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Screening of Biological Potential of *Pleurostylia opposita* (Wall.) Alston. - A Rare Medicinal Plant of Eastern Ghat

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Abstract

The present study aims to evaluate the phytochemical, antioxidant, and antibacterial properties of *Pleurostylia opposite* leaf and bark crude extracts in various solvent. Preliminary phytochemical and antibacterial screening by agar well diffusion method was conducted. Further, the estimation of total phenolic and flavonoid contents along with antioxidants properties by DPPH method was carried out in various solvents extracts. The phytochemical study revealed that the maximum secondary metabolites such as alkaloids, polyphenols, sterols, terpenoids flavonoids, and saponins were present in methanol extracts followed by other extracts. Likewise, antibacterial screening showed the maximum growth of inhibition in methanol leaf and bark extracts against *Escherichia coli* (23mm), *Salmonella typhi* (23mm), *Bacillus subtilis* (24mm), *Staphylococcus aureus* (19mm), and *Pseudomonas aeroginosa* (24mm). The minimum inhibitory concentrations of the leaf and bark extracts were in the range of 19.5 mg/L – 625 mg/L while gentamycin is 9.75 mg/L. The results indicated that the methanol extracts of leaf and bark showed potent antibacterial efficacy. Further, the antioxidant activity by DPPH assay showed that all the extracts exhibited significant antioxidant properties in a dose-dependent manner. Among the tested extracts, methanol exhibited excellent radical scavenging activity which was significant compared to gallic acid. Likewise, each extract also showed excellent sources of phenolic and flavonoid contents. This study reveals that the plant contains a considerable amount of phenols and flavonoids, including antioxidant properties which are also possessing antibacterial efficacy. This study offered a new avenue for the potential use of this species as a source of natural antioxidants.

Keywords: *Pleurostylia opposita*, Phytochemical, Antibacterial, Antioxidants, DPPH

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1. Introduction

Nature has provided fruitful opportunities for mankind in many aspects since ancient times. Plants are the major source of many bioactive compounds for thousands of years (Motaleb, 2011; Prakash et al., 2014). Most of the present-day synthetic drugs have been developed from natural sources based on their traditional usage. Since ancient times, innumerable plant species have been used to treat countless diseases all over the world (Sajem et al., 2008; Sofowora et al., 2013). Green plants produce a diverse range of bioactive molecules which enrich the source of active compounds, and they continued to play a dominant role in the maintenance of

the quality of human health (Cragg and Newman, 2013; Nisa et al., 2013). Along scientific lines, biological screening tests are very much crucial to substantiate the therapeutic properties of natural resources. A maximum percentage of drugs originated from natural resources while the plant kingdom represents an extraordinary reservoir of countless novel molecules. The potential usage of higher plants, which are the major source for a new drug and needs to be explored (Girish and Satish 2008). These are the reservoirs of potentially useful chemical compounds which gives hints for the present day's modern drug design (Yadav, and Agarwala, 2011). Most importantly, these bioactive compounds from plant origin are the constituents of alkaloids, tannins, flavonoids, and phenolic compounds. Keeping the above mentioned facts, *Pleurostylia opposita* - an important medicinal plant species that belongs to the family *Celastraceae* also known as the bitter-sweet family was undertaken for the present study. There are about 90-100 genera and 1,300 species have been reported, in India, especially found in the Western Ghats region of Kerala, South Sahyadri, Malabar, and the Eastern Ghats region of Karnataka (Gamble, 1997). The plant contains a large number of chemically complex and biologically active compounds (Neam suvan et al., 2012). Traditionally, the oral administration of the root and bark decoction of this plant is used for the treatment of malaria, toothache and the wound healing process (Uma Maheswari et al., 2012). Apart from the identification of compounds, no reports are available on the comparative biological studies of this plant. Therefore the study was conducted to evaluate the phytochemical analysis, antibacterial activity, and antioxidant properties in bark and leaf various solvent extracts of *P. opposita*.

Materials and Methods

2.1. Plant Collection and Extracts Preparation: The bark and leaf material of *P. opposita* were collected in Chamundi hill, Mysore, Karnataka, India. Plant material was thoroughly washed in sterile distilled water and shade dried. The dried leaves and bark materials were powdered and subjected to crude extraction using hexane, petroleum ether, chloroform, ethyl acetate, and methanol. About 50g of each material was taken in a 250 ml conical flask containing respective solvents and placed on a rotary shaker at 120rpm for 24h and extracts were stored in an airtight glass bottle until further use (Sadika et al., 2012).

