Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 6(5); 215-221

ISSN: 0976 822X

Research Article

Antioxidant Activity of Different Solvent Extracts of *Jacaranda mimosifolia* D. Don Bark and Leaf

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Available Online:4th August, 2015

ABSTRACT
In the present study, the total phenolic content and antioxidant activities of different solvent extracts of bark and leaf parts of *Jacaranda mimosifolia* D. Don were investigated. Among the different extracts studied, the ethyl acetate extract of *J. mimosifolia* bark showed higher level of total phenolic concentration (16.42 mg FAE/L). However, the ethanol extract of *J. mimosifolia* bark registered high antioxidant activity in terms of phosphomolybdate reducing power (1508 FAEA), ferric reducing power (0.99 Abs units), radical scavenging activity against DPPH (51.56%), superoxide (69.23%), hydrogen peroxide (87.47%) and hydroxyl radicals (75.41%). Between the analyzed materials of *J. mimosifolia*, the bark revealed high antioxidant power than leaf sample. Due to notable phenolic content and remarkable antioxidant effect, the ethanol extracts of *J. mimosifolia* bark could be considered for further studies on preventing oxidative stress induced diseases.

Key words: *Jacaranda mimosifolia,* bark, leaf, total phenols, antioxidant.

INTRODUCTION

Reactive oxygen species (ROS) are produced in the body as a result of incomplete reduction of oxygen during oxidative phosphorylation[1]. Free radicals could attack DNA and results in mutation and cancer[2]. It also can react with proteins, carbohydrates and lipids and plays a significant role in the pathogenesis of numerous disorders and pathophysiological processes including cardiovascular diseases, diabetes, and cancer[3]. Oxidative stress occurs, when the system loses its ability to neutralise the excessively produced free radicals[4]. The redox homeostasis, i.e. the balance between the free radicals and antioxidants is necessary for maintaining good health. This balance is maintained by a number of antioxidants (vitamin E and ascorbic acid) and enzymes like superoxide dismutase, catalase, glutathione peroxidise, etc. Under severe conditions, above-mentioned antioxidant system is not sufficient to prevent the oxidative stress. Hence, intake of external anti-oxidants is necessary for maintaining homeostasis in the body. When we are looking for natural source of antioxidants, the medicinal plants from Bignonaceae family received attention, because some of their members are well known for medicinal effects[5]. In this connection, the bark and leaf materials of *Jacaranda mimosifolia* D. Don were chosen and investigated for their antioxidant effect in the present study.

Jacaranda mimosifolia D. Don (Common name: Blue trumpet tree) is a common sub-tropical ornamental tree found worldwide and is known for its beautiful foliage and attractive flowers. It is sub-tropical tree native to South America, grows to a height of 50 m. It is usually grown as ornamental tree in public and home gardens, parks and avenues in India. Its bark is thin and grey-brown in colour, smooth when the tree is young though it eventually becomes scaly. The leaves are large, alternate, bipinnately compound, obovate and grow about 6 cm long. Flowers are purple in colour, bell shaped with pleasant fragrance and pods are dry and hard with 1 – 3 inches length. Miyajima et al.[6] reported the propagation methods of this plant, while other researchers investigated the optimal light, temperature, water, fertilizer and substrate conditions for the germination of *J. mimosifolia* seeds[7-9]. Olowoyo et al.[10] investigated *J. mimosifolia* tree bark as bio-monitor for atmospheric trace metals. Further, effect of application of bio-solids from waste water treatment on the growth of *J. mimosifolia* and the changes on physical and chemical conditions of a degraded soil was investigated by Ramirez et al.[11]. The leaves, flowers and bark of *J. mimosifolia* are traditionally used for the treatment of a number of diseases like hypertension, amoebic infections, blood purification, syphilis, blennorrhagia, venereal diseases, wound, dermatitis, urinary tract problems, ulcer, diarrhoea, and dysentery[12-16]. This plant species is reported to exhibit hypotensive[13], cytotoxic[17], antimicrobial[18,19] and antioxidant properties[20]. The flowers are used as a substitute for the Unani herb, Gul-e-Gaozabaan in Pakistan[16]. The *J. mimosifolia* seed oil containing jacaric acid was analyzed for anti-obesity property in animal model[21].

