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In-Vitro antioxidant potential of *Jasminum mesnyi Hance* (Leaves) extracts

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ABSTRACT

Antioxidant activity of 90% methanol and aqueous extracts of Jasminum mesnyi leaves and total phenolic and flavonoids contents were investigated for free and peroxyl radical scavenging activity, reducing capacity by adopting in vitro models. DPPH scavenging activity showed IC_{50} of 90% methanol and aqueous extracts (25.27±0.6 µg/ml and 71.84±0.06 µg/ml respectively) when compared with ascorbic acid and rutin (8.84±0.05 and 3.78±0.153 µg/ml respectively). The reducing power of extracts increased in concentration dependent manner in FRAP method. The IC_{50} value of lipid peroxidation assay for 90% methanol, aqueous extracts and BHT were 84.69±0.008 µg/ml, 145.62±0.007 µg/ml, and 48.89±0.01 µg/ml respectively. 90% methanol extract of J. mesnyi leaves have more antioxidant potential in comparison to the aqueous extract and are at par with standard antioxidants in all assay techniques. The results obtained in the present study indicate that the leaves of J. mesnyi are a potential source of natural antioxidants.

Keywords: Antioxidant, DPPH, FRAP, Jasminum mesnyi, TBARS

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INTRODUCTION

The term 'antioxidant' refers to the activity of numerous vitamins, minerals, and other phytochemicals to protect against the damage caused by ROS. By their ability to react with and damage many structures in the body, ROS are involved in various physiological processes and diseases such as ageing, cancer, diabetes and atherosclerosis etc. Several studies have demonstrated that plant(s) produce potent antioxidants [1].

Jasminum mesnyi Hance (Jasminum primulinum Hemsley) also known as "Primrose Jasmine" or "Japanese Jasmine" is found in tropical, sub-tropical and warm temperate regions of Asia. It is an open evergreen, rambling shrub with long and slender arching stems that climb like a sprawling vine if given support. Leaves are opposite, trifoliolate, and attached at base of branchlets. Flowers are usually solitary, axillary or rarely terminal, yellow coloured, having 6-10 petals arranged in a semidouble whorl [2-4].

Leaves are reported to have secoiridoids (jasmoside, jasmesoside, 9"-hydroxyjasmesoside, 9"- hydroxyjasmesosidic acid, jasminin 10"-O-β-D-glucoside, 2"-hydroxyjasminin, isojasminin, jasminin, 4"-hydroxyisojasminin and jasmosidic acid), a phenolic glucoside (syringin) and rutin [5-9].

There is no reported literature about the ethnobotanical uses & antioxidant potential of Jasminum mesnyi. Studies have shown that Jasminum sambac (related member of Jasminum) has a strong antioxidant activity [10] which forms the base for the conduction of antioxidant activity on Jasminum mesnyi.

MATERIAL AND METHODS

Plant material

Jasminum mesnyi was collected in its flowering phase in the month of February, 2008 from Solan, Himachal Pradesh, India and was authenticated by Dr. H.B. Singh; Director, Department of Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. NISCAIR/RHMD/Consult/-2008-09/1048/79). The leaves were separated, shade dried, powdered and stored in an air tight container till use.

Extraction

Coarsely powdered leaves (100 g) were extracted with petroleum ether (60°-80°C) to remove fat and dried marc was further extracted with 90% methanol using Soxhlet apparatus. The 90% methanol extract so obtained was freed from solvent in a vacuum evaporator (Equitron Roteva, Mumbai). Dried marc was re-extracted three times with distilled water at room temperature for 24 h and the aqueous extract was dried by rotary evaporator. Both the extracts were kept in desiccator till further use.

Quantitative estimation and screening for antioxidant activity

Chemicals and reagents

DPPH, gallic acid, Folin Ciocalteu's reagent and L - ascorbic acid were obtained from Sigma–Aldrich Co., St. Louis, USA. Rutin was obtained from Acros Organics, NJ, USA. TBA, TCA, potassium ferricyanide, potassium chloride and ferric chloride were purchased from CDH, New Delhi, India. BHT was purchased from Rankem, New Delhi, India. The other chemicals and solvents used in this experiment were of the analytical grade.