2.2. Phytochemical Analysis and Thin Layer Chromatography: The preliminary phytochemical screening was carried out for the detection of secondary metabolites in each crude solvents extracts of leaf and bark (Harborne, 1973). Further, the maximum positive test showed methanol leaf and bark extracts were subjected to thin-layer chromatography (TLC) analysis for the detection of specific compound (Ramaswamy et al., 2013). Each sample was spotted on previously activated TLC plates and placed in a saturated chromatography chamber using a suitable solvent system. Each TLC plate was removed after a successful one-fourth running of solvents. The visualization of the secondary metabolites was done by spraying different reagents such as iodine vapours, Dragendroff's, Benedict's, Liebermann-Burchard's, and Folin- Ciocalteu's (FC) reagents. The plates were activated at 100º C for 10 min. and observed under a UV light (366 - 254 nm) for colour development and the mean Rf values were recorded.

2.3. Antibacterial Activity by Well Diffusion Assay: The antibacterial screening was conducted by agar well diffusion methods (Joshi et al., 2011). Each crude extract was tested against bacterial pathogens such as B. *subtilis* (MTCC121), *Staph. aureus* (MTCC7443), *E. coli* (MTCC7410), *P. aeruginosa* (MTCC1688), and *S. typhi* (MTCC733) procured from Microbial Type of Culture Collection (MTCC), Chandigarh. A 24h. old fresh inoculum (106

CFU/ml) was uniformly spread in Petri dishes containing nutrient medium. Wells were made by using a sterile cork borer (6mm) and each well was filled with 50 µl (50 mg/L) and gentamycin (1mg/L) and respective solvents served as a positive and negative control. Plates were incubated at 37°C overnight and the zone of inhibition was measured. Minimal inhibitory concentration (MIC) was also determined by broth microdilution method using an ELISA multi-plate reader. Each extract was tested at concentrations ranging between 5- 0.002mg mL-1 from the stock solution of 100 mg mL-1 along with controls and the assay was performed in triplicates.

2.4. Total Phenolic Content: Each solvent extract of leaf and bark was subjected to measure the total phenolic content by the method of Folin- Ciocalteu's method (Muthukrishnan et al., 2018). Each sample was tested at 100 µg mL-1 and the absorbance was measured at 765nm using a spectrophotometer. The obtained results were compared with that of the standard drug gallic acid, and expressed as milligrams of gallic acid equivalence (GAE, µg mL-1).

2.5. Determination of Total Flavonoid: The amount of flavonoid contents in leaf and bark extracts were determined by the method of Muthukrishnan et al., (2018). About 0.5 mL of 2% $AlCl_3$ solution was mixed with 0.5 mL (100 µg mL-1) of each extract. The reaction mixture was kept in at room temperature for 1h. and the absorbance was measured at 420nm using spectrophotometer. The yellow colour in the reaction mixture indicates the presence of flavonoids and the quercetin calibration curve (50 - 250 µg mL-1) served as a reference standard. The obtained results are expressed as milligrams of Quercetin equivalence per gram of dry weight.

2.6. Antioxidant Activity: The DPPH (2, 2-diphenyl-1- picrylhydrazyl) free radical scavenging activity was performed using a 96 well plate (Yamasaki et al., 1994). Samples were tested at 20, 40, 60, 80, and 100µg mL-1 concentrations and the gallic acid was used as a standard. The reaction mixture was incubated at room temperature in dark conditions for 30 min. The OD was measured at 517nm using ELISA multi-plate reader. The assay was performed in triplicates and the percentages of free radical scavenging activity and the IC50was calculated by using a formula.

$$\% \text{ scavenging activity} = ((\text{Abs control} - \text{Abs sample}))/(\text{Abs Control}) \times 100$$

2.7. Statistical Analysis: Each assay was performed in triplicates and the data were analyzed using one-way analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were tested by Tukey's HSD test separated and determined by F values ($p \le 0.05$).

