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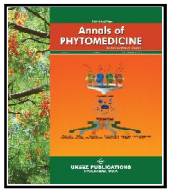

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Exploration of antioxidant activity of *Plumeria obtusa* L.

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Abstract

Due to a lack of scientific evidence, the study is aimed to explore the antioxidant potential of *Plumeria obtusa* L. (*P. obtusa*) using several *in vitro* chemical-based assays such as DPPH, ABTS, FRAP, *etc*. Each activity was performed on constant concentration to achieve the roust and most precise findings. The results of the study showed that *P. obtusa via* exhibiting a significant scavenging effect against the varieties of free radicals. It was found that the IC_{50} of *P. obtusa* for DPPH, ABTS and iron chelating effect found as 178.6 ± 3.274, 146.0 ± 3.045 and 237.3 ± 4.328 µg/ml, respectively. Hence, it can be concluded that *P. obtusa* exhibits a significant role as an antioxidant agent which can mitigate the diversity of free radicals and thus reduce oxidative damages to the normal function of vital organs. It can be used as adjuvant therapy for the reduction of oxidative stress induced by any exogenous or endogenous agents.

1. Introduction

Herbal medicines or their parts such as roots, leaves, barks, seeds, berries, or flowers can be used to maintain health and treat various acute and chronic diseases and because of their essential function, these have been acknowledged as herbal medicines, sometimes known as botanical medicines or phytomedicines (Kiran *et al*., 2021). Since history, medicinal plants have been playing a major role for treating disease and gaining exponential growth in developed and developing countries (Farahmandfar *et al*., 2019).

As per the record of World Health Organization (WHO) reports that in India, traditional or Ayurvedic medicines are used as an alternative and supplementary form of treatment for 65% of the people living in rural regions. There have always been issues with the uneven chemical makeup of herbal remedies and the sporadic ingestion of hazardous or adulterated substances (Vecchiato, 2019; Zyoud *et al*., 2016).

Furthermore, it has been reported that natural-derived active medicines are essential in treating a variety of pathophysiological onsets or even physical illnesses (Mehrotra, 2020). The majority of medications used in modern therapies, perhaps even more than 50%, are made from natural materials (Rana *et al*., 2021). The finest medications to treat various diseases are also generated from several naturally occurring ingredients (Gaurav *et al*., 2022).

For quality control, safety and their regulatory aspects, many variables, including as seasonal variations, harvesting dates, growing locations, post-harvest processing, adulterants or replacements of raw ingredients, and extraction and preparation techniques, can have an impact on the overall quality of herbal medicine (Amrutanand *et al*., 2021). Phytoconstituents play an essential role in assessment of quality control of herbal medicines from harvesting to the production of any herbal formulation (Gaurav *et al*., 2020). In addition, the study of phytoconstituents has applications in a wide range of research fields, such as authenticity, method optimization for extraction and purification, structure elucidation, and purity assessment, new drug discovery, *etc*. Systematic research employing phytoconstituents analysis may result in novel drug development (Bräm and Wolfram, 2017). The determination of the active principle acknowledged as the crucial step from separation science to biological science is the optimization of selected bioactive phytoconstituents from an herbal medication (Juszczak *et al*., 2019).

However, thousands of medicinal plants used to treat a variety of disorders are represented in ancient scripture (Pan *et al*., 2014; Sharma and Ahmad 1992). As a result, quality control analysis is a significant obstacle to the reasonable use of therapeutic herbs. Many therapeutic plants have an unknown active ingredient, and factors like genetics and the environment may have an impact on the quantity of plant secondary chemicals. Often, a marker ingredient is chosen and utilized to assess the potency of the herbal remedy as well as for development of the formulation (Ansari *et al*., 2020).