Although reports are available on the medicinal properties including antioxidant activity of different *Jacaranda* species such as *J. puberula*[22]; *J. acutifolia*[23]; *J. decurrens*[24] and *J. micrantha*[25], not much deeper studies are available on the antioxidant activity of *J. mimosifolia*. Hence, the

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Figure 1. Total phenolic concentration of different solvent extracts of bark and leaf of *J. mimosifolia*

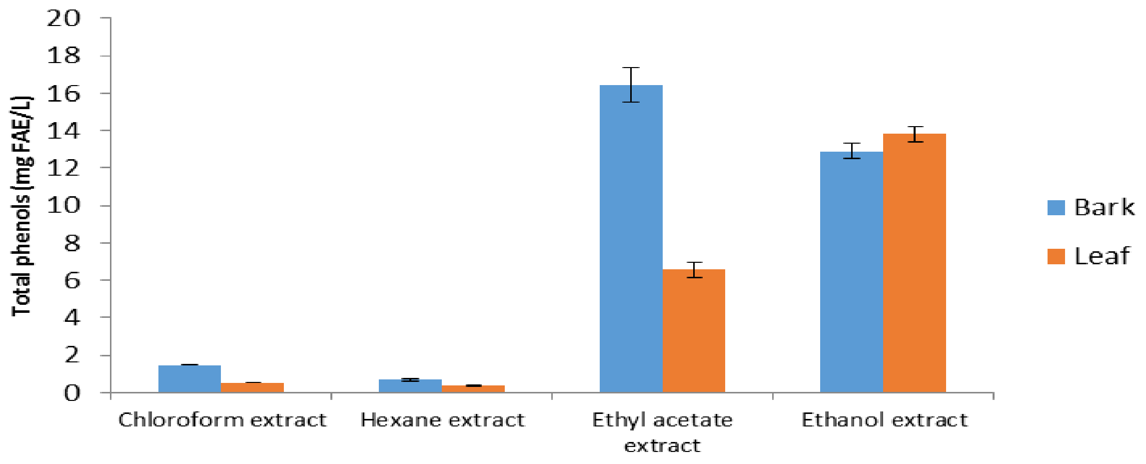


Figure 2. Phosphomolybdate reducing power of different solvent extracts of bark and leaf of *J. mimosifolia*

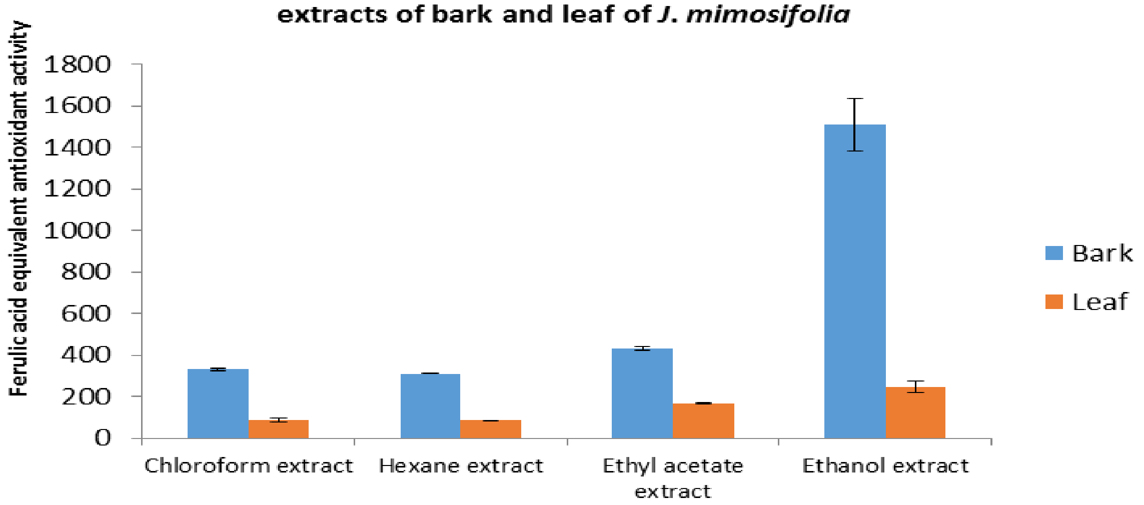


Figure 3. Ferric reducing power of different solvent extracts of bark and leaf of *J. mimosifolia*

