drops of acetic anhydride and 1 mL of concentrated sulphuric acid was added along the sides of the test tube. Development of a deep red ring at the junction of two layers confirmed the presence of steroids.

**2.4.9 Tests for detection of saponins**

**Froth test:** Approximately 200 mg of the extract was shaken with 5 mL of water. Persistence of foam produced for ten minutes indicated the presence of saponins.

**2.5 *In vitro* antioxidant activity:** Assessment of antioxidant activity of methanol extract of leaves of *P. daemia* was performed by DPPH and superoxide anion radical scavenging assays.

**2.5.1 DPPH radical scavenging activity:** 2, 2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Karthishwaran and Mirunalini (2012) [12]. With few modifications. Four millilitres of the reaction mixture containing one mL of DPPH (0.1 mM in methanol), 3 mL of test solution at 2.5. 5, 10, 20, 40, 80, 160 and 320 µg/mL concentrations of plant extract was incubated at 37 º???C for 30 min and absorbance of the resulting solution was measured at 517 nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore). Ascorbic acid was used as the reference standard. The per cent inhibition of DPPH radical was calculated by comparing the absorbance of the test with those of control using the following equation:

Per cent inhibition = (1- AT/AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/standard

**2.5.2 Superoxide anion radical scavenging assay:** Superoxide anion free radical scavenging activity was measured according to the method of Robak and Gryglewski (1988) with some modifications [13]. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). One millilitre of nitroblue tetrazolium (NBT, 156 µM), 1 mL of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) and 3mL of test solution at 2.5. 5, 10, 20, 40, 80, 160 and 320 µg/mL concentrations of plant extract was mixed. The reaction was initiated by adding 100 µL of phenazine methosulphate (PMS, 60µM). The reaction mixture was incubated at 25 ºC for 5 min followed by measurement of absorbance at 560 nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore). Ascorbic acid was used as the reference standard. The per cent inhibition was calculated by using the equation

Per cent inhibition = (1- AT/AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/ standard

**2.6. Statistical analysis:** All results were expressed as Mean ± SE with 'n' equal to the number of replicates. The $IC_{50}$ values of the extract were calculated using the Graphpad Prism Version 5. All the statistical analysis was conducted using SPSS software version 24. Analysis of variance (ANOVA) in a completely randomized design followed by Duncan's multiple range tests was used to compare any significant differences among various concentrations of the extract and independent sample t-test was performed to compare the $IC_{50}$ concentration of the extract and the standard ascorbic acid.

## 3. Results and Discussion

**3.1 Phytochemical screening:** The methanol extract of *Pergularia daemia* yielded a variety phytochemical constituent such as steroids, glycosides, tannins, flavonoids, diterpenes, triterpenes and saponins. The extract did not show positive results for alkaloids and phenolic compounds. The results of the qualitative phytochemical analysis are described in the table 1.

Similar phytochemical results were also obtained by Nithyarani and Kavitha (2008) who performed phytochemical studies in leaves of *P. daemia* and found the presence of alkaloids, steroids, terpenoids, saponins, glycosides, amino acids and proteins, carbohydrates, reducing sugars, phenols and tannins in methanol extract [12]. Another study performed by Karthishwaran *et al.* (2010) [13]. and Martin *et al.* (2011) [14]. obtained similar results where they detected the presence of flavonoids, steroids, carbohydrates, tannins, cardiac glycosides, alkaloids and terpenoids in methanol extract of leaves of *P. daemia*.

The variations in the results of phytochemical screening obtained from the studies conducted by different authors could be due to various factors such as part of the plant extracted, climatic conditions, geographical area, type of soil, stress factors, weather conditions and seasonal variations. So also, the phytochemicals showing negative results in qualitative screening does not necessarily indicate its absence, rather, it could be due to the presence of very small quantities which was not detected by the tests performed.

**Table 1:** Qualitative phytochemical analysis of methanol extract of leaves of *Pergularia daemia*

| Phytochemcial Test | Methanol extract of leaves of *Pergularia daemia* |
|---|---|
| **Steroids** | |
| • Salkowski's test | + |
| • Liebermann Burchardt test | + |
| **Alkaloids** | |
| • Dragendroff's test | - |
| • Mayer's test | - |
| • Wagners's test | - |
| • Hager's test | - |
| **Glycosides** | |
| • Sodium hydroxide test | + |
| • Benedict's test | + |
| **Tannins** | |
| • Ferric chloride test | + |
| • Gelatin Test | + |
| **Flavonoids** | |
| • Lead acetate test | + |
| • Ferric chloride test | + |
| **Phenolic Compounds** | - |
| **Saponins** | |
| • Foam test | + |
| **Diterpenes** | + |
| **Triterpenes** | |
| • Salkowski's test | + |
| • Liberman Burchardt test | + |