3. Results and Discussion

3.1. Phytochemical Screening: The preliminary qualitative phytochemical test revealed various classes of secondary metabolites in both leaf and bark extracts of *P. opposita.* The phytochemicals such as sterols, triterpenoids, carbohydrates, glycosides, tannins, flavonoids, resins, and carbohydrates are the major constituents present in the extracts. These phytochemicals have played the most important role in providing various nutraceutical products (Das et al., 2012). The results obtained in this study were summarized (Supp. table 1) and it showed that all the extracts possess several phytochemicals. The comparative phytochemical screening revealed that the maximum tests were positive in methanol bark extract than leaf methanol extract, while the remaining extracts showed presence of less number of phytochemicals. Similar results were reported by Misra et al., (2011) wherein the maximum phytochemicals were present in methanol extracts of Alstonia scholaris.

Further, the maximum phytochemicals showed methanol leaf and bark extracts subjected to TLC analysis. The results revealed the presence of secondary metabolites such as reducing sugars, alkaloids, saponins, and phenols after spraying Dragendroff's, Benedict's, Liebermann Burchard, and FC reagents. The results were presented in figure 1 and the corresponding Rf value of confirmed phytochemicals were 0.4, 0.38, 0.59, 0.98, and 0.84, 0.65, 0.57, 0.56 for reducing sugars, alkaloids, saponins, and phenols appeared orange, light brown, and bluish respectively. Likewise, Ramaswamy et al., (2014) also investigated various classes of phytochemicals in leaves and petioles of Oroxylum indicum through TLC analysis. Cassia et al., 2011 have also reported the presence of phytochemicals in many other species of the Celastraceae family due to their massive pharmacological activities. The study demonstrated that biological interest which is associated with the presence of flavonoids, alkaloids, triterpenes, phenols, and glycosides (Dantanarayana et al., 1982).

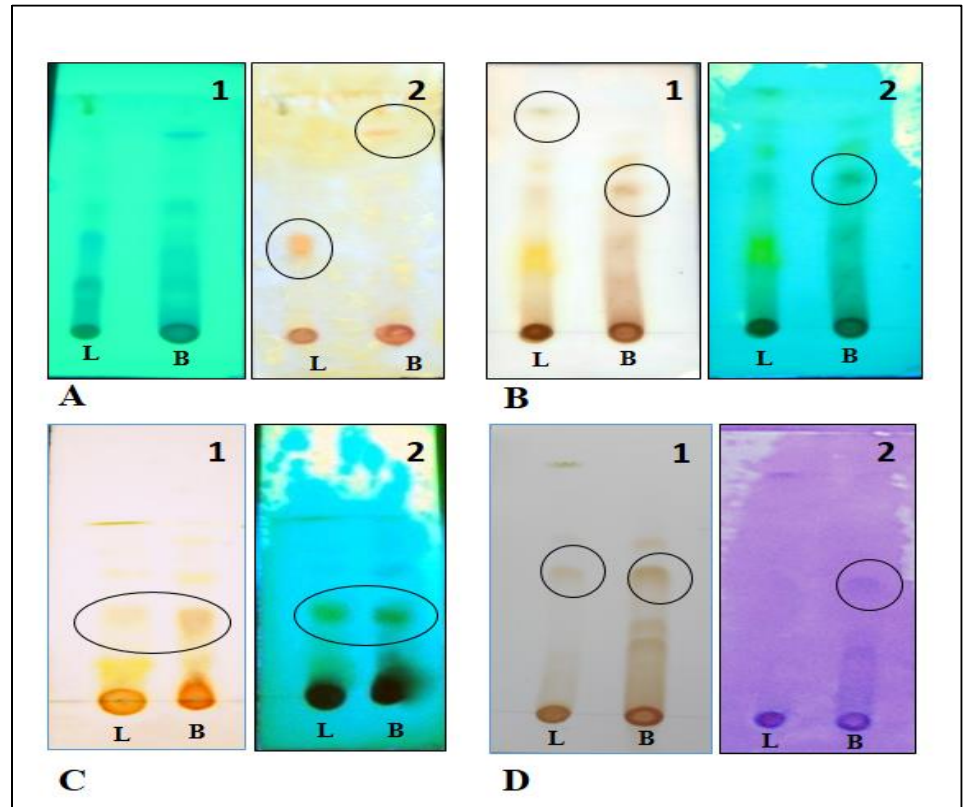


Figure: 1. A-Dragendroff's test for alkaloids, **B-** Benedict's test for reducing sugars, **C-** Liebermann Burchard test for sterols and saponins, **D-** FC reagent test for phenols. (**L-** Leaf extract, **B-** Bark extract, **1-** Before spray, **2-** After spray).