The increasing incidence and significant prevalence in development of herbal medicine as complementary and alternative therapy (CAT) for people are at their peak for the treatment ailments including liver and its associated complication (Gahlot *et al*., 2021). Furthermore, a new therapeutic option is generated through herbal medicines for patients associated with liver disease and provide an effective regimen for mitigating liver dysfunction and associated

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ailments. In such condition, liver transplant relics only option for the patient associated with progressive or chronic liver disease. Herbal medicines attributed to normalizing the liver function *via* ameliorating biomarkers of the liver and preventing progressive distortion of hepatocytes with an overall improvement of the architect of the liver (Aher and Wahi, 2011; Rather *et al*., 2016).

P. obtusa (Apocynaceae) is one of the most known Indian traditional medicinal plants. It is mostly acknowledged as the most deciduous as well as an ornamental plant. It is a tropical plant and grown in premises, gardens, parks and graveyards as it looks most attractive and fragrant flowering. It has been used traditionally for treating various acute and chronic ailments such as wounds and skin disease, diabetes mellitus, inflammation, oxidative damages, *etc*. It is well defined for its diuretic and purgative effect as well as used in abortion. Mostly, it is used for cosmetics purposes such as aromatherapy, necklaces and offerings (Bihani *et al*., 2021).

P. obtusa possess numerous chemical constituents of different category such as iridoids, cardiac glycosides, terpenoids, flavonoids, steroids, phenolic acids, fatty acid esters, cardenolide, coumarin, cinnamic acid derivate and volatile oils. Based on the complex matrix of phytoconstituents, the plant possesses a huge role in different pathophysiological onsets of the body and thus exhibits a protective effect for the well-being of humans (Ali *et al*., 2008; Bihani and Mhaske, 2020; Gupta *et al*., 2021; Siddiqui *et al*., 1989; Singh and Verma, 2019). Although, it cannot be denied that the antioxidant activity of such medicinal plants provides an essential role to evade the deleterious effect of oxidative stress-induced free radicals on the normal function of the vital organs. Since history, the medicinal plant has been used to alleviate many diseases or disorders by exhibiting its multi-mechanistic action. Due to the lack of their quality, efficacy and safety assessment, medicinal plants are still reported to be least prompted by people for the treatment of any acute and chronic ailments. Taking all these facts into consideration, the study is associated to explore the antioxidant effect of *P. obtusa* using various *in vitro* approaches and thus validating the scientific acts on the antioxidant activity of *P. obtusa*.

2. Material and Methods

2.1 Chemicals and reagent

The Folin-Ciocalteu reagent was acquired from Mumbai, India's Loba Chemie Pvt. Ltd. Purchases were made from Sisco Research Laboratories Pvt. Ltd. for gallic acid and quercetin (SRL). Ascorbic acid, 2,22 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, as well as 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were bought from Sigma Aldrich Co. in St. Louis, Missouri, in the United States. We bought ethanol (95%) from Ranbaxy Fine Chemicals Ltd. Aluminum chloride, sodium acetate, and sodium carbonate. Every solvent that was bought was of analytical grade.

2.2 Collection, authentication and preparation of plant material extract

Fresh leaves of *P. obtusa* were collected from the Garden located in Karkarduma area and authenticated by Dr. Sunita Garg, Head Botanist at RHMDCSIR-NIScPR,. The specimen of the plant material was submitted to the institutional herbarium laboratory for future record purposes. Furthermore, the plant material was extracted in ethanol by using 1:8, w/v ratio of plant drug and solvent. The extraction procedure was performed using the Soxhlet extraction method till complete extraction would not be achieved. After extraction, the obtained content was filtered using Whatman's filter paper and the filtrate was concentrated over the reduced pressure. The percentage yield of the extract was calculated as stored in an airtight container at room 4°C temperature for further use.

2.3 Total phenolic content

The Folin Ciocalteu (FC) method was followed to determine the total phenol concentration in *P. obtusa* (Gaurav *et al*., 2020). The medication was produced as a stock solution in ethanol at a concentration of 5 mg/ml. 2.5 ml of sodium bicarbonate solution (7.5%), 500 l of the stock solution, and 2.5 ml of FC (1:10, v/v) were then combined. The resultant mixture was held for 30 min while being occasionally shaken. At 765 nm, the solution was spectrophotometrically measured. Gallic acid was used as the reference medication to calculate the total amount of phenols. The results were given in units of gallic acid equivalent (GAE) per milligramme of extract (g/mg).).