### 3.2 *In vitro* antioxidant activity

**3.2.1 DPPH radical scavenging assay:** DPPH radical scavenging assay was performed to predict the antioxidant's property to inhibit lipid oxidation. The methanol extract of leaves of *Pergularia daemia* showed inhibition of DPPH radical significantly ($p<0.05$) in a concentration-dependent manner. The highest concentration of 320 µg/mL produced maximum inhibition of 73.19±1.34 per cent indicating the extract's potential to inhibit free radicals responsible for lipid oxidation in biological system. The $IC_{50}$ concentration was found to be 100.67±7.9 µg/mL and was significantly higher when compared with standard ascorbic acid whose $IC_{50}$ value was found to be 21.98±0.54 µg/mL. Table 2. and Fig. 1. describes the per cent inhibition of DPPH radicals with various concentrations of the extracts. Similar results were also obtained by Balaji *et al.* (2013) who investigated *in vitro* antioxidant activity of aqueous extract of aerial parts of *P. daemia* and found that the extract produced 72.43 per cent inhibition of DPPH radicals at 400 µg/mL [15].

**Table 2:** DPPH free radical scavenging assay: the per cent inhibition of DPPH free radical generation by methanol extract of leaves of *Pergularia daemia*

| Concentrations (µg/mL) | % inhibition of DPPH free radical | |
|---|---|---|
| | MPD | Ascorbic acid (Standard) |
| 2.5 | $4.54±0.65^{a}$ | $7.77±1.03^{a}$ |
| 5 | $6.97±1.23^{a}$ | $12.45±1.39^{a}$ |
| 10 | $8.25±1.22^{a}$ | $27.81±1.35^{b}$ |
| 20 | $15.68±1.43^{b}$ | $55.99±2.41^{c}$ |
| 40 | $21.32±0.60^{c}$ | $67.35±3.00^{d}$ |
| 80 | $28.75±3.01^{d}$ | $85.15±1.60^{e}$ |
| 160 | $44.14±1.21^{e}$ | $97.87±0.34^{f}$ |
| 320 | $73.19±1.34^{f}$ | $97.71±0.44^{f}$ |
| $IC_{50}$ (µg/mL) | $100.67±7.9^{A}$ | $21.98±0.54^{B}$ |

Values are expressed as Mean±SE (n=6). Means bearing the different superscript (a-f in columns) and (A-B in row) vary significantly at $p<0.05$

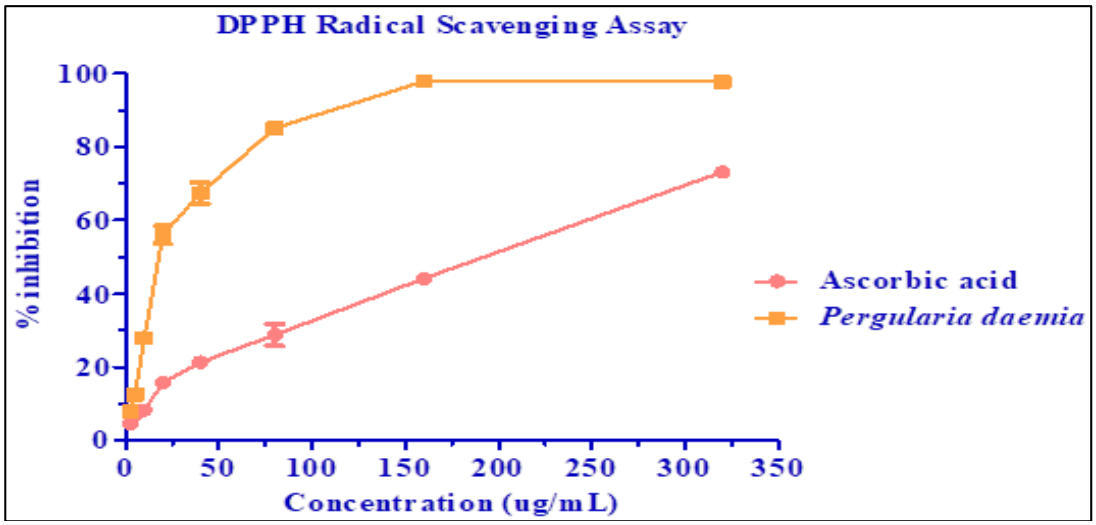


**Fig 1:** DPPH free radical scavenging assay: the per cent inhibition of DPPH free radical generation by methanol extract of leaves of *Pergularia daemia*

