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Phytochemical analysis and antioxidant activity of *Prosopis Juliflora* thorn extract

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ABSTRACT

The bioactive principles of the plants have potent therapeutic value. Thus plants serve the humans and maintain their health. In the present study, the ethanolic and aqueous thorn extracts of *Prosopis juliflora* were assessed for antioxidant activity and nitric oxide scavenging activity. The phytochemical analysis revealed the presence of flavonoids, saponins, glycosides, terpenoids, coumarins in ethanolic and aqueous thorn extracts of *Prosopis juliflora*. At higher concentrations, aqueous thorn extracts of *Prosopis juliflora* exhibited significant antioxidant activity by DPPH (1, 1 diphenyl 1-2-picric hydrazine) radical scavenging method whereas ethanolic extracts of *Prosopis juliflora* thorns exhibited significant antioxidant activity by ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging method. Also the ethanolic thorn extracts of *Prosopis juliflora* showed a significant activity by Nitric oxide scavenging method. The presence of the secondary metabolites attributes the antioxidant activities.

Keywords: Antioxidant activity, Nitric oxide*, Prosopis juliflora,* Secondary metabolites, Phytochemical.

1. INTRODUCTION

Plants are environmental friendly clean factories that produce a wealth of novel and biologically active chemicals [1]. Several human diseases including cancer, diabetes, cardiovascular diseases and aging are caused due to free radical mediated oxidative damage. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases [2].The quality of life can be improved by intake of plant derived antioxidants, which helps in scavenging the free radicals. The antioxidant activities of medicinal plants may be - due to the presence of phenolic compounds, containing the hydroxyl groups that confers the hydrogen donating ability [3].The bioprospecting of plants will be helpful for many pharmaceutical firms.

Prosopis juliflora belong to the family Fabaceae and the Mimosoideae subfamily. *Prosopis juliflora* has soothing, astringent and antiseptic properties [4, 5]. It is also known as Mesquite and has been used to treat open wounds, eye problems, digestive problems and dermatological ailments. Aqueous extracts of Mesquite are antibacterial and has antibiotic activity.

Prosopis juliflora leaf extracts are richest source of secondary metabolites like alkaloids, phenolic compounds, flavonoids, glycosides, steroids, tannins and triterpenoids, these phytochemicals may help in protection against chronic diseases [6]. *Prosopis juliflora* containing a diverse group of secondary metabolites has a unique and multifactorial medicinal properties [7]. The aim of the present study is to screen the phytochemicals and analysis of the antioxidant and nitric oxide scavenging activity of the ethanolic and aqueous thorn extracts of *Prosopis juliflora*.

2. MATERIALS AND METHODS

2.1 Sample collection

The thorns of *Prosopis juliflora* were collected from Thirukalikundram, Kanchipuram District. The plants were identified by Dr.Sasikala Ethirajulu, Research Officer (Pharmacognosy) and Dr.S.Jega Jothi Pandian, Research Officer In charge, Siddha Central Research Institute, Arignar Anna Government Hospital Campus, Arumbakkam,Chennai-600106 (Central Council for Research in Siddha, Department of AYUSH, Ministry of Health & family welfare, Govt. of India). With fresh water, the thorns of *Prosopis juliflora* were thoroughly washed. Then the thorns were shade dried at room temperature and were powdered using pulverizer and used for extraction.

2.2 Preparation of Ethanolic & Aqueous Thorn Extracts

Ethanolic thorn extract: 20 grams of the powdered thorns of *Prosopis juliflora* was extracted successively with 250ml of ethanol using Soxhlet extractor for 15 refluxes. After complete extraction, the extract was condensed using rotary evaporator. The thorn extract was labelled and stored at 5°C for further use.

Aqueous thorn extract: 20 grams of the powdered thorns of *Prosopis juliflora* were soaked in 250 ml double distilled water and kept in orbital shaker for 24 hours in a closed Erlenmeyer flask for continuous agitation. The extract was then filtered using Whatmann No.1 filter paper. The solvent from the extract was removed by using rotary vacuum evaporator. The thorn extract obtained was labelled and stored at 5°C for further use.

2.3. Phytochemical Screening

The thorn extracts of *Prosopis juliflora* was qualitatively screened for the phytoconstituents using the standard methods [8, 9]. The phytochemicals like flavonoids, steroids, tannins, saponins, glycosides, alkaloids, terpenoids, anthraquinones and coumarins were screened.