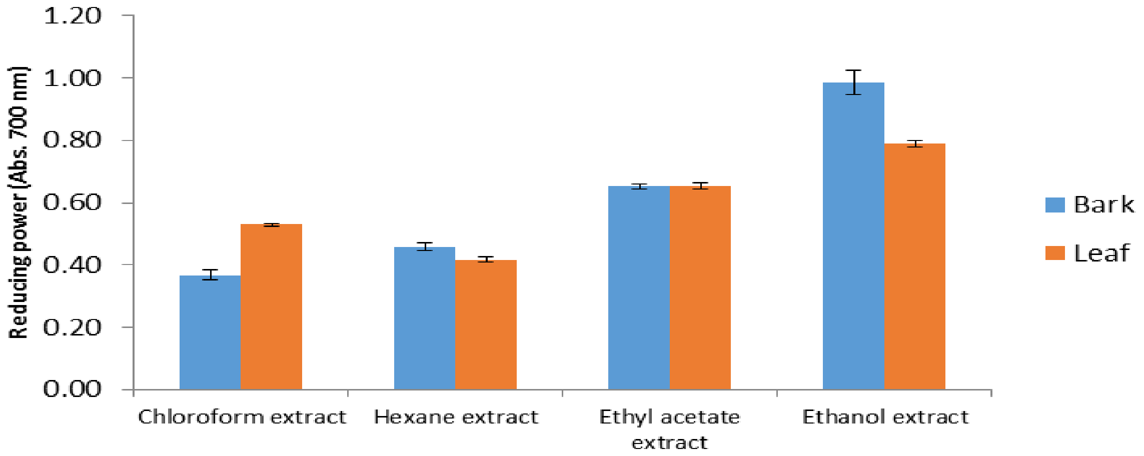


Figure 4. DPPH radical scavenging activity of solvent extracts of bark and leaf of *J. mimosifolia*

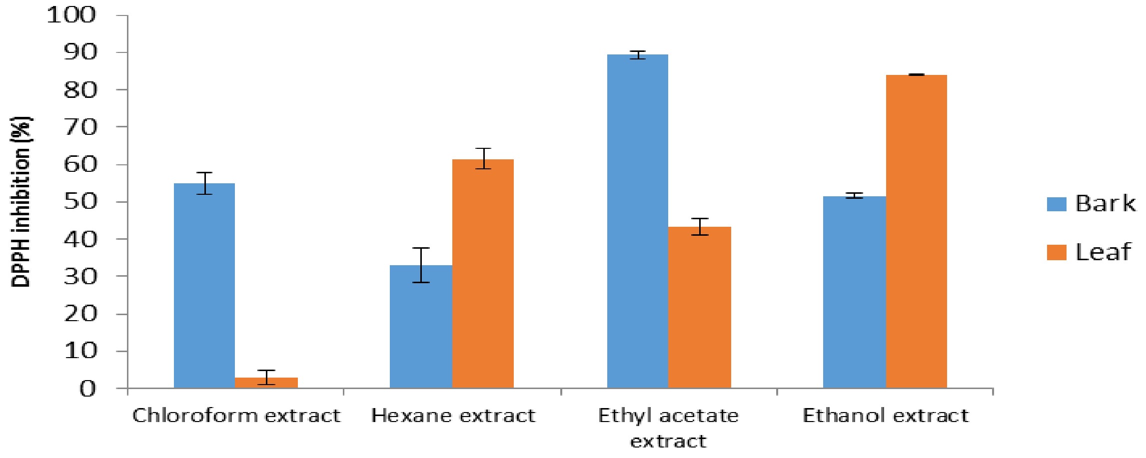


Figure 5. Superoxide scavenging activity of different solvent extracts of bark and leaf of *J. mimosifolia*