3.2. Antibacterial Activity: The evaluation of the antibacterial activity of the crude extracts of leaf and bark against selected bacteria through agar well diffusion assay. The outcome of this study and the zone of inhibition were presented in supporting table 2 along with minimum inhibitory concentrations (MIC). Among the extracts tested, the methanol extract of both leaves and bark was highly sensitive to tested pathogens. Followed by ethyl acetate, and chloroform extracts also showed growth inhibition against tested pathogens excluding hexane and petroleum ether extracts. Overall, in the comparative study of antibacterial activity, the bark extracts showed more significant antibacterial activity than leaf extracts due to the presence higher number

of phytochemicals in methanol extracts. Similar results were also reported by Moteriya et al., (2014) where they have noticed the remarkable difference in antibacterial properties in Maytenuse marginata leaf and stem extracts. The highest zone of inhibition observed in P. aeruginosa and B. subtilis is 24mm with a MIC concentration of 19.5 µg mL-1, while the standard antibiotic shows a zone of inhibition of 22-28 mm with MIC concentrations of 9.75 µg mL-1 against tested pathogens. The results presented here is in accordance with the earlier report of Selvamohan et al., (2012); Borges et al., (2017) wherein leaf and bark extracts of Brosimum gaudichaudii against Staph. aureus and P. aeroginosa.

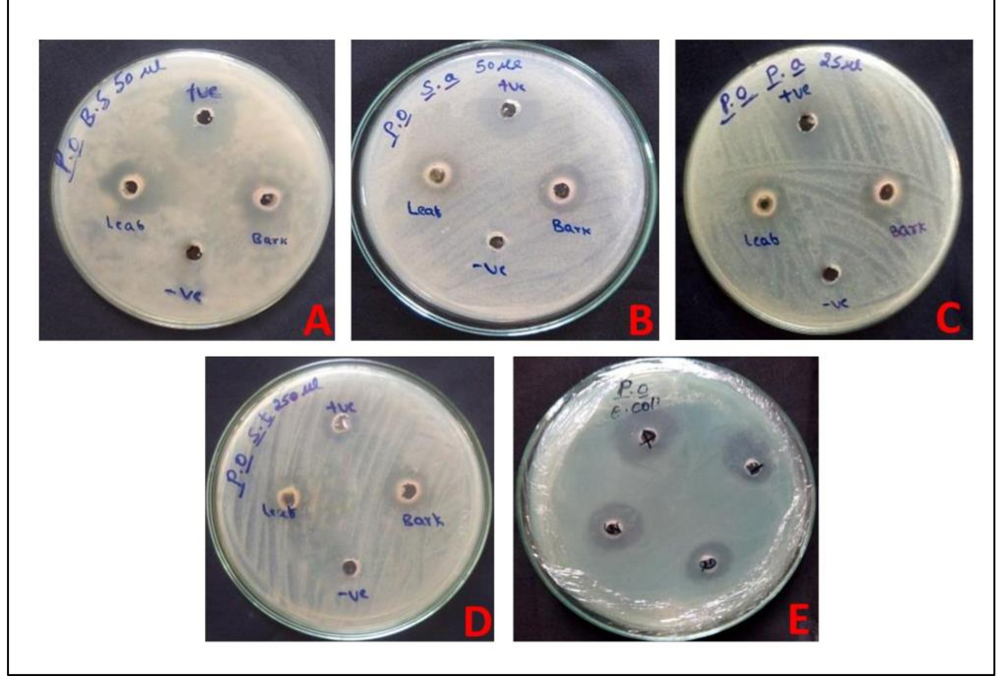


Figure: 2. Antibacterial activity of methanol leaf and bark extracts of *Pleurostylia opposite* against; A – *B. subtilis*, **B** – *Staph. aureus*, **C** – *P. aeroginosa*, **D** – *S. typhi*, **E** – *E. coli.*