2.4. DPPH Radical Scavenging Activity

DPPH (1, 1 diphenyl 1-2-picric hydrazine) radical scavenging activity was used for evaluating the antioxidant activity [10]. 0.1ml of methanol was taken in the control tube (C) and 0.1ml of varying concentrations (50 to 250 µg/ml) of ethanolic and aqueous thorn extracts of *Prosopis juliflora* were added in the tube (T) respectively. 2.0ml of 0.1mM of methanolic DPPH was added to all the tubes which include control, test and standard. Ascorbic acid was taken as standard. For 20 minutes, the tubes were incubated in dark and then were read at 517nm by spectrophotometer. By making use of the following formula, the percentage of inhibition was calculated and expressed as percent scavenging of DPPH radical [11]. The tests were repeated three times.

$$\% \text{ DPPH inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.5. ABTS free radical scavenging activity

The free radical scavenging activity was determined using ABTS radical cation 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) decolorization assay [12]. ABTS radical cation ($ABTS^{*+}$) was produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulphate (final concentration) and was stored in the dark at room temperature for 12-16 hours before use. $ABTS^{*+}$ solution was then diluted with absolute ethanol to obtain an absorbance of 0.70 (±0.02) at 734 nm. Reagent blank reading was taken (A_0). After addition of 2.0 ml of diluted $ABTS^{*+}$ solution ($A734\ nm$ = 0.70 (±0.02) to 20µl of varying concentrations (50 to 250µg/ml) of the ethanolic and aqueous thorn extracts, the absorbance was measured exactly 6 minutes after initial mixing (A_t). Appropriate solvent blanks were run in each assay. Ascorbic acid was taken as standard. All the tests were carried out in triplicates. Absorbance was measured at 734 nm. The scavenging activity was calculated as percentage inhibition using the formula.

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

Where, $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the sample(thorn extracts) at t = 6 min.

2.6. Nitric Oxide Radical Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH was measured by Griess IlIosvoy reaction [13, 14]. The reaction mixture of 3ml containing 2ml Sodium nitropruside (10mm) in 0.5ml Phosphate buffer saline (0.025 M, and pH 7.4) and 0.5ml of the ethanolic and aqueous thorn extracts of various concentrations (50 to 250µg/ml) was incubated at 25°C for 150minutes . A control experiment without the sample but with an equivalent amount of buffer was conducted in an identical manner. After incubation, 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% napthylethylene diamine dihydrochloride) was added. The same procedure was conducted with ascorbic acid which was used as standard. The absorbance of the chromophore formed was read at 546 nm. Percentage inhibition of nitric oxide scavenging activity was calculated using the formula. The tests were repeated for three times.

% inhibition

$$= \frac{\text{Absorbance of control- Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.7. Statistical Analysis

The samples were taken in triplicates and analyzed, the results were expressed as mean±standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

The ethanolic and aqueous thorn extracts of *Prosopis juliflora*, showed the presence of the phytochemicals like flavonoids, saponins, glycosides, terpenoids, coumarins. Steroids were present in the ethanolic thorn extracts of *Prosopis juliflora*. Tannins and alkaloids were present in the aqueous extracts of *Prosopis juliflora*. A study supports that *Prosopis juliflora* leaves showed the presence of tannins, glycosides, flavonoids and alkaloids in the preliminary phytochemical screening [15]. It was suggested in a study that *Prosopis juliflora* contained large amounts of the flavonoids in its heartwood [16]. The phytochemical analysis of the extracts revealed the presence of tannins, phenolics, flavonoids, alkaloids, terpenes and steroids in most parts of *Prosopis juliflora* [6]. A study suggested that the phytochemical analysis of the methanolic extracts of *Prosopis juliflora* showed the presence of alkaloids, flavonoids, glycosides, phenolics and tannins [17]. It was reported in a study that phytochemical screening of the *Prosopis juliflora* leaf extract showed the presence of alkaloids, flavonoids, steroids, phenolics and tannins [18]. A study revealed that leaf extracts of *Prosopis juliflora* Swartz DC., is a rich source of natural constituents, especially for flavonoids, alkaloids and saponins, signifying the importance of the plant as a potential candidate for deriving phytomedicines [19].

3.2 Antioxidant activity by DPPH radical scavenging method

It is one of most commonly used methods for determining the antioxidant activity of drugs derived from plants. Among the ethanolic and aqueous thorn extracts of *Prosopis juliflora*, the aqueous thorn extracts unveiled significantly higher values than its ethanolic thorn extracts. At 250µg/ml concentration of aqueous thorn extracts, the free radical scavenging potential was 83.81±0.14%. The presence of flavonoids, alkaloids and terpenoids may confer the antioxidant activity. Earlier studies suggest that the DPPH scavenging effect of the phenolic extracts of *Prosopis juliflora* leaves increased with the increasing concentration. Also it was mentioned that antioxidants have been the centre of focus in chronic disease prevention research [20]. It was reported in a study that *Prosopis juliflora* contained flavanols which was responsible for the strong antioxidant activities of crude extracts obtained [21]. According to a study the antioxidant activities of methanolic leaf extracts of *Prosopis juliflora* (Swartz) DC showed significant results [22]. Also in a study, it was reported that methanolic bark extract of *Prosopis juliflora* have a lot of chemical compounds with antioxidant properties which can inhibit the oxidative effect of free radicals [23]. It was revealed in a previous study that *Prosopis juliflora* roots exhibited higher antioxidant activity and leaves showed lower antioxidant activity by DPPH assay [24].