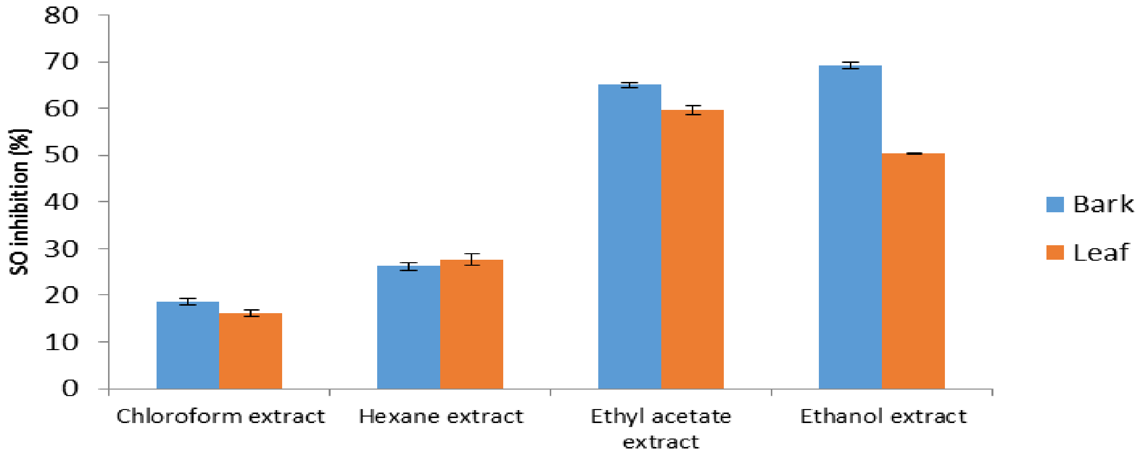
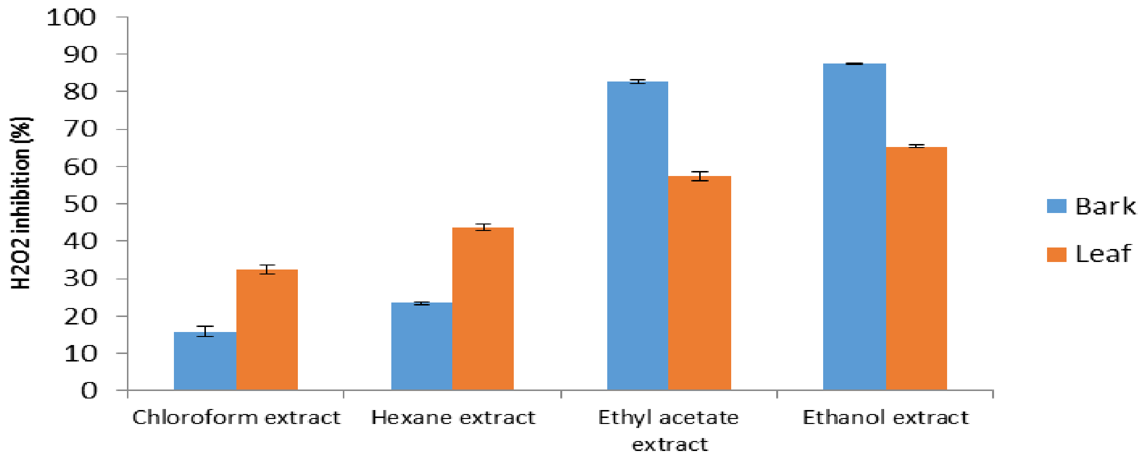


Figure 6. H2O2 inhibition capacity of different solvent extracts of bark and leaf of *J. mimosifolia*



present study was carried out with a view to evaluate the antioxidant potential of different solvent extracts of bark and leaf of *Jacaranda mimosifolia*.

MATERIALS AND METHODS

Preparation of the extract

The bark and leaf materials of *Jacaranda mimosifolia* plant were collected from Rajasthan State of India. The materials were shade dried and powdered into 1 mm particle size using a lab mill. For the preparation extract, different solvents such as chloroform, hexane, ethyl acetate and ethanol were used. *Jacaranda mimosifolia* bark (200 g) and leaf (300 g) were taken separately in 1000 ml beaker and 550 ml solvent was added and kept at room temperature for 48 h. Then the contents were filtered and the filtrate volume was noted and then allowed to evaporate at room temperature and the remaining residue weight was recorded. The dried extract was re-constituted with respective solvent at 10 mg/ml ratio and used for further experiments. Throughout the experimental period, the extracts were maintained at refrigerated condition and they brought to room temperature before 2 h of each experiment.

Analysis of total phenolic content

The total phenolic content was analyzed using Folin-Ciocalteu reagent method[26] with some modifications. The sample (50 µl opportunely diluted) is added to 250 µl of Folin-Ciocalteau reagent in a test tube and vortexed. Then, 4.7 ml of 2.2% sodium carbonate solution are added and the mixture is vortexed again. A blank is prepared with 50 µl of the sample solvent instead of the sample. The tubes are incubated at 40°C for 30 min in the dark. The absorbance is read at 750 nm against the blank using Spectrophotometer (Perkin-Elmer, Model). A calibration curve was prepared with standard ferulic acid (200 – 1600 mg/L, R^2 = 0.9978) and used to express the results as ferulic acid equivalents (FAE). The total phenolic content of the sample was then calculated and expressed on dry weight and fresh weight basis.

