

Antioxidant activity and phytochemical screening of *Prosopis juliflora* leaves extract

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Accepted 26 July, 2017

ABSTRACT

P. juliflora belongs to the family Fabaceae and is commonly known as mesquite. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat, and in the healing of wounds. Its antibacterial, antioxidant, antifungal, antitumor, and anthelmintic activities are also reported and it contains phytochemicals such as flavonols, alkaloids, tannins, ellagic acid, glycosides, steroids, and various phenolic compounds. It is reported to be used as feed for cattle worldwide. The present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay) and Preliminary phytochemical screening of ethanol extract of *P. juliflora*. The ethanolic leaves extract of *P. juliflora* was screened for antioxidant screening using 2,2-Di (4-tert-octylPhenyl)-1-Picryl-Hydrazyl (DPPH), compared with p. galate as standard antioxidant. The tested antioxidant activity gave (61.55 ± 1.02 RSA %) in comparison to the control of propyl galate levels (88 ± 0.07 RSA %) and the preliminary phytochemical screening of the leaves revealed that the plant contains Alkaloids, Flavonoids, Saponins, Tannins, Anthraquinone Glycoside and Coumarins.

Keywords: *In-vitro*, antioxidant, phytochemical screening, *Prosopis juliflora*.

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INTRODUCTION

The genus *Prosopis* belongs to the family Leguminosae (Mimosaceae). About 45 species are known uses in folklore medicine (Saidman and Vilardi, 1987; Nadeem, 1992). It is an important species because of its high nitrogen fixing potential in very dry areas and in drought seasons and also it provides shelter and food to many species of animals on its nectar, pollen, leaves and fruits (Almaraz-Abarca et al., 2007), *P. juliflora* are rich source of piperidine alkaloids. Many alkaloids such as juliflorine, julifloricine and julifloridine (Ahmad et al., 1978), juliprosine (Daetwyler et al., 1981), juliprosinene and juliflorinine (Ahmad et al., 1989), 3'-oxojuliprosopine, sceojuliprosopino I, 3-oxojuliprosine and 3'-oxojuliprosine (Nakano et al., 2004) have been isolated from leaves and have proven to be pharmacologically active (Ahmad et al., 1989; Aqeel et al., 1989), *P. juliflora* is used as a traditional medicine on different continents for

curing catarrh, colds, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat, and for the healing of wounds (Hartwell, 1971). Its juice is used in folk remedies for the cancerous condition. Pain and inflammation are common complaints in many patients suffering from acute conditions (Vikrant and Arya, 2001). Anti-inflammatory agents inhibit the synthesis of prostaglandin synthesis which is one of the most important mediators of inflammation. Other mechanism of anti inflammatory activity the stabilization of lysosomal membrane in leucocytes (lysosomal enzymes destroy cartilage and other issues and perpetuate inflammation) and antagonism of certain actions of bradykinin (Tripathi, 1994). The aim of the present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay) and preliminary phytochemical screening of ethanol extract of *Prosopis juliflora*.

MATERIALS AND METHODS

Plant materials

The *Prosopis juliflora* (leaves) were collected from Elsunut Forest Khartoum State Sudan. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute. The *P. juliflora* (leave) were air-dried, under the shadow with good ventilation for three days and then ground finely in a mill to powder for extracts preparation.

Preparation of crude extracts

Extraction was carried out according to the method described by Harbone (1984). Occasionally 50 g were macerated in 250 ml of ethanol at room temperature with occasional shaking for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was determined (% of dry weight) and stored at -20°C for further analysis in tightly sealed glass vial.

Antioxidant activity of plant extracts

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Shimada et al. (1992) with some modification. In 96-wells plate, the test samples were allowed to react with (DPPH) for half an hour at 37°C. The working concentration of DPPH was 300 µM. The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and P.Gallate. All tests and analysis were run in triplicate.

Preparation of the extracts

In this study the preliminary phytochemical screening was conducted by Haborne (1984). 10 g of the plant powder was soaked in 100 ml of ethanol for about twenty four hours at room temperature. The extract was filtered through filter paper and solvent was evaporated under reduced pressure using rotary evaporator apparatus. This extract was used for phytochemical screening.

Phytochemical screening of *Prosopis juliflora* (leaves)

Test for unsaturated sterols and triterpenes

10 ml of the prepared extract (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample (Harbone, 1984).