**3.2.2 Superoxide anion radical scavenging assay:** Superoxide radical scavenging assay was performed to assess the plant extract's antioxidant potential to scavenge superoxide anion free radicals. The extract produced a significant ($p<0.05$) concentration-dependent inhibition of superoxide radicals with $IC_{50}$ value of 39.63±1.41 µg/mL. Superoxide radical scavenging activity of the extract was comparable with that of standard ascorbic acid whose $IC_{50}$ was found to be 14.17±0.22 µg/mL. The highest concentration of the extract used at 320 µg/mL produced maximum inhibition of 68.69±0.56 per cent. Table 3 and fFig. 2 describes the per cent inhibition of superoxide radicals with various concentrations of the extracts. Bhaskar and Balakrishnan (2009) [10] also obtained similar results with ethanol and aqueous extract of *P. daemia* with superoxide radical scavenging inhibition of 74.20 and 62.69 per cent respectively [10].

Phytochemicals such as flavonoids and phenolic compounds present in the plants are proved to have potent antioxidant properties. The extract's antioxidant activity could be mainly attributed to the presence of flavonoids in it. Flavonoids are oxidised directly by free radicals thereby forming a more stable or less reactive radical. Certain flavonoids can directly scavenge the superoxide radicals whereas other flavonoids scavenge highly reactive oxygen derived radicals called peroxynitrite [16].

**Table 3.** Superoxide anion free radical scavenging assay: the per cent inhibition of superoxide free radical generation methanol extract of leaves of *Pergularia daemia*

| Concentrations (µg/mL) | % inhibition of superoxide anion free radical | |
|---|---|---|
| | MPD | Ascorbic acid (Standard) |
| 2.5 | 3.24±0.45$^{a}$ | 68.25±1.36$^{a}$ |
| 5 | 8.32±0.34$^{b}$ | 70.57±1.39$^{ab}$ |
| 10 | 16.94±2.80$^{c}$ | 73.34±1.24$^{bc}$ |
| 20 | 25.06±0.76$^{d}$ | 75.96±1.12$^{cd}$ |
| 40 | 36.54±0.66$^{e}$ | 76.80±1.15$^{cde}$ |
| 80 | 43.03±0.32$^{f}$ | 78.04±0.94$^{de}$ |
| 160 | 58.82±0.74$^{g}$ | 78.94±0.94$^{de}$ |
| 320 | 68.69±0.56$^{h}$ | 79.85±0.98$^{e}$ |
| $IC_{50}$ (µg/mL) | 39.63±1.41$^{B}$ | 14.17±0.22$^{C}$ |

Values are expressed as Mean±SE (n=6). Means bearing the different superscript (a-h in columns) and (A-B in row) vary significantly at $p<0.05$