2.4 Total flavonoid content

P. obtusa flavonoid content was determined using aluminum chloride assay with some changes in the reference protocol (Gaurav *et al*, 2020). Briefly, *P. obtusa* stock solution (5 mg/ml) was prepared in ethanol. 500 ml of stock solution, 1.5 ml methanol followed by the addition of 0.1 ml aluminum chloride (10%), 0.1 ml sodium acetate (1 M), as well as 2.8 ml water were mixed together. The mixture was incubated for 40 min and measured spectrophotometrically at 415 nm. Quercetin was taken as the standard drug to determine the total content of flavonoids. The outcomes were expressed as µg quercetin equivalent/mg of extract (µg quercetin/mg extract).

2.5 DPPH radical scavenging activity

P. obtusa antioxidant activity was performed against DPPH free radical probe using reference protocol with some modifications (Gaurav *et al*., 2020; Khan *et al*., 2022a). In this method, a 1 mg/ml stock solution of the plant extract was prepared in ethanol and vortexed for proper dissolution of the plant extract. The obtained solution was centrifuged at 10000 rpm for 10 min to separate the undissolved litters present in the solution. After centrifugation, the supernatant was collected in another fresh vial to perform the antioxidant activity. Different concentrations of the sample solution such as 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were prepared for antioxidant activity. Thereafter, a methanolic solution of DPPH (0.01 M) was prepared for the analysis.

In brief, 20 µl of sample and 180 µl of DPPH solution was mixed to evaluate the effect of the drug against DPPH free radicals. After 30 min, the mixture was evaluated spectrophotometrically at 517 nm. Triplicate reading of the sample was recorded.

2.6 ABTS radical scavenging activity

P. obtusa ABTS scavenging effectwas examinedby following referenced method with some changes (Polu *et al*., 2017). ABTS

reagent (7 mM) was prepared using 2.45 mM potassium persulfate solution. The obtained solution was kept to stand at room temperature for 15 h and in the dark. The solution was diluted with methanol to achieve the absorbance of 0.7 ± 0.2 units at 750 nm after being kept for the night and then the developed solution was termed as ABTS solution. In brief, 20 ml of the test solutions from the different concentrations such as 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were added to 180 ml of the ABTS free radical solution after the standard/extract solutions had been made in methanol at varied concentrations. At 750 nm, the absorbance was measured after a 20 min incubation. The positive control was ascorbic acid. The measurements were taken in triplicate.

2.7 Iron chelating activity

P. obtusa iron chelating effect was examined as per referenced method with some changes (Polu *et al*., 2017). In brief, 0.16 g of ferric ammonium sulphate, 2 ml of 1 M hydrochloric acid, and 0.198 g of 1, l0-phenanthroline monohydrate were mixed in 100 ml of water to develop the 1, l0-phenanthroline-iron (III) or FRAP reagent. In brief, 0.2 ml of each concentration such as 31.25, 62.5, 125, 250, 500 and 1000 µg/ml, respectively, 0.6 ml of methanol, as well as 4 ml of water were combined with 0.2 ml of 1, l0-phenanthroline-iron (III) reagent, and after 30 min at 50°C. Each sample was spectrophotometrically measured at 510 nm. Ascorbic acid was used as positive control. The effect of drug was measured in proportion of the drug response. The measurements were made three times.

2.8 Statistical analysis

The Tukey test was used to compare all of the pairwise comparisons between the columns after the data were reported as mean standard deviation (SD) (n=3). The p-value of 0.05 was used to determine the significance of the difference.