Phosphomolybdate assay

The antioxidant activity of extracts was evaluated according to the method of Prieto et al[27]. An aliquot of 100 µl of extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a screw-capped vial. The vials were closed and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results expressed as ascorbic acid equivalent antioxidant activity.

Ferric reducing power

The reducing power of extract was determined according to the method of Oyaizu[28]. Samples (2.5 ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 650 x g (rpm) for 10 min. The supernatant (5.0 ml) was mixed with ferric chloride (5.0 ml, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. Based on the absorbency value, the ferric reducing power of extract was expressed.

DPPH radical scavenging activity

The DPPH radical scavenging activity was analyzed for each by following Sanchez-Moreno et al.[29] method. The extract (100 µl) was added to 3.9 ml of DPPH solution (0.025 g/L) and the reactants were incubated at 25°C for 30 min. Different concentrations of ferulic acid was used as a positive control and ethanol was used instead of extract in blank. The decrease in absorbance was measured at 515 nm with a spectrophotometer. The radical scavenging activity of tested samples was calculated and expressed on percentage basis.

Superoxide radical scavenging activity

The capacity of extracts to scavenge the superoxide anion radical was measured according to the method described by Zhishen et al[30]. The reaction mixture was prepared using 3 x 10^{-6} M riboflavin, 1 x 10^{-2} M methionine, 1 x 10^{-4} M nitroblue tetrozolium chloride and 0.1 mM EDTA in phosphate buffered saline (pH 7.4). For the analysis, 3.0 ml of the reaction mixture was taken with 100 µl of extract in closed tubes and illuminated for 40 min under fluorescent lamp (18 W). The absorbance was then read at 560 nm against the un-illuminated reaction mixture. Results are expressed as superoxide radical scavenging activity on percentage basis.

Hydrogen peroxide scavenging activity

The effect of extract on hydrogen peroxide was analyzed according to the method proposed by Ruch et al[31]. The extract (100 microliter) was mixed with 5 ml of 45 mM hydrogen peroxide solution in 0.1 M phosphate buffer (pH 7.4). The reaction mixture was vortexed and incubated for 30 min at room temperature and then the absorbency was measured at 230 nm. The extract with phosphate buffer is used as a blank and the level of hydrogen peroxide remaining in the solution was calculated using a calibration curve. The hydrogen peroxide inhibition effect of extract was calculated and expressed on percentage basis.

