***Original Research Article***

**DPPH and ABTS Radical scavenging assay and Antibacterial Efficacy of Methanol Flower Extract of *Peltophorum* Pterocarpum (DC.) Backer ex K. Heyne**

**ABSTRACT:** **Aim** of the present study is to investigate the anti-radical activity and antibacterial potential of the methanol flower extract of *Peltophorum pterocarpum.* **Method:** Anti-radical activity was done through the DPPH and ABTS radical scavenging assay. The antibacterial activity of methanol flower extract was tested against *Bacillus subtilis* and *Escherichia coli* bacteria by agar well diffusion method. **Results:** The methanol flower extract of *P. pterocarpum* showed mild DPPH anti-radical activity and for ABTS radical scavenging assay presented remarkable antioxidant activity. The crude flower extract showed a significant zone of inhibition (1.5 mm) against *E. coli* (a Gram-negative bacterium) at 30 μg/well, compared to a smaller zone of inhibition (1.2 mm) against *B. subtilis* (a Gram-positive bacterium). The positive control, streptomycin, produced zones of inhibition of 2.2 mm and 2.5 mm. respectively. **Conclusion:** The present findings confirms the existence of mild and remarkable antioxidant activity and significant antibacterial potential in the flower extract of *P. pterocarpum* and this data could pave the way for further research.

*Keywords: DPPH, ABTS, IC50, P. pterocarpum, Quercetin, B. subtilis, E. coli, Streptomycin*

**INTRODUCTION**

Plants play a vital role in human health by supporting environmental sustainability and planetary health through ecosystem services like oxygen production, carbon dioxide absorption, climate regulation, and providing habitat and food for other species (Rawat & Agarwal, 2015). Medicinal plants are nature’s hidden resources, serving as a valuable natural pharmacy for humanity. Medicinal plants are important for their potential to heal wounds, relieve pain and prevent infections. That implies that in ancient times, people worshipped plants due to their strong reliance on plant-based medicine guided by experience. The indigenous system of medicine, Ayurveda—the science of life—dates back to 1500–800 BC, Angiosperms are key sources of plant medicines, and herbal remedies (Stepp, 2004). Advances in chemistry led to the discovery of more pharmacologically active compounds in medicinal plants (Atanasov, et al., 2015). Later, this interest in the plants was mainly on their bioactive compounds produced by the plants, that is found to have therapeutic effects on human physiology. Medicinal plants contain secondary metabolites, such as alkaloids, glycosides, and steroids have significant pharmaceutical effects like anticancer, antioxidant, antimicrobial, antidiabetic activities etc. Plants are the rich source of antioxidants that help to encounter the negative effects of oxygen free radicals which arise during oxidation process in cells of our body. Free radicals are unstable atoms that carries an unpaired electron, due to the insufficient stable number of electrons in outer most shell, these atoms are constantly searching for other molecules to become stable. In a living system free radicals are produced during an uncontrolled mitochondrial respiration or as by-products of other metabolic processes. These are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis (Dwivedi, et al., 2020). Antioxidants are the substances that delays or inhibits oxidative damage to a target molecule (Yamagishi & Matsui, 2011). The main characteristic of an antioxidant is its ability to trap free radicals (Wu, et al., 2011). Many plants contain antioxidant compounds and these compounds protect cells against the free radicals. Plant-derived products may prevent the microbial growth, especially in disease treatment. Many studies have explored their chemical makeup and how they inhibit microbes, alone or with conventional drugs. The antimicrobial effects of plants, shown through essential oils and extracts, suggest they could aid to develop new medicines to fight antibiotic-resistant bacteria (Davis & Bryson, 1994). *Peltophorum pterocarpum* is one of the important ornamental as well as medicinal plant belongs to the family Fabaceae (Sub family – Caesalpinioideae), native to Malay Peninsula, Andamans and North Australia, commonly called copper pod or yellow flame tree. It is a very attractive tree because of its bright yellow coloured flowers and pinnately compound leaves with oblong leaflets. Ecologically, flowers of this plant serves as a nectar source and attracts insect pollinators such as honey bees, bumble bees and several economically important vasps (Kiruba, et al., 2008), (Kiruba, et al., 2008). Apart from these *P. pterocarpum* also having effective medicinal significance. Conventionally, Paliyar tribe in Tamil nadu used the bark of the tree to treat wounds (Duraipandian, et al., 2006). and for oral healthcare practices. In Malaysia, Orang Asli tribe of Kampung Bawong, used the powdered bark of this plant to cure Psoriasis (Ganesan, 2008). Latest studies revealed that the plant leaves and bark parts subjected to antimicrobial (Samuelet al., 2010), (Voravuthikunchai, et al., 2004), (Voravuthikunchai, et al., 2006), (Voravuthikunchai & Khla, 2005), (Limsuwan, et al., 2005), (Ravikumar, et al., 2007), (Ravikumar & Rathinam, 2009), antioxidant (Ling et al., 2010), anticancer bioactive assays etc, (Husain, et al., 2009). Bioactive assays of decoction method of methanol flower extraction of *P. pterocarpum* has been not done so far. Hence, in the present investigation methanol flower extract obtained from decoction method to screen the DPPH, ABTS and antibacterial efficacy.