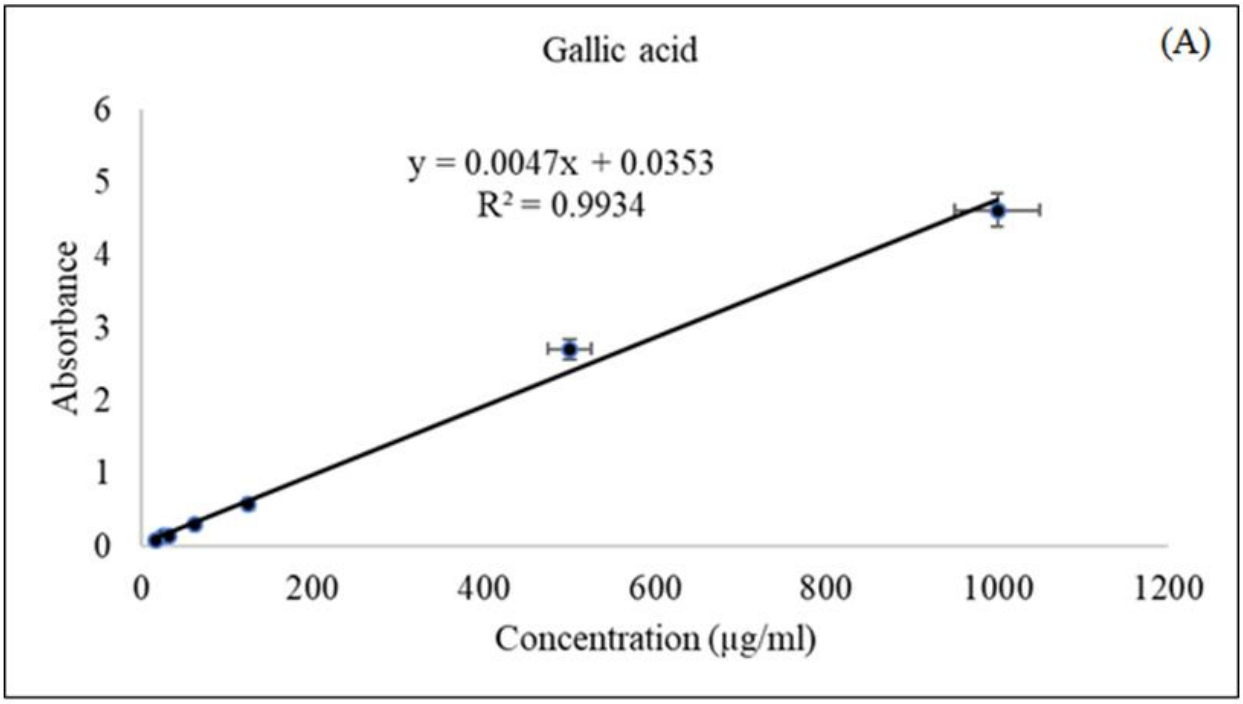
3. Results

The extraction process for the development of ethanolic extract of *P. obtusa* was performed successfully. the obtained extractive yield of the plant extract was found to be 7.374 ± 0.654%. The results were strongly supported as per the limit mentioned in the Ayurvedic pharmacopeia of India. After the extraction process, the quantitative as well as qualitative screening of phytochemical in plant extract and the scavenging effect was performed to validate the antioxidant activity of *P. obtusa*.

3.1 Total phenolic and flavonoid content

P. obtusa total phenolic content was determined as per the referenced protocol, successfully. The study was performed on different concentrations of the sample to determine the concentration-dependent free radical scavenging effect of *P. obtusa*. The outcome of the study showed *P. obtusa* phenolic content was found to be 11.412 ± 0.371 %, w/w, which was supported by the previous studies conducted on the same species (Dawood and Hassan, 2015).

Furthermore, total flavonoid content in *P. obtusa* extract was determined as per the referenced protocol, successfully. The study was performed on different concentrations of the sample to determine the concentration-dependent scavenging effect of *P. obtusa*. The outcome of the study showed that the flavonoid content in ethanolic extract of *P. obtusa* was found to be 7.565 ± 0.242 %, w/w,which was supported by the previous studies conducted on the same species (Dawood and Hassan, 2015). The calibration curve for gallic acid and quercetin has been represented in Figure 1.