3.3. Antioxidant Activity by DPPH Assay: The antioxidant property of each extract was evaluated by DPPH assay. The free radical scavenging activities measured by DPPH assay, it is a widely used method to analyze the antioxidant properties in most of the plant extracts. The test samples are directly involved in the inhibition of the generation of reactive oxygen species (ROS). The results obtained from this experiment revealed that all the tested extracts showed significant antioxidant properties which are in a dose-dependent manner. The outcome of this study was depicted in the figure 3 and 4. The methanol extract of leaf and bark showed excellent antioxidant activity compare to other solvent extracts. The IC50was calculated by plotting the graph of radical scavenging activity against the different concentrations of extracts by using standard gallic acid. It is also observed that bark methanol extract showed the maximum free radical scavenging activity which is nearly equal to the standard gallic acid with the half maximal inhibitory concentration (IC50) value of 16.97µg mL-1 while the IC50 value of gallic acid was 15.95µg mL-1. Likewise, leaf methanol extract also showed significant antioxidant properties with an IC50 value of 18.83 µg mL-1. Therefore the results revealed that the plant possesses excellent antioxidant properties and each extract effectively scavenged free radicals which are varied among the different solvent extracts. Similar results were reported by Jhade et al., (2012); Nisa et al., (2013) where the extracts possess

plant metabolites that effectively inhibit the generation of free radicles. The results obtained are due to the higher number of secondary metabolites in the extracts which are responsible for antioxidant activity. These findings are also supported by earlier reports by Gupta et al., (2011); Yuan et al., (2011); Mayakrishnan et al., (2012) wherein the plant metabolites such as flavonoids, tannins, catechins, and other phenolic compounds are the major constituents which are responsible for good sources of antioxidant activity.

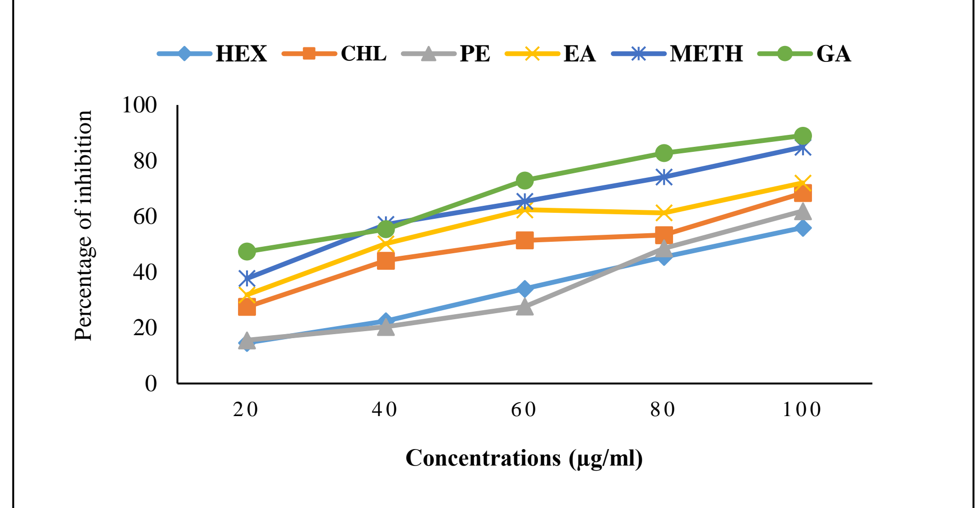


Figure: 3. DPPH antioxidant activity of different solvent extracts of leaf in *P. opposita.*

