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In Vitro Antioxidant and Anti-diabetic Activity of Bark, Flower and Leaf Extracts of *Jacaranda mimosifolia* D. Don

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Abstract

Jacaranda mimosifolia D. Don. (Bignoniaceae) is an ornamental tree with opposite bipinnate leaves and tender lavender-blue flowers. Knowledge of the phytochemicals present in any plant is important for understanding its biological significance which may lead to the discovery of new alternative therapeutic drugs. This paper aims to investigate the phytochemical profile, antioxidant potential and *in vitro* antidiabetic activity of the bark, flowers and leaves of *J. mimosifolia,*extracted using the cold extraction method with various solvents, *viz.* hexane, acetone, 100% ethanol and 70% hydroethanol. Preliminary phytochemical test of all extracts was performed to determine their chemical constituents. The total phenolic content (TPC) and total flavonoid content (TFC) were calculated using the Folin-Ciocalteu method and aluminium chloride colorimetric assay, respectively. Similarly, the *in vitro* antioxidant and antidiabetic potentials of all extracts were determined using the diphenylpicrylhydrazyl (DPPH) assay and α-glucosidase inhibition assay, respectively. The qualitative phytochemical test showed the presence of flavonoids, steroids, glycosides, anthocyanins, terpenoids, tannins and carbohydrates (at least in one part of plant) whereas alkaloids and proteins were completely absent in all the plant extracts. In this study, the highest TPC and TFC were shown in acetone bark extract (219.06 ± 19.54 mg GAE g^{-1}) and hexane leaf (449.76 ± 28.89 mg QE g^{-1}) respectively, whereas the highest IC_{50} for antioxidant activity and anti-diabetic activity were found to be in 100% ethanol bark (56.15 μg mL^{-1}) and hexane flower (92.5% enzyme inhibition), respectively. Due to notable phenolic content, remarkable antioxidant and antidiabetic effects, the phytochemicals present in leaf and flower extracts of *J. mimosifolia* could be considered for the discovery of a new drug against oxidative stress-induced diseases and diabetes.

Keywords: Bignoniaceae, Diphenylpicrylhydrazyl, Medicinal plant, α-glucosidase

Introduction

Since ancient times, plants have been used for therapeutic purposes due to the presence of bioactive compounds and antioxidant properties (Velu et al., 2018). According to the World Health Organization (WHO), approximately 75% of the world's population, especially in developing countries depends on phytotherapeutic agents for primary healthcare (Pan et al., 2014). Phytochemicals have been reported to have various health-promoting effects, including the prevention of the onset of chronic diseases such as cancer, atherosclerosis, neurodegeneration, obesity, articular rheumatism, skin aging and diabetes (Bansal et al., 2021). It has also been reported that they are involved in the pathway of molecular signal transduction, inflammation, defense mechanisms; they can reduce oxidative stress by scavenging deleterious free radicals, or decomposing peroxides, and they help in the regulation of carbohydrate metabolism too (Bansal et al., 2021; Mishra & Tripathi, 2015).

The genus "*Jacaranda*" is an ornamental, woody representative of the Bignoniaceae family having 49 species and found mainly in tropical and subtropical geographical areas (Gachet & Schühly, 2009; Zaghloul et al., 2011). *Jacaranda mimosifolia* D.Don, also called blue jacaranda, black poui, fern tree, is native to Brazil, Bolivia, and Argentina (Xie et al., 2021) and is considered as pioneer tree with opposite bipinnate leaves and tender, deciduous foliage having soft, delicate, dense terminal clusters of lavender-blue, lightly fragrant, trumpet-shaped

flowers (El-Marasy et al., 2020; Gilman & Watson, 1993; Mostafa et al., 2014). The pharmaceutical interest of *J. mimosifolia* is increasing due to its richness in bioactive metabolites like alkaloids, flavonoids, polyphenols, iridoid glycosides, tannins, saponins, steroids, triterpene, and acteoside (Adelanwa & Habibu, 2015; El-Marasy et al., 2020; Mostafa et al., 2014).