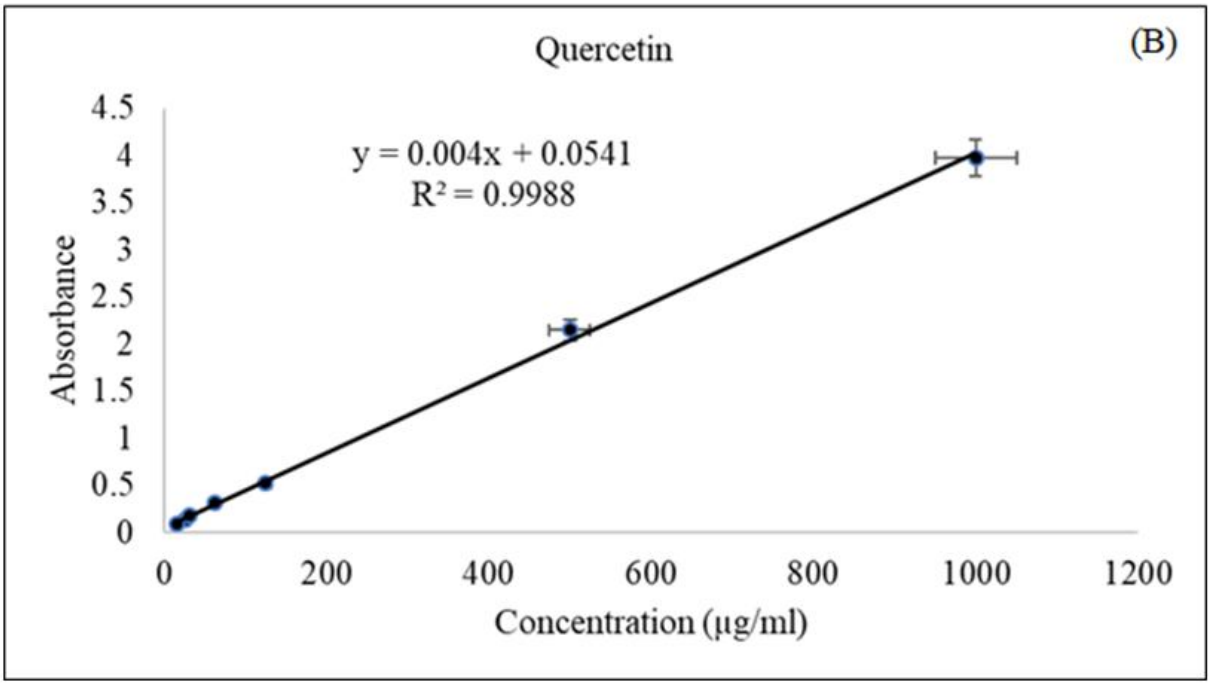


Figure 1: Calibration curve and equation of the gallic acid and quercetin.

3.2 DPPH radical scavenging activity

DPPH is one of the most and precise methods for determination of scavenging effect of medicinal plants or naturally derived medicine or their formulation. It is a widely acceptable and economic method for determination of antioxidant activity. Antioxidant effect of *P. obtusa* was determined by the DPPH scavenging method, successfully. the outcome of the study showed that the effect of *P. obtusa* was found in the dose-dependent method as the concentration of the sample increased. The effect of *P. obtusa* and standard drug (ascorbic acid), was expressed in IC_{50} value was found to be 178.6 ± 3.274 and 87.25 ± 2.004 µg/ml, respectively. The outcome of the study has been depicted in Figure 2. The results revealed no significant effect in scavenging activity of the drug then standard drug. In this analysis, the antioxidant effect of the drug was determined in proportion to the color changes from dark purple to yellow color. A more yellow color represents the significant antioxidant potential of the drug (Ali *et al.*, 2013).