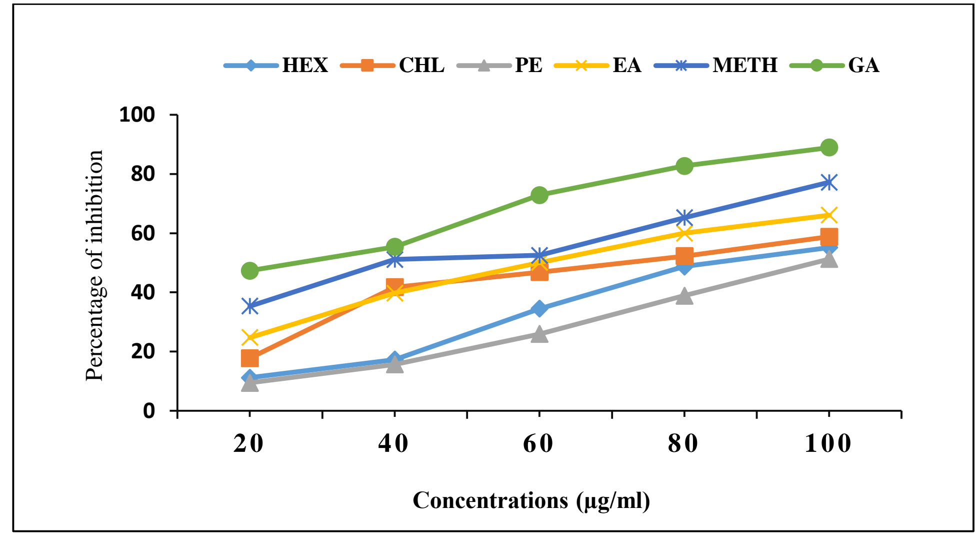
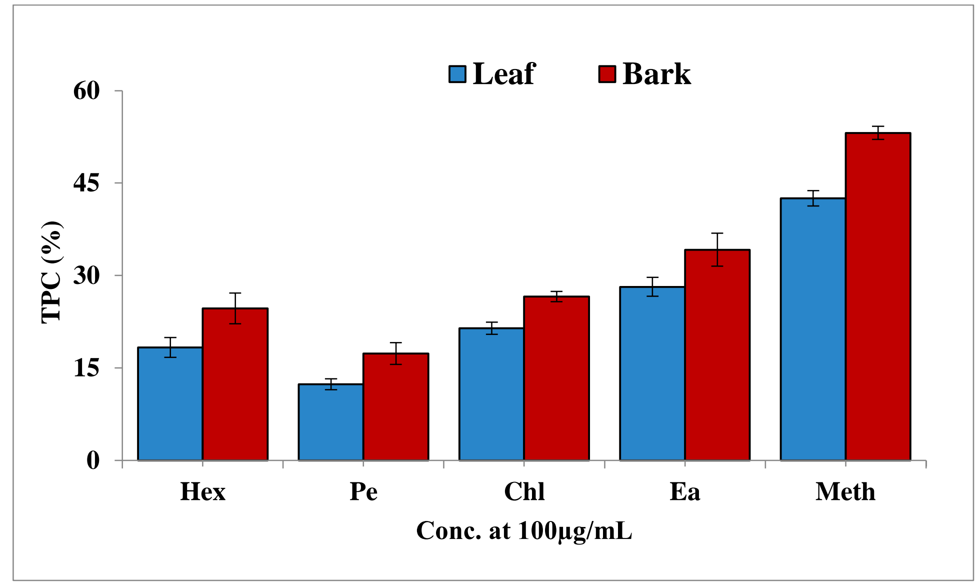


Figure: 4. DPPH antioxidant activity of different solvent extracts of bark in *P. opposita.*

3.4. Total phenolic Content: The summary of the total phenolic content (TPC) of various solvent extracts of leaf and bark was estimated by the Folin-Ciocalteu (FC) method by using standard drug gallic acid. Phenolic compounds are very important secondary metabolites with potent antioxidant activity, which chelate redox-active ions and prevent the conversion of reactive oxygen species (Khorasani Esmaeili et al., 2015). The TPC of P. opposita leaf and bark different solvent extracts is presented in figure 5. Overall the TPC value was higher in methanol extracts of bark (53.14%) and leaf

(42.53%) followed by other solvent extracts. The lowest TPC content was observed in petroleum ether extract and it was also noticed that leaf extracts were found to possess less percentage of phenolic contents than bark extracts. The obtained results were correlated with standard gallic acid by plotting the linear regression (y = 0.0153x; R2= 0.9953). The differences in the phenolic contents between leaf and bark solvent extract is may be due to the presence of complex biopolymers such as carbohydrates and proteins in the leaf extract. The result presented here was in accordance with the recent reports of Phuyal et al., (2020); Muhammad et al., (2012) in Zanthoxylum armatum, where the ethanolic extracts of bark and fruit possess a higher amount of TPC than leaf extracts.