Traditionally the bark of *J. mimosifolia* had been used to treat venereal diseases like syphilis and to purify the blood in Ecuador (Acosta Solís, 1992; Gachet & Schühly, 2009). The extracts were recorded to treat hypertension, ulcers, wounds, diarrhea, dysentery, amoebic infections in Bangladesh, India and Pakistan, as well as skin problems like, fungal infections, boils, eczema, and psoriasis in Africa(Serra et al., 2020; Sidjui et al., 2014). It possesses antiulcer (Shoukry et al., 2023), antileishmanial, antiprotozoal, anti-inflammatory, and anticancer activities (Naz et al., 2020).Various researchers have found its effective antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Salmonella typhii* and *Staphylococcus aureus* (Adelanwa & Habibu, 2015; Aguirre-Becerra et al., 2020). The hydromethanolic extract of leaf was also found to have hypothermic and hypotensive properties via a blockage of alpha-adrenergic receptors (Nicasio & Meckes, 2005; Rahmatullah et al., 2010).

Knowledge of the phytochemicals present in any plant is important for understanding its biological significance which may lead to the discovery of new alternative therapeutic drugs. Though some research has been carried out on phytochemical screening but complete research on various solvent extracts of different parts of *J. mimosifolia* has not been carried out yet. This study aimed to conduct qualitative phytochemical screening, as well as quantitative estimation of total phenolic contents (TPC), total flavonoid contents (TFC) and antioxidant activities with radical scavenging effects of various extracts of *J. mimosifolia* bark, leaves and flower. Also, *in vitro* antidiabetic activity of various parts of *J. mimosifolia* was evaluated.

Materials and Methods

Instruments and chemicals

Rota evaporator from Hahnshin Scientific Co. (Korea) and Bio Tek Epoch-2 Microplate Reader from Biotech (USA) were used. Ascorbic acid, *α*-p-nitrophenyl α-D-glucopyranoside (nPG) and acarbose were obtained from Sigma- Aldrich (China), 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma- Aldrich (Germany), quercetin from Sigma- Aldrich (India) and gallic acid from Loba Chemie Pvt. Ltd (India). All other chemicals used were of analytical grade.

Collection and extraction of plant materials

The bark, flowers and leaves of *J.mimosifolia* were collected from Thapathali, Kathmandu, Nepal (latitude 27°41'15.28224" N, longitude 85°19'19.1892" E) at an elevation of 1285 meters above sea level. Then the plant was identified and authenticated at National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur, Nepal and deposited for future reference. All the laboratory work was carried out at Natural Products Research Laboratory (NPRL), Thapathali, Kathmandu, Nepal. The extracts were prepared by cold extraction method as mentioned by Harborne (1998), Seyfe et al. (2017) and Nortjie et al. (2022). The bark, flowers and leaves were cleaned of extraneous materials, shade dried and ground into fine powder using an electric blender separately. A 200 gm portion of powdered material from each plant part was sequentially macerated at room temperature in 1000 mL of 100% ethanol, followed by 70% ethanol, then acetone, and finally hexane, each for 72 hours with intermittent agitation. Then, the solution was filtered through Whatman grade 1 filter paper, and the residues were macerated further two times with the respective solvents. The filtrates, thus obtained, were concentrated separately using a rotary vacuum evaporator under reduced pressure at 40-45°C to obtain the respective extracts, which were then stored in a refrigerator at 4°C until further use.

Qualitative phytochemical screening

The qualitative phytochemical screenings of twelve extracts of *J. mimosifolia* (bark, flower and leaf extracts obtained using four different solvents) were performed to detect the presence of alkaloids, glycosides, flavonoids, tannins, phenols, saponins, carbohydrates and steroids using the standard methods (Harborne, 1998; Mishra & Tripathi, 2015; Shaikh & Patil, 2020).

Determination of total phenolic contents (TPC) and total flavonoid contents (TFC)

The TPC was determined using the Folin-Ciocalteu's method, taking gallic acid as standard for the calibration curve as described by Singleton and Rossi (1965) with a slight modifications. Briefly, 20 µL of 1 mg mL^{-1} of different extracts was loaded in triplicate in a 96-well plate. Gallic acid at concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 µg mL^{-1} was also loaded in triplicate and used as standard. Then, 100 µL of 10% Folin-Ciocalteu (FC) reagent was added to each well containing either gallic acid or extract. An initial reading of the absorbance was taken at 765 nm using a microplate reader (Epoch2, BioTek, Instruments, Inc, USA). After initial reading, 80 µL of 7% sodium carbonate was added to each well and incubated in dark for 30 minutes. The final absorbance was also taken at 765 nm using the microplate reader. The TPC was expressed in milligrams per gram (mg g^{-1}) of dry plant extract, in gallic acid equivalent (GAE) (Fombang & Mbofung, 2015; Mujic et al., 2009; Seyfe et al., 2017).