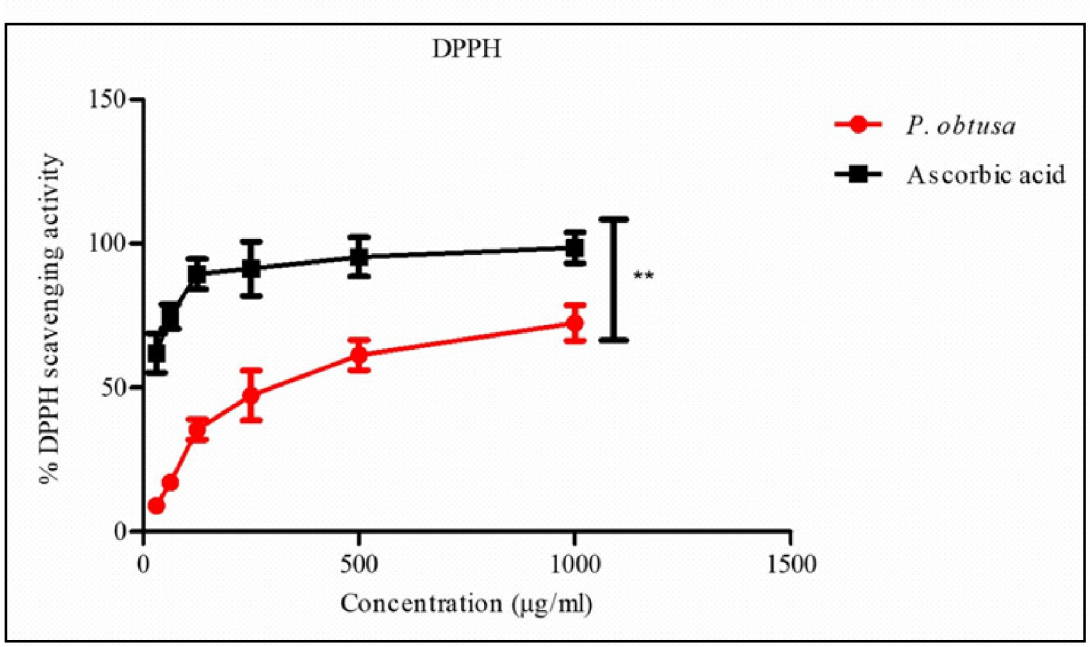


Figure 2: DPPH activity of *P. obtusa* and ascorbic acid.

3.3 ABTS radical scavenging activity

ABTS scavenging activity is also a most precise assay for determination of scavenging effect of medicinal plants and their derived products. This assay is generally targeting the nitrogen species of the free radicals which are quenched by the antioxidant drug. In the present study, ABTS analysis of *P. obtusa* was performed against the ABTS free radicals, successfully. The results of the study showed a concentration-dependent effect of *P. obtusa* against ABTS radials. The effect of *P. obtusa* and ascorbic acid at its higher concentration (1000 µg/ml) was found to exhibit the inhibition of 57.328 ± 5.235 and 91.2340 ± 7.237, respectively. The IC_{50} value of *P. obtusa* and ascorbic acid was found as 146.0 ± 3.045 and 112.6 ± 4.029 µg/ml, respectively. The results of the study are represented in Figure 3.