Figure; 5. The total phenolic content of leaf and bark different solvent extracts of *P. opposita.*

3.6. Total Flavonoid Contents: Presence of total flavonoid contents in the leaf and bark extracts of P. opposite was determined spectrophotometrically. The flavonoid contents of each extract was calculated by plotting the linear regression calibration curve of the standard drug quercetin (y = 0.0146x; R^2 = 0.9918) and expressed as mg quercetin equivalent per gram of dry weight (mg/g). The assay revealed that the flavonoid content was much higher in bark methanol extracts (64.58%) than in leaf (51.56%) followed by other extracts and the result is presented in figure 6. Contrary to phenol contents overall, the flavonoid content was comparatively lower in leaf extracts than in bark extracts and the result was compared with that of the standard drug quercetin. The obtained results revealed major variations in the percentage of flavonoid contents may be because of the presence of vast number secondary metabolites present in the plant extracts. The result is in agreement with the previous report of Saeed et al., (2012) wherein the Torilis leptophylla plant extracts, and Sulaiman, and Balachandran, (2012) have also revealed the amount of total flavonoid content present in some medicinal plants inhabited in India. Further, Iqbal et al., (2015) also observed rich flavonoid contents in bark and leaf extracts of Goniothalamus velutinus and justified that the plants manifest themselves as good sources of antioxidants

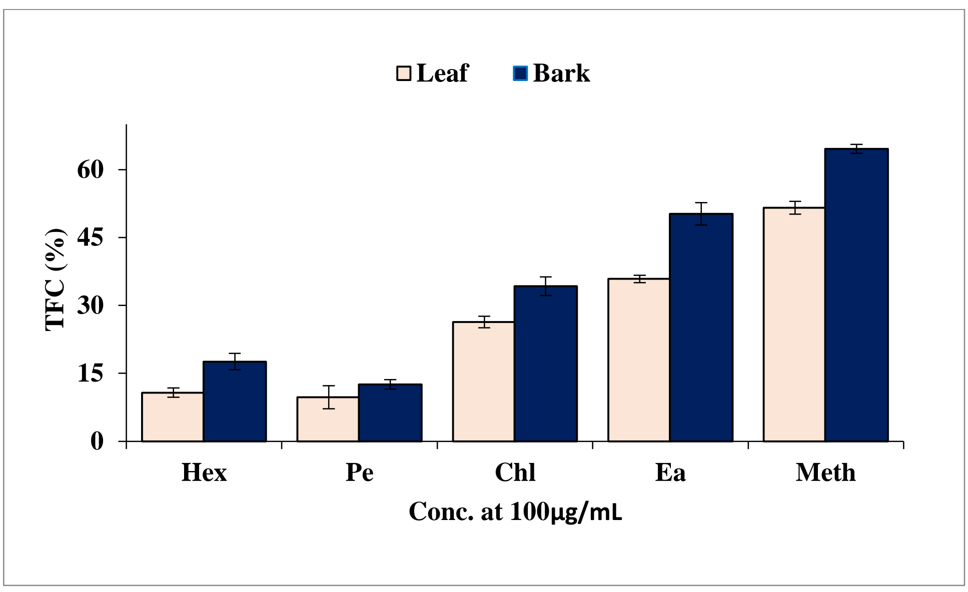


Figure 5. The total flavonoid content of leaf and bark different solvent extracts of *P.opposita.*

3. Conclusion

The present study summarises that this plant possesses a good source of phytochemicals. The difference in the presence of number of phytochemicals between the solvent extracts is remarkably varied. However, these parameter is better in bark extracts than compared to leaf extracts. The plant extracts proved to be potentially inhibit the tested bacterial strains and comparatively methanol extract of both leaf and bark was found to possess the most effective antibacterial properties. The differential total phenolic, flavonoid content, and antioxidants properties from different solvent extracts of bark and leaf may due to the variations in the phytochemical constituents. Our investigation will provide the basis for the selection of potent medicinal plant species for the investigation of desired new bioactive molecules from herbal resources. With this result, we concluded that further studies are needed for insight into the investigation, and isolation of bio-active compounds from this plant for future health applications.

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Conflict of Interest

The authors declare that there are no conflict of interest and agree to publish the data.

Author contribution

Mahendra C., Savitha R. S. and Ravindra K. N. collected the plant, prepared the samples, performed all the experiments, and analyzed the data. All the researchers wrote the manuscript, reviewed it together. Theyt read and approved the final manuscript.

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