**MATERIALS AND METHODS**

**Plant material**

*Peltophorum pterocarpum* twigwith inflorescence were collected from Department of Botany, Jnanabharathi campus, Bangalore university (Latitude: 12° 56' 11.74" N and Longitude: 77° 30' 6.86" E). The plant was authenticated by Dr. L Rajanna, Senior Professor, Department of Botany, Bangalore university, Karnataka. Voucher specimen (twig with inflorescence) was deposited in the Herbaria, Department of Botany, B.M.S College for women, Bugle rock road, Basavanagudi, Bengaluru for further studies.

**Preparation of extract by decoction method**

Fresh and healthy flowers of Peltophorum pterocarpum were collected and thoroughly washed with running tap water to remove dust and other debris. Excess water was removed by decanting and wiping the flowers with dry cotton cloth and also allowed the plant material for shade dry. Then the dried flowers were ground into powder using an electric mixer grinder to obtain fine powder which was stored in air-tight container. 15g powder was weighed and extracted using 150ml methanol solvent for 4 hrs. in a water bath at 50°C by decoction method. The content was filtered through the Whatman No.1 filter paper. The extract thus obtained was allowed to evaporate the solvent in a hot air oven at 60°C for 3-4 days. The condensed extract was stored in microcentrifuge vials at 4°C for antioxidant and antimicrobial assay.

***In vitro* Antioxidant activity by DPPH and ABTS Radical scavenging assay**

**Protocol**

The DPPH (2,2, ̶ diphenyl-picryl hydrazyl) radical scavenging assay of *P. pterocarpum* flower extract was determined by using the method followed by RajKumar *et al*. (2011). Various concentrations of extract (6.25, 12.50, 25, 50, 100, 200 µg/ml) in methanol were mixed with 75µl of DPPH and incubated at 25°C for 15 min. in dark. After incubation the absorbance was measured at 517 nm using semi-auto analyser against control. DPPH with methanol without the extract in the reaction mixture served as blank and Quercetin (0.35, 0.61, 1.25, 2.50, 5 µg/ml) used as reference standard (Rajkumar, et al., 2011).