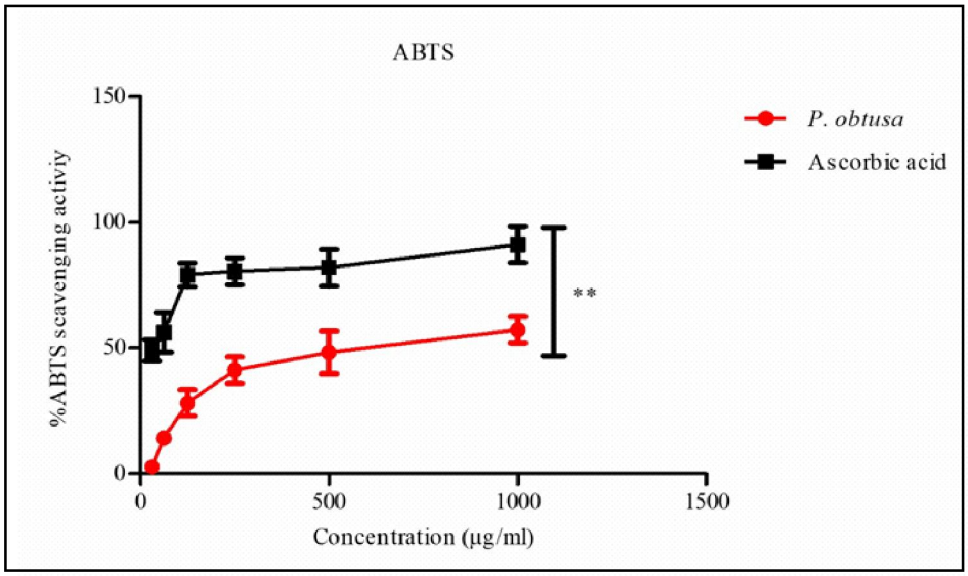


Figure 3: ABTS scavenging activity of *P. obtusa* and ascorbic acid.

3.4 Iron chelating activity

Iron ions are known to accelerate the conversion of less reactive species like H_2O_2 or lipid peroxides to more reactive ones like hydroxyl, peroxyl, and alkoxyl radicals. Because the release of iron from cellular injury can enhance oxidative damage, substances with iron chelating properties can function as strong antioxidants. In this study, the iron chelating effect of *P. obtusa* was determined against ferrous ions (Halliwell, 2009). The study was performed on different concentrations to determine the concentration-dependent effect. The results showed that *P. obtusa* significantly scavenge the ferrous ions and the outcome found significant changes in the effect of ascorbic acid and showed a concentration-dependent effect of *P. obtusa* against Fe+ radials. The IC_{50} value of *P. obtusa* and ascorbic acid was found as 237.3 ± 4.328 and 130.1 ± 3.998 µg/ml, respectively. The outcomes of the study have been represented in Figure 4.

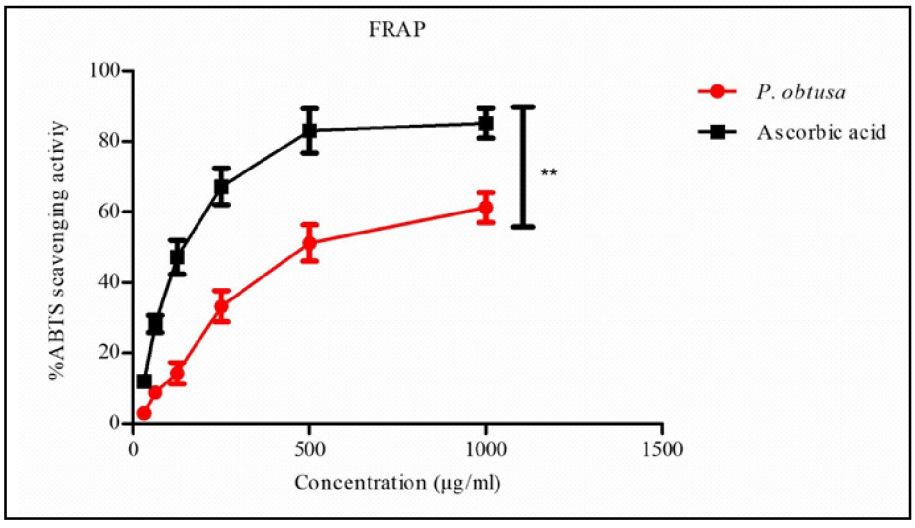


Figure 4: Iron chelating scavenging activity of *P. obtusa* and ascorbic acid.