Test for alkaloids

Aliquot of 7.5 ml of PE was evaporated to dryness on a water bath.

Thereafter, 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 min, then was cooled, was filtered and was divided into two test tubes. To one test tube few drops of Mayer's reagent (Lab tech- Sudan) were added, while to the other tube few drops of Valser's reagent (Lab tech - Sudan) were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harbone, 1984).

Test for flavonoids

Aliquot of 17.5 ml of the PE was evaporated to dryness on a water bath, was cooled and the residue was defatted with petroleum ether. The defatted residue was dissolved in 30 ml of methanol (80%) and was filtered. The filtrate was used for the following test: To three ml of the filtrate in a test tube was added 1 ml of 1% aluminum chloride (methanolic solution). Formation of yellow color indicated the presence of flavonoids (Harbone, 1984).

Test for tannins

Five ml of saline solution (following n-hexane application on the prepared extract) was treated with few drops of gelatin salt reagent (Lab tech - Sudan). Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for saponins

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 s. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of saponins.

Test for anthraquinone glycosides

10 g of the powdered plant sample were boiled with 10 ml of 0.5 N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline solution was found to have assumed pink or red color.

Test for coumarins

3 g of the original powdered plant sample was boiled with 20 ml of distilled water in a test tube and filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5 N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarins was indicated if the spot has found to be absorbed the UV light.

Statistical analysis

All data were presented as means ± standard deviation (SD). Statistical analysis for all the assays results were done using Microsoft Excel program (2010).

RESULTS AND DISCUSSION

As shown in Table 1, the yield (22.1%) results from antioxidant activity of *P. juliflora* (leaves) showed moderate antioxidant activity against the DPPH free radical (61.55 ± 1.02 RSA%) in comparison to the control of propyl gallate levels (88 ± 0.07 RSA%) was used as standard drug level.

Phytochemical analysis of *Prosopis juliflora* (leaves)

The phytochemical analysis of crude ethanolic extract of *P. juliflora* (leaves) performed by the method described earlier and then analyzed for phytochemicals like

steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed, showed the presence of alkaloids, anthocyanins, flavonoids, polyphenols, saponins and tannins except saponins and Unsaturated Sterol And/or Triterpenes were absenced (Table 2).

Nowadays antioxidants had been the centre of focus in chronic disease prevention research. Phytochemical study on *P. juliflora* has been earlier reported (Rastogi and Mehrotra, 1993) certain important metabolites are shown to be present, not only in the leaves, but also in other plant parts, such as tannin reported in root, alkaloids reported in leaves, are also evident in flower and pod. flavonoids are present in most part of the plant, as mentioned in earlier reports (Ahmad, 1989b).

Table 1. Antioxidant activity of *Prosopis juliflora* (leaves) extract.

No.	Name of plant	Yield (%)	Part	%RSA* \pm SD (DPPH)
1	<i>Prosopis juliflora</i>	22.1	Leaves	61.55 ± 1.02
2	*Control		PG	88 ± 0.07

Key: RSA* = Radicals scavenging activity, *Control = P.G = propyl galate.

Table 2. Phytochemical analysis of *Prosopis juliflora* (leaves).

No.	Tested	<i>P. juliflora</i> (leaves)
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	-
4	Tannins	+
5	Anthraquinone glycoside	+
6	Coumarins	+
7	Unsaturated Sterol and/or Triterpenes	-

+ = Present; - = Absent.

CONCLUSION

The present study suggests that qualitative phytochemical screening of ethanolic extract of *P. juliflora* L. supports the presence of bioactive compounds such as flavonoids, alkaloids, tannins, phenolic compounds, and saponins in the medicinal plant and thus responsible for the antioxidant activities. The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents.

ACKNOWLEDGEMENTS

The authors are grateful to Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI),

National Center for Research, Khartoum, Sudan and Department of Microbiology, Faculty of Medical Laboratory Sciences, International University of Africa, Khartoum, Sudan.

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Citation: Badri AM, Garbi MI, Gmaraldeen SM, Magzoub AA, Ibrahim IT, Saleh MS, Kabbashi AS, Mohamed SG, 2017. Antioxidant activity and phytochemical screening of *Prosopis juliflora* leaves extract. *Adv Med Plant Res*, 5(3): 37-40.