**% inhibition = Absorbance (control) ̶ Absorbance (sample) × 100**

**Absorbance (control)**

**ABTS Radical scavenging assay protocol**

The free radical scavenging activity of *P. pterocarpum* flower extract for ABTS (2, 2’̶ azinobis ̶ 3 ethyl ̶ benzothiazoline ̶ 6 ̶ sulphonic acid) assay was performed as per Auddy *et al*., (2003). 10 ml of ABTS (7Mm) and 10 ml of Ammonium per sulfate (2.45 mM) solutions were mixed and allowed to incubate at room temperature in dark for 16 hrs. The solution thus obtained is further diluted with Phosphate buffered saline to give an absorbance of 1.000 at 734. Fresh ABTS solution was prepared for each assay. Various concentrations of extract (6.25, 12.50, 25, 50, 100, 200 µg/ml) in methanol were allowed to react with 1ml of the ABTS solution and the reaction mixture was allowed to incubation for 10 min in dark, later absorbance was taken at 734 nm using UV ̶ spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of Quercetin standard (0.35, 0.61, 1.25, 2.50, 5 µg/ml) and control reaction was carried out without the test sample. The percentage inhibition of ABTS radical scavenging assay was calculated by using the following formula and IC50 value is calculated by Graph pad prism software (Auddy, et al., 2003).

**% inhibition = Absorbance (control) ̶ Absorbance (sample) × 100**

**Absorbance (control)**

***In vitro* Antimicrobial Assay**

During the present study the crude methanol flower extract was prepared using organic solvent methanol. Thus the extract obtained was subjected to antibacterial activity.

**Organisms used:** ***Bacillus subtilis* (**Gram positive bacterium**), *Escherichia coli***(Gram negative bacterium)procured from Plant tissue culture and phytochemistry laboratory, Department of Botany, Jnanabharathi campus, Bangalore university, Bengaluru.

**Antibacterial activity protocol**

The antibacterial activity was determined by well diffusion method (Holder & Boyce, 1994). 25mL of molten Mueller Hinton agar was poured into 2 different sterile Petri plates and were allowed to solidify, later 100μl of 18 hrs. old cultures of two different strains of Gram positive and Gram negative pathogenic bacterial suspension were transferred onto the two different petri plate contained agar medium and culture lawn by using sterile L – rod spreader and allowed to settle on the agar medium. Using sterile cork borer 5 mm well was made on the agar medium. Before loading the test (plant) samples to wells, samples were dissolved in sterile saline and loaded into the wells with various concentrations such as 10 μg/well, 20 μg/well and 30 μg/well. Sterile saline and streptomycin were served as negative and positive control respectively. The plates were incubated at 37˚ C in a bacteriological incubator for 24-48hrs. The antibacterial activity was determined by measuring the diameter of the zone of inhibition (ZOI) around the well using antibiotic zone scale.