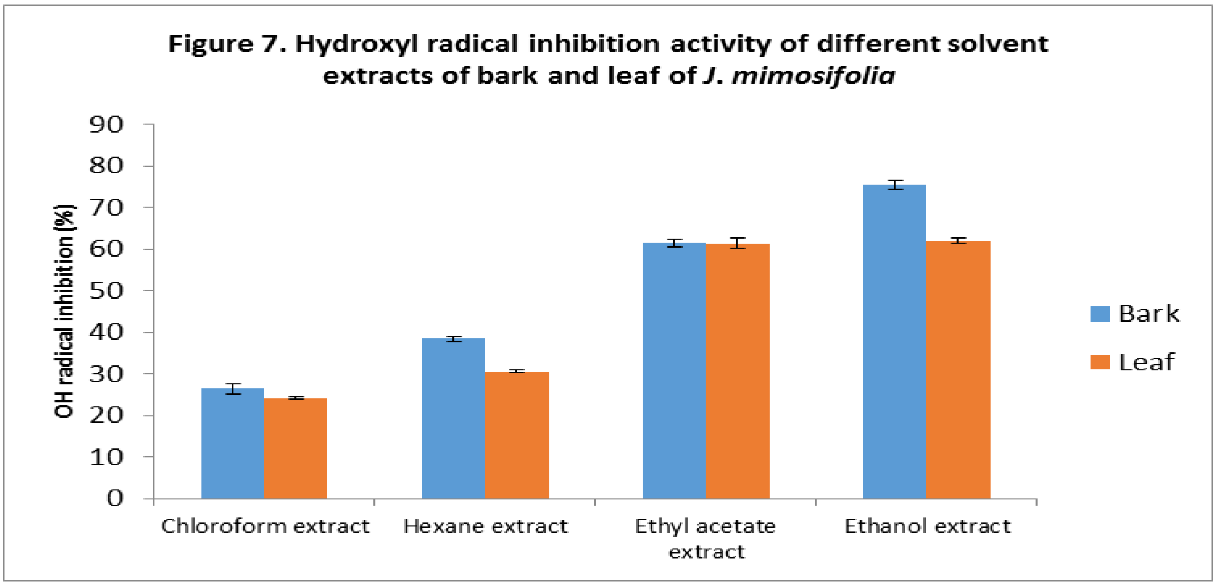
Hydroxyl radical scavenging activity

The hydroxyl radical quenching activity of extracts was evaluated according to the method of Hagerman et al[32]. The reaction mixture consists of 10 mM phospate buffer (pH 7.4), 2.8 mM Deoxyribose, 2.8 mM H2O2, 0.025 mM FeCl3, 0.1 mM EDTA and 0.1 mM ascorbic acid in a total volume of 3 ml. With the reaction mixture, 100 microliter of extract was added and incubated at 37°C for 15 min. Then the reaction was terminated by the addition of 1 ml of 2.5% ice-cold TCA and 1% TBA. The reactants were mixed well and heated at 90 C for 15 min in a water bath and cooled to room temperature. The chromogen was extracted with 1-butanol and absorbency was measured at 530 nm. Based on absorbency value, the hydroxyl radical scavenging activity of extracts was calculated and expressed on percentage basis.

RESULTS AND DISCUSSION

Total phenolic concentration

The total phenolic compounds were quantified by Folin-Ciocaltue reagent method. This assay is based on the reduction of Folin's reagent by the phenolic compounds.



Under alkaline pH, phenols dissociate into phenolate anion and proton, hence phenolate anion can reduce the Mo (VI), which is a major component of Folin's reagent, by single electron transfer. Due to this reduction, a blue coloured complex $(PMoW_{11}O_{40})^{4-}$ is formed with absorption max at 750 nm. The analysis of presently investigated *J. mimosifolia* samples revealed higher level of total phenolics in ethyl acetate extract in the case of bark (16.42 mg FAE/L) and ethanolic extract in the case of leaf (13.82 mg FAE/L) (Figure 1). On comparison, ethyl acetate extract of *J. mimosifolia* bark exhibited higher amount of phenolics than that of leaf material.

Antioxidant activity

When the molybdenum (VI) is reduced to Mo (V) by an antioxidant, it forms a green coloured complex at acidic pH in the presence of phosphorous with the absorption maxima at 695 nm. This assay evaluate the reducing or electron donating power of the antioxidant to Molybdenum and the intensity of PMo(V) complex is proportional to antioxidant power of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant. Figure 2 reveals the phosphomolybdate reducing power of different solvent extracts of *J. mimosifolia* bark and leaf. Among the investigated extracts, the ethanol extract of bark registered higher level of reducing power (1508 FAEA) than all other extracts of both bark and leaf sample of *J. mimosifolia*. Similarly, the antioxidant effect of *Jacaranda micrantha* extract was proved through phosphomolybdate assay[25].

In ferric reducing assay, Fe (III) is reduced to Fe (II) by the antioxidant compound through electron transfer. The reduced Fe (II) forms the Pearl's blue complex, which can be measured at 700 nm. On examining ferric reducing power, it can be seen that the ethanol extract of *J. mimosifolia* bark (0.99 Abs units) and leaf (0.79 Abs units) have higher antioxidant power among the investigated extracts (Figure 3). However, these values were found to be lower when compared to an earlier report on ethyl acetate extract of *Jacaranda acutifolia*[23].

The evaluation of the antioxidant power by DPPH radical scavenging activity has been widely in use for different plant extracts. DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a stable radical, methanolic solution of which has dark purple colour with maximum absorption at 515 nm**.** Antioxidants can reduce DPPH through hydrogen transfer into its non-radical form (DPPH-H) and hence the absorption disappears at 515 nm. The decrease in absorbency at 515 nm may be due to the reaction between phytochemicals and DPPH, which indicates the antioxidant power. On performing the DPPH radical scavenging assay for the different extracts, higher level of antioxidant activity was noted in ethyl acetate extract (89.28%) and ethanolic extract (83.99%) of *J. mimosifolia* bark and leaf, respectively (Figure 4). Similarly, ethyl acetate extract has revealed high DPPH radical scavenging activity in *Jacaranda puberula*[22] and *Jacaranda acutifolia*[23].