Similarly, the TFC were determined by aluminium chloride colorimetric assay, taking quercetin as standard as described by Fombang and Mbofung (2015) and Nobossé et al. (2018). In brief, 20 µL of different concentrations i.e., 10, 20, 40, 60, 80, 100 µg mL^{-1} of quercetin and 20 µL of 1 mg mL^{-1} plant extracts were loaded separately in triplicate in a 96 well plate. Then, 110 µL of distilled water and 60 µL of ethanol were added to each well. The initial absorbance was taken at wavelength 415 nm in a microplate reader. Then, 10 µL of 5% aluminium chloride and 10 µL of 0.5M potassium acetate was added to each plate, followed by incubation in dark for 45 minutes and the final absorbance was taken at the same wavelength i.e., 415 nm. The TFC of each extract was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QEg^{-1}) of the extract.

Evaluation of antioxidant activity

The anti-oxidant activity was evaluated using the DPPH assay, following the method developed by Brand-Williams et al. (1995) with some modifications. Briefly, 10 µL of sample extracts (1 mg mL^{-1}) and ascorbic acid (Vitamin C) of various concentrations as standard were loaded in triplicates in a 96-well plate. Methanol (90 µL) was added and initial absorbance was taken at 517 nm. Then,150 µL of 0.1 mM DPPH solution was added to each well. After incubation for 30 minutes in the dark, absorbance was taken again at 517 nm. The linear plot of percent inhibition versus concentration was analyzed as equation Y = mX + b where, X is the concentration of the measured substance and Y is the percent inhibition. Meanwhile, the IC50 value was determined as the X value of this equation when Y was equal to 50%.

The percentage inhibition of DPPH radicals by the plant extract was calculated using the following formula (Baba & Malik, 2014; Nobossé et al., 2018):

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} * 100$$

Whereas, A_c = absorbance of blank control,
A_s = absorbance of tested sample extract

In vitro antidiabetic assay

The anti-diabetic activity of *J. mimosifolia* extracts was evaluated by α-glucosidase inhibitory activity using α-p-nitrophenyl α-D-glucopyranoside (pNPG) (Sagbo et al., 2018), which is hydrolyzed by α-glucosidase to release p-Nitrophenol (a yellow-colored agent) and acarbose taken as positive standard as mentioned by Bhatia et al. (2019), El Omari et al.(2019) and Marmouzi et al. (2017). For this assay, 20 µL of DMSO as negative standard/

blank, 20 µL of 1 mg mL^{-1} stock solution of plant extract and 20 µL of different concentrations, i.e. 100, 200, 400, 600, 800, 1000 µg mL^{-1} of acarbose were loaded separately in triplicate in a 96-well plate. Then, 20µL of α-glucosidase enzyme (0.5 units mL^{-1}) and 70 µL of phosphate buffer (6.8 pH) were loaded in each plate and left for 15 minutes at 30 °C. The initial absorbance was taken at 405 nm wavelength. Then, 40 µL of pNPG (1.24 mM) was added and reincubated for 30 minutes at 37 °C. The final reading was also taken at 405 nm wavelength after the addition of 50 µL of sodium carbonate (0.2M). The α-glucosidase inhibitory activity was expressed as percentage inhibition.

$$\% \text{ inhibition} = \frac{A_c - A_s}{A_c} * 100$$

Whereas, A_c = absorbance of blank control,
A_s = absorbance of sample extract

Statistical analysis

Data were expressed as mean ± SEM using Microsoft Excel 2021. IBM SPSS (Statistical Package for Social Sciences) version 26 was used for further statistical analyses. One-way ANOVA (Analysis of Variance) test was performed to determine overall mean differences among the various extracts at a 0.05 level of significance (α). Significant ANOVA results were followed by post hoc Dunnett's two-sided test, treating one group as the control and comparing all other extract groups against it.