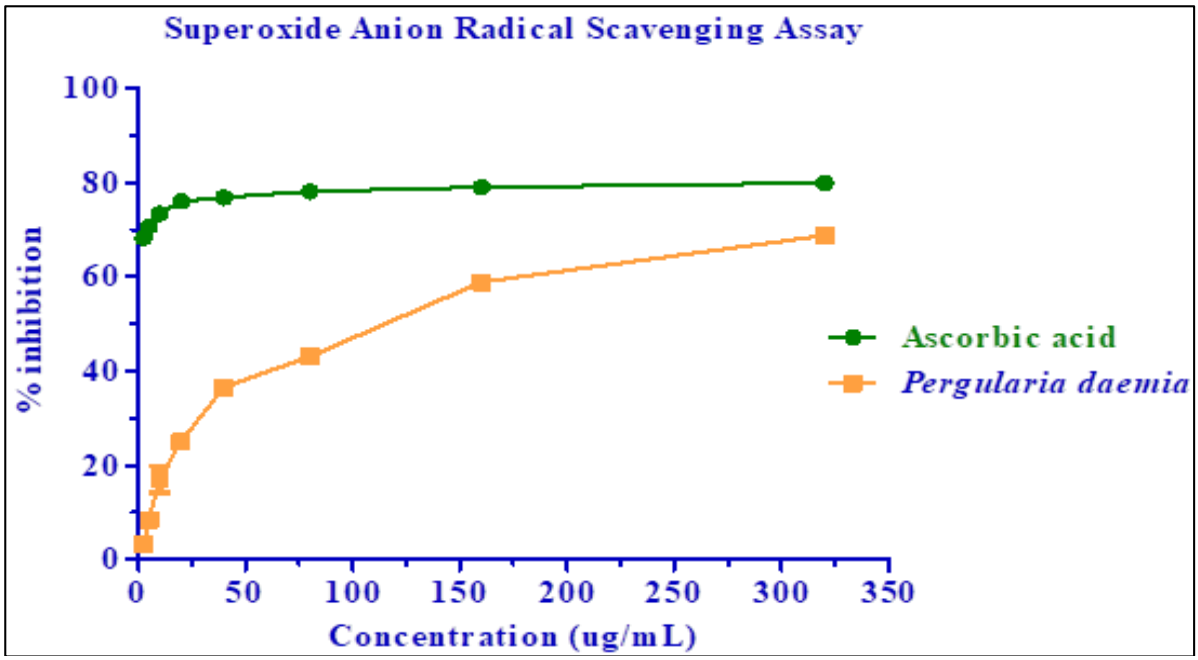


**Fig 2:** Superoxide anion free radical scavenging assay: the per cent inhibition of superoxide free radical generation methanol extract of leaves of *Pergularia daemia*

## Conclusion

In conclusion, the methanol extract of leaves of *Pergularia daemia* upon qualitative phytochemical screening yielded majority of the potent phytochemicals that include steroids, glycosides, tannins, flavonoids, diterpenes, triterpenes and saponins. The extract proved a significant concentration dependent antioxidant activity in both DPPH and superoxide radical scavenging assay which could be responsible for its therapeutic usage in ayurvedic preparations.

## References

1. Kourounakis AP, Galanakis D, Tsiakitzis K, Rekka EA, Kourounakis PN. Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Development Research. 1999; 47(1):9-16.
2. Gülçın İ, Oktay M, Kıreçcı E, Küfrevıoğlu Öİ. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food chemistry. 2003; 83(3):371-382.
3. Oktay M, Gülçin İ, Küfrevioğlu Öİ. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT-Food Science and Technology. 2003; 36(2):263-271.
4. Vanacker SA, Tromp MN, Haenen GR, Vandervijgh WJ, Bast A. Flavonoids as scavengers of nitric oxide radical. Biochemical and biophysical research communications. 1995; 214(3):755-759.
5. Chang WS, Lee YJ, Lu FJ, Chiang HC. Inhibitory effects of flavonoids on xanthine oxidase. Anticancer research. 1993; 13(6A):2165-2170.
6. Sorata Y, Takahama U, Kimura M. Protective effect of quercetin and rutin on photosensitized lysis of human

erythrocytes in the presence of hematoporphyrin. Biochimica et Biophysica Acta (BBA)-General Subjects. 1984; 799(3):313-317.
7. Karthishwaran K, Mirunalini S. Therapeutic potential of *Pergularia daemia* (Forsk.): the Ayurvedic wonder. Int J Pharmacol. 2010; 6(6):836-843.
8. Jain SC, Jain R, Mascolo N, Capasso F, Vijayvergia R, Sharma RA, Mittal C. Ethnopharmacological evaluation of *Pergularia daemia* (Forsk.) Chiov. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 1998; 12(5):378-380.
9. Sutar NG, Pal SC. Finger printing analysis of the flavonoid from leaves Pergularia daemia Forsk using HPTLC analysis. J Pharmacogn Phytochem. 2015; 3:157-161.
10. Bhaskar VH, Balakrishnan N. Veliparuthi (*Pergularia daemia* (Forsk.) Chiov.) as a phytomedicine: a review. Int. J. Pharm. Tech. Res. 2009; 1(4):1305.
11. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer science & business media; 1998.
12. Nithyatharani R, Kavitha U. Phytochemical Studies on the Leaves of Pergularia Daemia Collected from Villupuram District, Tamil Nadu, India. IOSR J. Phar. 2018; 8(1):9-12.13.
13. Karthishwaran K, Mirunalini S, Dhamodharan G, Krishnaveni M, Arulmozhi V. Phytochemical investigation of methanolic extract of the leaves of Pergularia daemia. J Biol Sci. 2010; 10(3):242-246.
14. Martin S, Kavitha PD, Rathi MA, Kumar DG, Gopalakrishnan VK. Cytotoxic activity of Pergularia daemia against ovarian cancer cell lines OAW-42 and PA-1. Journal of Natural Pharmaceuticals. 2011; 2(4):203.
15. Balaji K, Manasa G, Reddy AA, Nagaraju M, Srikanth T, Ramesh S. Evaluation of *in vitro* anti-oxidant and cytotoxicity activity of aqueous extract of Pergularia daemia. Sch. Acad. J. Pharm. 2013; 2:125-129.
16. Nijveldt RJ, Van Nood EL, Van Hoorn DE, Boelens PG, Van Norren K, Van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. The American journal of clinical nutrition. 2001; 74(4):418-425.