The superoxide radical scavenging activity of samples was investigated by generating superoxide through photo-induced reduction of riboflavin, which can generate superoxide radical in the presence of methionine. The generated superoxide radical reduce the NBT into purple colour formazan, which was measured at 560 nm. In presence of antioxidant, the generated superoxide radicals were scavenged and hence, formation of purple colour formazan is minimum or nil. The superoxide scavenging activity of various extracts of *J. mimosifolia* bark and leaf was shown in the Figure 5. The ethanolic extract of bark (69.23%) and ethyl acetate of leaf (65%) exhibited high antioxidant effect.

The effect of different solvent extracts on the hydrogen peroxide inhibition was illustrated in the Figure 6. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removing hydrogen peroxide is very important for protection of cellular system. The hydrogen peroxide can decompose into water by accepting two electrons and protons. The level of hydrogen peroxide in buffer solution can be detected spectrometrically at 230 nm. If antioxidants (electron donors) are added to the reaction mixer, they can accelerate the conversion of hydrogen peroxide into water. The results revealed that the ethanolic extract of bark (87.47%) as well as leaf (65.40%) of *J. mimosifolia* have recorded higher hydrogen peroxide inhibition activity.

Hydroxyl radicals are produced by the Fenton reaction between Fe(II)-EDTA and hydrogen peroxide. The hydroxyl radicals (OH.) degrade Deoxyribose and produce MDA, which can be measured by TBARS reaction. The TBA can react with MDA in acidic medium to form pink colour chromogen, which could be extracted with 1-butanol and read at 530 nm. OH radicals may attack various biomolecules including proteins, lipids, and DNA and cause oxidative damage to the cellular components and hence it is considered to be biologically dangerous free radical. Hydroxyl radical inhibition assay performed for different solvent extracts of *J. mimosifolia* indicates that the ethanolic extract was more effective in scavenging the hydroxyl radicals in bark (75.41%) and leaf (62.05%) (Figure 7).

Based on *in vitro* studies, ethanol extract of *J. mimosifolia* was found to have high antioxidant effect when compared to other solvent extracts. This is in agreement with earlier report on its related species *Jacaranda decurrens*[24]; *J. acutifolia*[23]; *J. micrantha*[25] and *J. puberula*[22]. The remarkable antioxidant effect of ethanol extract of *J. mimosifolia* might be due to the presence of notable level of phytochemical constituents, especially polyphenols as demonstrated by the total phenolic content of the present study. Already, there are certain phenolic constituent were reported such as jacaranone, verbacoside and other flavonoids such as scutellarin, apigenin, luteolin, isoquercitrin, campneoside, jacraninoside and isovitexin from the leaf of *J. mimosifolia* with anticancer and sedative activities[20]. Lupenone, beta-sitosterol, ursolic acid and oleanolic acid were reported constituents from root bark[33-36] and the flavonol glycoside isoquercitrin was identified in the flower[16]. The chemical composition of floral nectar of *J. mimosifolia* has also been analyzed[37]. Hence, due to the presence of such bioactive compounds, the solvent (ethanol) extract of stem bark of *J. mimosifolia* displayed maximal antioxidant property.

CONCLUSION

The results of the present investigation revealed the presence of remarkable levels of total phenolic compounds with good antioxidant property in *J. mimosifolia* bark and leaf materials. In the case of *J. mimosifolia* bark, the ethyl acetate extract recorded maximum level of phenolic compounds, but higher antioxidant effect was observed in ethanol extract when compared to other solvent extracts. It is clear that the presence of high polar compounds soluble in ethanol is responsible for the observed antioxidant effect of *J. mimosifolia*. In *J. mimosifolia*, bark is found to be more efficient than leaf in exhibiting high antioxidant power, which gives scientific evidence for the use of stem bark of this plant in traditional system of medicine in India rather than leaf. Since the ethanol extract of *J. mimosifolia* bark having good antioxidant power, they might prevent the oxidative stress induced diseases like cancer, atherosclerosis, diabetes etc., which should be investigated using suitable animal models in future. Exploring the medicinal value of such indigenous plants to combat the chronic diseases will be beneficial for the human society.

ACKNOWLEDGEMENT

Authors are thankful to the management and administrative authorities of SASTRA University, Thanjavur, Tamilnadu for their support and encouragement to conduct this research project.

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