Results and Discussion

Qualitative phytochemical screening

In this study, flavonoids, steroids, glycosides, anthocyanins, terpenoids, tannins and carbohydrates were detected in the plant extracts (in at least any one part of the plant) whereas alkaloids and proteins were completely absent as shown in Table 1. Naz et al. (2020) also revealed the presence of phenolics, flavonoids, tannins and saponins in all the leaf extract fractions. However, in this study, flavonoids were found in all plant extracts except the acetone and alcohol extracts of bark and the hexane extracts of flowers. Similarly, tannins were found in 70% ethanol extract of leaves and in all flower extracts, except the hexane flower extract. Saponins were detected only in the 70% ethanol extract of flowers and 100% ethanol extract of bark. Previously, methanolic flower extracts of *J. mimosifolia* were found to be rich in phenolics, flavonoids, terpenoids and quinones (Joselin et al., 2013). Additionally, the methanolic leaf extracts have been reported to contain tannins, flavonoids, carbohydrate and to lack triterpenoids and alkaloids (Adelanwa & Habibu, 2015), which is consistent with the findings of our study.

Table 1: Result of phytochemical test of various extracts of *J. mimosifolia*

S.N.	Phytochemicals screening	Methods	70% Hydroethanol			Acetone			Hexane			100% Ethanol		
			Br	Fl	Lf	Br	Fl	Lf	Br	Fl	Lf	Br	Fl	Lf
1	Alkaloids	Hager´s test	-	-	-	-	-	-	-	-	-	-	-	-
		Mayer´s test	-	-	-	-	-	-	-	-	-	-	-	-
2	Flavonoids	NaOH test	++	++	+++	-	+	+	+	-	++	-	+	+
		Shinoda test	++	++	+++	-	+	+	+	-	++	-	+	+
3	Steroids	Salkowski's test	-	-	-	+	+	-	+	+	+++	+	+	-
4	Glycosides	Fehling's test	-	++	-	+	-	-	+	++	+	+	+	-
5	Anthocyanins	Anthocyanin test	++	++	++	++	++	-	-	-	+	+	+	+
6	Terpenoids	Chloroform test	-	-	-	+	+	-	+	+	+	+	+	-
7	Saponin	Froth test	-	+	-	-	-	-	-	-	-	+	-	-
8	Tannins/Phenolics	$FeCl_3$ test	-	++	++	-	++	-	-	-	-	-	+	-
9	Carbohydrates	Molisch test	++	++	++	+	+	+	+	+	+	+	+	+
10	Proteins	Ninhydrin test	-	-	-	-	-	-	-	-	-	-	-	-

Note: + = positive, ++ = mildly positive, +++ = strongly positive, - = negative, Br = bark, Fl = flowers, Lf = leaves

Total phenolic contents (TPC) and total flavonoid contents (TFC)

The quantification of TPC of *J. mimosifolia* revealed that the acetone bark extract had the highest value of TPC (219.06 ± 19.54 mg GAE g^{-1}) followed by 100% ethanol bark (173.32 ± 5.74 mg GAE g^{-1}), while hexane bark extract showed the lowest TPC (19.65 ± 5.4 mg GAE g^{-1}) as shown in Figure 1.The TPC values reported in the present study are higher than the value reported by Aguirre-Becerra et al. (2020) for aqueous flower extract, but lower than the value for methanolic flower extract. In contrast, Rana et al. (2013) found a slightly higher TPC in butanol extracts of leaf and flower than in our study.

Similarly, the quantification of TFC of *J. mimosifolia* showed the absence of flavonoid contents in 70% ethanol extracts of bark and flower, acetone extracts of bark and flower, hexane extract of flower, and 100% ethanol extracts of bark and flower as shown in Figure 1. Flavonoid contents were detected only in the leaf extracts, regardless of whatever solvent was used. Other extracts, except hexane bark extract, lacked flavonoids. TFC was found to be the highest in hexane leaf extract (449.76 ± 28.89 mg QE g^{-1}), while the lowest TFC was observed in 100% ethanol leaf extract (116.42 ± 30.77 mg QE g^{-1}) as shown in Figure 1. Findings are in agreement with TFC value range mentioned by Mostafa et al. (2014). However, Aguirre-Becerra et al. (2020) found higher flavonoid contents even in flower extract while using rutin as standard instead of quercetin.