**RESULTS AND DISCUSSION**

***In vitro* Antioxidant activity**

The methanol flower extract of *P. pterocarpum* showed mild antioxidant activity with an IC50 value of 103.5μg/ml with respect to the standard quercetin (3.64µg/ml) used. The result of DPPH assay is tabulated in **Table. 1 & Graph. 1.** Similar observation was recorded that (Rashid, et al., 2022)the ethanol leaf extract of *P. pterocarpum* had effective DPPH radical scavenging potential. Another study conducted on antioxidant activity for aqueous and ethanol leaf and bark parts of *P. pterocarpum.* Among them the ethanol extracts of *P. pterocarpum* showed more activity than the aqueous extract of the plant. Particularly, the ethanol bark extract of *P. pterocarpum* revealed more anti-oxidant capacity than that of the ethanolic leaf extract of the plant. Bergenin is a naturally occurring bioactive compound found in various plants of *Bergenia* genus belongs to the family Saxifragaceae. It is used for various pharmacological activities viz., antioxidant, anti-inflammatory, immunomodulatory properties and it is a potent bioactive molecule used in treating conditions like tuberculosis, bronchitis and even as a component in cosmetics and nutritional supplements (Jerline Babu, et al., 2021). Studied an antioxidant activity by isolating Bergenin diethyl ether and its derivative from the bark of *Peltophorum pterocarpum* and bark crude extracts such as ethanol, methanol & water. From the result it can be determined that all the bark crude extracts and bergenin proven significant antioxidant activity (Htwe, et al., 2020). The present investigation has not focussed on the isolation of phytoconstituents from the *Peltophorum pterocarpum*. But the research study conducted by Htwe *et al*., clearly suggested that the presence of bergenin an important bioactive phytochemical from the bark extract. In *Peltophorum africanum*, free radical scavenging activity of ethyl acetate stem bark extract exhibited more DPPH scavenging potential when compared to the standard used (Okeleye, et al., 2015) The detection of reactive oxygen species (ROS) was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the ethanol leaf extract of *P. pterocarpum* revealed the dose-dependent activity with respective IC50 value (Shafie, 2023). The DPPH radical scavenging potential for methanol pod extract of *P. pterocarpum* found to be possessed effective scavenging activity with respective IC50 value (Khan, et al., 2013). The DPPH scavenging activity of stem extract of *P. pterocarpum* obtained from Petroleum ether, dichloromethane, ethyl acetate and methanol solvents. Among the different solvents, ethyl acetate stem extract of *P. pterocarpum* exhibited remarkable antioxidant potential with respective IC50 value (Jain, et al., 2012). In the present study the methanol flower extract of *P. pterocarpum* showed noteworthy ABTS+ scavenging activity with an IC50 value of 48.60μg/ml with respect to the standard quercetin (1.71µg/ml) used. The result of ABTS assay is tabulated in **Table. 2 & Graph. 2.** Similar study was reported that ethanol leaf extract of *P. pterocarpum* signifies strong ABTS radical scavenging potential when compared to the aqueous leaf extract (Manaharan, et al., 2011). Another study conducted by (Oliveira, et al., 2017) bergenin isolated from the bark part of *Peltophorum dubium* for testing the ability to eliminate the ABTS+ radicals in *in-vitro* conditions and also bergenin’s ability to protect the RBCs from the oxidative damage. The study concluded that bergenin had better antioxidant and anti-haemolytic ability. Researchers performed antioxidant efficacy of flower extract obtained from Petroleum ether, dichloromethane, ethyl acetate and methanol solvents. Among the different crude flower extracts, methanol flower extract showed highest antioxidant ability (Jain, et al., 2011). Antioxidant activity for aqueous and ethanol leaf and bark parts of *P. pterocarpum,* among them the ethanol extracts of *P. pterocarpum* showed more ABTS radical scavenging activity than the aqueous extract of the plant. Particularly, the ethanol bark extract of *P. pterocarpum* revealed more anti-oxidant capacity than that of the ethanol leaf extract of the plant (Jerline Babu, et al., 2021). Several studies have been reported that flower extract gained from methanol solvent was the most effective solvent for antimicrobial study. Former research study on antimicrobial activity revealed that plant extracts were most effective against gram positive than the gram negative pathogens. On the view of previous work, in the present investigation whole flower extract obtained from methanol solvent subjected for well diffusion antibacterial activity. The current findings were compared with the older results that methanol flower extract of *P. pterocarpum* designated significant zone of inhibition (1.5 mm) against *E. coli* (a Gram-negative bacterium) at 30 μg/well, compared to a smaller zone of inhibition (1.2 mm) against *B. subtilis* (a Gram-positive bacterium). The positive control, streptomycin, produced zones of inhibition of 2.2 mm and 2.5 mm. respectively. Theresult of antibacterial assay istabulated in **Table. 3 and fig. 1 & 2.**Similar observation was made by (Vadlapudi, 2010), the methanol flower extract of *P. pterocarpum* demonstrated higher level of antimicrobial activity against gram negative bacterium *Xanthomonas compestries.* Antibacterial activity of ethanolic flower extract of *P. pterocarpum* signified effective against gram positive bacterium *Streptococcus pyogenes* studied by (Sethuraman, et al., 1984). The above studies clearly confirmed that the flower extract of *P. pterocarpum* effectual against the both gram positive and gram negative bacteria.