3.3 Antioxidant activity by ABTS radical scavenging method

At higher concentrations, the ethanolic thorn extracts of *Prosopis juliflora* exhibited significantly higher values than the aqueous thorn extracts. 87.17±0.39% of inhibition was obtained at 250µg/ml concentration of ethanolic thorn extracts of Prosopis juliflora. In a study, it was reported that in *Prosopis juliflora*, with the increase in concentration of bark extracts, both

Table 1: Phytochemical Analysis of *Prosopis Juliflora* Thorn Extracts

S.No.	Phytochemicals	Ethanolic Thorn Extract	Aqueous Thorn Extract
1	Flavonoids	+	+
2	Steroids	+	-
3	Tannins	-	+
4	Saponins	+	+
5	Glycosides	+	+
6	Alkaloids	-	+
7	Terpenoids	+	+
8	Anthraquinones	-	-
9	Coumarins	+	+

+ = Present - = Absent

Table 2: Percentage inhibition by DPPH Radical Scavenging Method

S.No	Concentration (µg/ml)	Ethanolic Thorn Extract of *Prosopis Juliflora*	Aqueous Thorn Extract of *Prosopis Juliflora*
1	50	55.48±0.26	78.50±0.48
2	100	58.70±0.62	79.70±0.27
3	150	61.67±0.52	81.57±0.51
4	200	63.66±0.41	82.78±0.26
5	250	65.67±0.57	83.81±0.14

Table 3: Percentage inhibition by ABTS Free Radical Scavenging Method

S.No	Concentration (Mg/Ml)	Ethanolic Thorn Extracts of *Prosopis Juliflora*	Aqueous Thorn Extract of *Prosopis Juliflora*
1	50	80.94±0.36	11.88±0.17
2	100	82.33±0.33	17.08±0.28
3	150	85.25±0.37	34.34±0.47
4	200	86.45±0.50	39.78±0.41
5	250	87.17±0.39	42.87±0.14

Table 4: Percentage Inhibition by Nitric Oxide Radical Scavenging Method

S.No	Concentration (Mg/Ml)	Ethanolic Thorn Extracts of *Prosopis Juliflora*	Aqueous Thorn Extracts of *Prosopis Juliflora*
1	50	14.32±0.42	9.21±0.48
2	100	18.17±0.43	15.62±0.53
3	150	22.44±0.33	17.26±0.42
4	200	24.09±0.36	20.16±0.52
5	250	32.10±0.49	27.35±0.76

antioxidant and pro-oxidant properties increased [23]. A study supports that *Prosopis cineraria* leaf extracts have the ability in effectively scavenging ABTS radicals revealing the strong radical scavenging potential of the leaves [25]. The percentage inhibition values of the methanol extract of *Prosopis Africana* leaves were higher with the ABTS•+ assay [26].

3.4 Nitric oxide radical scavenging method

Using nitric oxide radical scavenging method, the ethanolic thorn extracts of *Prosopis juliflora* revealed significantly higher values than its aqueous thorn extracts. At 250µg/ml concentration of ethanolic thorn extracts, 32.10±0.49 % inhibition was obtained. It was suggested in a study that *Prosopis juliflora* showed a moderate level of nitric oxide scavenging activity [27]. In a study, it was suggested that antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and the methanolic extract of stem bark of Prosopis Cineraria (Linn.) showed antioxidant activity by nitric oxide scavenging [28]. The results of a study revealed that methanolic and ethyl acetate extracts of *Prosopis cineraria* leaves exhibited maximum inhibition of nitric oxide [25]. It was reported in a study that nitric oxide scavenging activity can be useful for the management of diseases in which radical nitrite is directly involved [29]. A study suggests that medicinal plants might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO [30].

5. CONCLUSION

Plants play a prominent role in day to day life. Plant derived antioxidants help in preventing and slowing the onset of degenerative diseases. Therefore plants are important source of medicinal agents. The phytochemical analysis of ethanolic and aqueous thorn extracts of *Prosopis juliflora* reveals the presence of flavonoids, saponins, glycosides, terpenoids, coumarins. At higher concentrations, the aqueous thorn extracts of *Prosopis juliflora* exhibited significant antioxidant activity by DPPH radical scavenging method. And ethanolic extracts of *Prosopis juliflora* thorns exhibited significant antioxidant activity by ABTS method and Nitric oxide scavenging method. The presence of phytochemical constituents might have contributed to the antioxidant and nitric oxide radical scavenging activity. The bioactive metabolites present in the plants might serve as leads for development of newer drugs which will be helpful for the treatment of oxidative stress generated diseases. Hence these compounds can be isolated and used as an alternate source for synthetic drugs.

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Conflicts of Interest

There are no conflicts of interest.

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