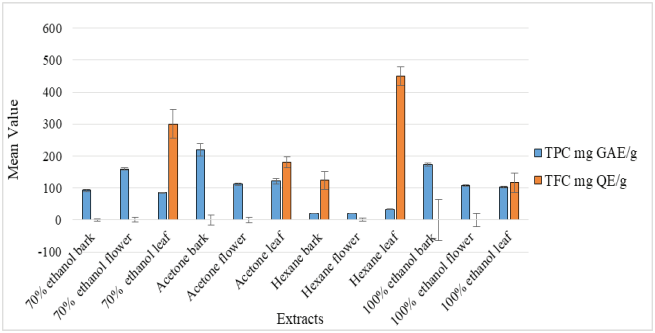

Figure 1: Quantitative value of TPC and TFC of *J. mimosifolia* extracts

Anti-oxidant activity (DPPH Assay)

The DPPH free radical scavenging activity of various extracts of *J. mimosifolia* showed that 100% ethanol bark extract inhibited DPPH free radicals even at low concentration, with an IC_{50} value of 56.15 µg mL^{-1}.This value is slightly lower than that of the ascorbic acid standard (60.26 µg mL^{-1}) as shown in Table 2. Furthermore, the difference between the IC_{50}values of the 100% ethanol bark extract and the positive control (ascorbic acid), was

Table 2: Result of percent inhibition of DPPH free radicals by various extracts of *J. mimosifolia*

Sample	**% of inhibition (Mean±SEM)**			**IC_{50}(µg/mL)**
Concentration (µg/mL)	**25**	**50**	**100**	
Positive control, Ascorbic acid	17.07±0.03	55.02±0.00	73.62±0.01	60.26±0.01
Concentration (µg)	**125**	**250**	**500**	**IC_{50}(µg/mL)**
70% ethanol bark	9.49±0.00	20.21±0.00	41.95±0.00	593.09±0.02
70% ethanol flower	19.56±0.00	43.91±0.00	75.50±0.01	316.83±0.00*
70% ethanol leaf	11.66±0.00	21.15±0.00	42.68±0.00	590.16±0.00*
Acetone bark	26.01±0.00	42.89±0.01	76.08±0.00	304.15±0.00*
Acetone flower	11.88±0.00	25.07±0.00	49.13±0.00	507.12±0.00*
Acetone leaf	20.50±0.00	42.31±0.00	76.30±0.00	316.31±0.00*
Hexane bark	4.61±0.00	6.26±0.00	8.79±0.00	4,245.54±0.00*
Hexane flower	14.23±0.01	15.37±0.01	15.55±0.00	11,493.59±0.01*
Hexane leaf	19.54±0.00	18.65±0.00	20.49±0.00	9,745.34±0.00*
100% ethanol bark	50.85±0.02	68.31±0.01	77.41±0.00	56.15±0.01
100% ethanol flower	33.71±0.00	44.52±0.00	60.08±0.01	348.00±0.00*
100% ethanol leaf	30.92±0.03	44.84±0.03	73.30±0.01	294.44±0.02*

Note: Asterisk (*) indicate statistically significant differences from acarbose at $p < 0.05$ (one-way ANOVA followed by Dunnett's post hoc test)