4. Discussion

Phenols and flavonoids are the most abundant group of phytoconstituents which can be seen in numerous medicinal plants. The chelation of the compound form of many phenols compounds has been termed as polyphenolic compounds. Phenols have an extensive contribution for treating various acute and chronic ailments such as inflammation, jaundice, obesity, immune-based pathophysiological onsets and used as antibacterial, antifungal, antidiabetic, anticancer, nephroprotective, cardioprotective, neuroprotective, hepatoprotective, *etc*. (Du *et al*, 2016; Ly *et al*, 2015; Man *et al*, 2020; Marmouzi and Ezzat, 2018; Miranda *et al*, 2018; Shen and Ji, 2016; Wang *et al*, 2021; Xiao and Hogger, 2014). Furthermore, it has been reported that polyphenols have a potential therapeutic effect in the alleviation of kidney disease and associated complications (Basist *et al*, 2022; Gautam *et al*, 2021; Khan *et al*, 2022a). Antioxidant effect of polyphenols such as quercetin, gallic acid, kaempferol, ellagic acid, apigenin, luteolin, rutin, hesperidin, etcor the dietary supplements enriched in polyphenols is the most effective therapeutic and nutraceutical medicinal regimen for alleviation of the pathophysiological dysfunction associated with the oxidative stress induced by free radicals (Gautam *et al*., 2021). It has been reported that the generation of the free radicals is the normal cellular metabolic process, if it is in excess it generates varieties of free radicals which harms the normal function of the vital organs and leads the severe disease such as oxidative stress-induced myocardial infarction, diabetes, nephrotoxicity, neurotoxicity senescence, *etc*. (De Oliveira *et al*, 2015; Gautam *et al*, 2021; Khan *et al*, 2022b; Subramaniyan and Natarajan, 2017).

DPPH free radicals, ABTS free radicals and iron chelating scavenging activity of *P. obtusa* was conducted to determine the antioxidant potential against oxidative stress induced pathogenesis and the determined the effectiveness of *P. obtusa* against oxidative stress. The findings revealed significant effect of *P. obtusa* against varieties of free radicals. Several studies have been published on the anti-oxidant activity of the medicinal plants which is evaluated by the DPPH assay. Dogra and his team determined the antioxidant activity of *P. obtusa* using the DPPH method and the results showed that *P. obtusa* has significant antioxidant activity against DPPH free radical with 46.32 ± 0.62% of inhibition at the higher dose (Dogra, 2016). The finding of the present study is supported by the reported database. Furthermore, the author declared that *P. obtusa* can be a good candidate for antioxidant activity and effectively mitigates oxidative stress-induced organ dysfunction. Furthermore, it has been reported that the compounds such as ellagic acid, ferulic acid, rutin and gallic acid are the most prominent constituents in *P. obtusa* (Bihani *et al*., 2022).

The antiradical elements are compared to the polyphenols' action in terms of their ability to fulfil the blue-green chromophore ABTS ions, which exhibit distinctive absorption at 734 nm. When antioxidants are introduced, the produced radical cation is converted to ABTS, and the activity is demonstrated by the test sample's decolorization. In this procedure, an antioxidant is introduced to an ABTS radical solution that has already generated, and the quantity of $ABTS^{+}$ that is left after a specific length of time is quantified spectrophotometrically at 734 nm (Polu *et al*, 2017). It is also reported that the constituents present in such as terpenoids, flavonoids, iridoids, cardiac glycosides, phenolic acids, fatty acid esters, coumarin, steroids, cardenolide are not only responsible for the antioxidant activity but also exhibit anti-inflammatory activity

It has been suggested that polyphenols, which contain several hydroxyl groups and function as antioxidants by scavenging free radicals, may demonstrate ABTS activity of the medicinal plants or their formulation. Additionally, polyphenols may function as reducing agents, metal chelators, hydrogen donors, and singlet oxygen quenchers due to their redox properties (Javanmardi *et al*, 2003). The findings revealed that *P. obtusa* has significant anti-oxidant potential against varieties of free radicals. Hence, it can be a promising herbal therapy for treating oxidative stress induced pathogenesis.

5. Conclusion

The present study concludes that *P. obtusa* exhibits a significant role as an antioxidant agent which can mitigate the diversity of free radicals and thus reduce oxidative damages to the normal function of vital organs. It can be used as adjuvant therapy for the reduction of oxidative stress induced by any exogenous or endogenous agents. However, further molecular and clinical investigations are needed to enhance the credibility of the present findings.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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