**Table 1: Results of 2, 2, -diphenyl-picryl hydrazyl (DPPH) antioxidant assay of methanol flower extract of *P. pterocarpum.***

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** |  **Conc. of standard (μg/mL)** | **Optical density (OD)** | **% of Inhibition** | **Sl. No.** | **Sample Conc. (μg/mL)** | **Optical density (OD)** | **% of Inhibition** | **IC50** **µg/ml** |
| **Control** | 0 | 0.32 | 0.00 | **Control** | 0 | 0.32 | 0.00 |  **103.5** |
| Standard**Quercetin****IC50 – 3.64** | 0.35 | 0.31 | 5.24 | **1.** | 6. 25 | 0.30 | 6. 25 |
| 0.61 | 0.29 | 9.30 | **2.** | 12.50 | 0.28 | 12.5 |
| 1.25 | 0.23 | 27.41 | **3.** | 25 | 0.25 | 21.87 |
| 2.50 | 0.18 | 42.97 | **4.** | 50 | 0.23 | 28.12 |
| 5.0 | 0.13 | 58.60 | **5.** | 75 | 0.21 | 34.37 |

**Graph 1: Peaks showing % inhibition of standard and methanol flower extract of DPPH radical scavenging assay**



**Table 2: Results of 2,2’ azinobis 3 ethyl benzothiazoline 6 sulfonic acid (ABTS) antioxidant assay of methanol flower extract of *P. pterocarpum***

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** |  **Conc. of standard (μg/mL)** | **Optical density (OD)** | **% of Inhibition** | **Sl. No.** | **Sample Conc. (μg/mL)** | **Optical density (OD)** | **% of Inhibition** | **IC50** **µg/ml** |
| **Control** | 0 | 0.75 | 0.00 | **Control** | 0 | 0.75 | 0.00 |  **48.60**  |
| Standard**Quercetin****IC50- 1.71**  | 0.35 | 0.64 | 14.53 | **1.** | 6. 25 | 0.71 | 5.33 |
| 0.61 | 0.54 | 27.57 | **2.** | 12.50 | 0.68 | 9.33 |
| 1.25 | 0.41 | 44.90 | **3.** | 25 | 0.65 | 13.33 |
| 2.50 | 0.25 | 66.81 | **4.** | 50 | 0.60 | 20 |
| 5.0 | 0.13 | 82.88 | **5.** | 75 | 0.54 | 28 |

**Graph 2: Peaks showing % inhibition of standard and methanol flower extract of ABTS radical scavenging assay**



**Table 3: Antibacterial activity of methanol flower extract of *P. pterocarpum***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pathogens** | **10 μg/well** | **20 μg/well** | **30 μg/well** | **Positive control Streptomycin (μg/well)** |
| ***Bacillus subtilis* (**Gram positive bacterium**)** | 0.8 mm | 1.0 mm | 1.2 mm | 2.5 mm |
| ***Escherichia coli***(Gram negative bacterium) | 0.9 mm | 1.0 mm | 1.5 mm | 2.2 mm |

  

 ***Bacillus subtilis* (**Gram positive**) *Escherichia coli***(Gram negative)

**Fig. 1 & 2: Well diffusion Antibacterial activity of the methanol flower extracts of *P. pterocarpum* against *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative)**

**CONCLUSION**

Review of literature of *P. pterocarpum* can be concluded that all the bark and flower crude extracts and bergenin isolated from this plant proven substantial antioxidant activity and in the present study, the flower extract define mild DPPH and remarkable ABTS radical scavenging potential. From the antibacterial point of view, it has been concluded that the flower extract of *P. pterocarpum* is effective against the both gram positive and gram negative bacteria. Eventually, the data attained from the current study could be the basic platform for further qualitative, quantitative and other bioactive studies in detail.

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**CONFLICTS OF INTEREST:** The authors declare that the research was done with no competing financial interest, directly or indirectly.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript

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