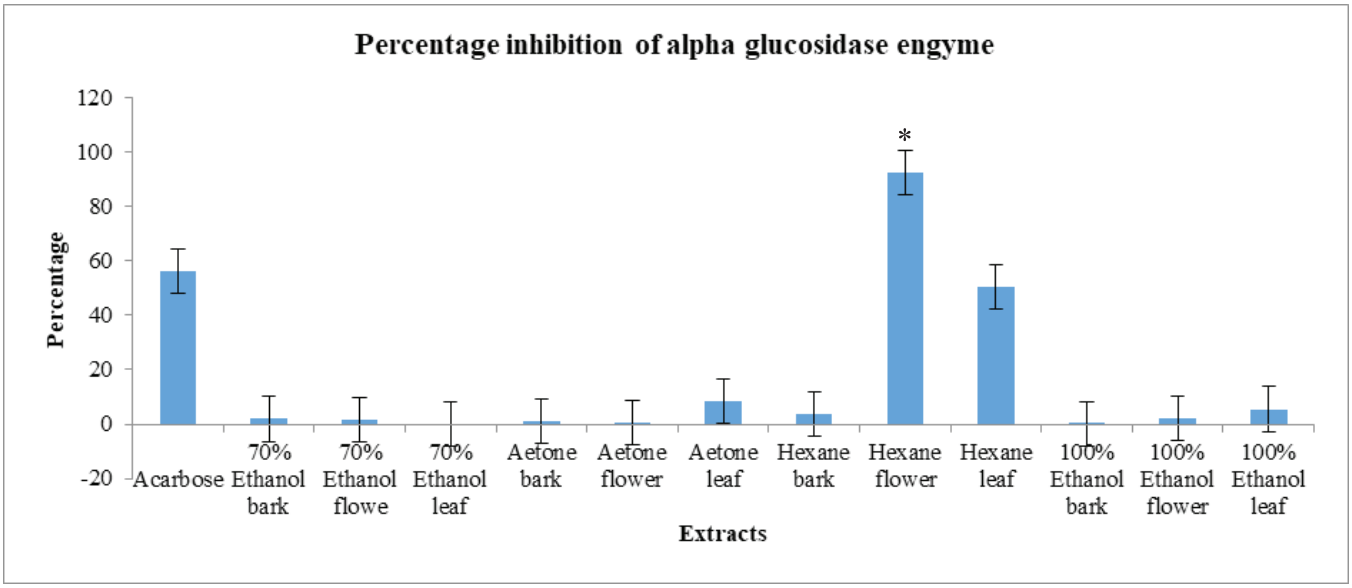

Figure 2: Percentage inhibition of *α*-glucosidase enzyme by *J. mimosifolia* extracts. The values are expressed as mean. Asterisk (*) indicates statistically significant differences from acarbose at p<0.05 (one-way ANOVA followed by Dunnett's post hoc test)

found to be insignificant at 5% level of significance, indicating that the antioxidant potential of 100% ethanol bark is comparable to that of ascorbic acid.

In vitro Anti-diabetic assay

The *in-vitro* anti-diabetic assay using the α-glucosidase inhibition test revealed that the enzyme was potently inhibited by hexane flower extract (1 mg mL^{-1}) and hexane leaf extract (1 mg mL^{-1}) of *J. mimosifolia* with inhibition percentage of 92.58 ± 0.19 and 51.47 ± 1.86 respectively (Figure 2). In comparison, the standard used, i.e. acarbose (1 mg mL^{-1}), exhibited an inhibition percentage of 56.21 ± 1.20. Moreover, the hexane flower extract exhibited a statistically significant difference when compared to the positive control, acarbose ($p < 0.05$), indicating a greater α-glucosidase inhibition capacity. In contrast, the hexane leaf extract did not show a statistically significant difference when compared with acarbose ($p = 0.232$), suggesting that its α-glucosidase inhibition capacity is comparable to that of the standard. Our findings are in agreement with those of Serra et.al (2020), who reported that acteoside (verbascoside), isolated from the *J. mimosifolia* leaves, had significant antidiabetic effects in a rat model of type 2 diabetes induced by streptozotocin-nicotinamide (STZ-NA).

Conclusion

Jacaranda mimosifolia showed strong positive reactions for several phytochemical groups like flavonoids, steroids, glycosides, anthocyanin, terpenoids and phenolics, indicating their potential presence in notable amounts. The highest TPC and TFC were shown in acetone bark extract (219.06 ± 19.54 mg GAE g^{-1}) and hexane leaf extract (449.76 ± 28.89 mg QE g^{-1}) respectively whereas the highest DPPH radical scavenging activity and anti-diabetic activity were found to be in 100% ethanol bark extract (56.15 µg mL^{-1}) and hexane flower extract (92.5% inhibition of *α*-glucosidase) respectively. All parts (bark, leaves and flower) of this plant have higher potential for discovery of new drugs. Due to notable phenolic content, remarkable antioxidant and antidiabetic effects, the leaves and flower extracts of *J. mimosifolia* could be considered for further studies on preventing oxidative stress induced diseases and treating diabetes. Therefore, isolation and purification of phytochemicals like jacaranone and acteoside responsible for antioxidant and antidiabetic properties respectively from various parts of *J. mimosifolia* are recommended for discovery of new drug against oxidative stress induced diseases and diabetes.

Author Contributions

All authors have contributed equally to bring the manuscript in this form.

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