Estimation of Total Phenolic Compounds

1 g of sample (leaves powder) was extracted three times with 15 ml of 50% methanol by maceration of 2 hours, then filtered and the final volume was made with 50% methanol up to 50 ml. Gallic acid was weighed (10 mg) and dissolved in 100 ml of 50% methanol to get 100 µg/ml stock solution. Lower concentrations of gallic acid (1, 2, 4, 6, 8 and 10 µg/ml) were prepared by serially diluting stock solution.

Sample and various concentrations of standard (1ml each) were taken in test tubes, diluted with 10 ml of distilled water, 1.5 ml Folin Ciocalteu's reagent was added and was kept at room temperature for 5 min to which 4 ml of Na_2CO_3 (20% w/v) was added, the final volume adjusted to 25 ml with distilled water, then agitated and allowed to stand for 30 min at room temperature Absorbance was measured at 765 nm for three parallel determinations. Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as µg gallic acid equivalents (GAE) and percentage w/w [11].

$$\textbf{Total phenolic contents (\%)} = GAE \times V \times D \times 10^{-6} \times 100/W$$

GAE - Gallic acid equivalent (µg/ml); V - Total volume of sample (ml); D - Dilution factor; W - Sample weight (g)

Total Flavonoids Content

90% methanol extract (1g) was dissolved in 25 ml of 80% methanol. 10 mg of rutin was dissolved in 100 ml of 80% methanol (100 µg/ml). Lower concentrations of rutin (10, 20, 40, 60, 80 and 100 µg/ml) were prepared by serially diluting stock solution.

Sample and various concentrations of standard (0.5 ml each) were mixed with 1.5 ml of 95% methanol separately. 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were added. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer (Shimadzu, Japan). Quantification was done on the basis of a standard curve of rutin. Results were expressed as µg rutin equivalents (RE) and percentage w/w [12].

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$$\text{Flavonoid content (\%)} = RE \times V \times D \times 10^{-6} \times 100/W$$

RE - Rutin equivalent (µg/ml); V - Total volume of sample (ml); D - Dilution factor; W - Sample weight (g)

DPPH Radical Scavenging Activity

90% methanol and aqueous extracts were weighed (50 mg each) and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions separately. Lower concentrations (10, 20, 40, 80, 160 µg/ml) of methanol and aqueous extracts were prepared by serially diluting stock solutions. Ascorbic acid and rutin were weighed (50 mg each) and dissolved in 500 ml of methanol to get 500 µg/ml stock solutions separately. Lower concentrations of ascorbic acid and rutin (5, 10, 15, 20, 25 µg/ml and 2, 4, 6, 8, 10 µg/ml respectively) were prepared by serially diluting stock solutions.

The stable DPPH radical was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extracts were added at an equal volume to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula

$$\%\text{Radicle Scavenging Power} = \frac{\text{Absorbance [Control - (Sample-Blank)]}}{\text{Absorbance of Control}} \times 100$$

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of J. mesnyi extracts, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The measurements were triplicated and their scavenging effect was calculated based on the percentage of DPPH scavenged [13-14].

Ferric Reducing Antioxidant Power (FRAP) Assay

90% methanol and aqueous extracts were weighed (50 mg each) and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions separately. Lower concentrations (25, 50, 100, 200, 400 µg/ml) of methanol and aqueous extracts were prepared by serially diluting stock solutions. Ascorbic acid was weighed (50 mg) and dissolved in 500 ml of methanol to get 500 µg/ml stock solutions Lower concentrations of ascorbic acid (25, 50, 100, 200, 400 µg/ml) were prepared by serially diluting stock solution.

Various concentrations of sample and standard solutions (1ml each), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were mixed separately and

allowed to incubate at 50 º C for 30 min and 2.5 ml of 10% TCA was added to the mixtures and centrifuged for 10 min at 3000 rpm. About 2.5 ml of the supernatant was diluted with 2.5 ml water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. All tests were performed in triplicate and the graph was plotted with the average of the three determinations [10].

***In-Vitro* Lipid Peroxidation Method**

90% methanol and aqueous extracts were weighed (50 mg each) and dissolved in 100 ml of methanol to get 500 µg/ml stock solutions separately. Lower concentrations (25, 50, 100, 200, 400 µg/ml) of methanol and aqueous extracts were prepared by serially diluting stock solutions. Similarly BHT stock solution (500 µg/ml) was prepared (50 mg BHT in 500 ml methanol). Lower concentrations (25, 50, 100, 200, 400 µg/ml) of BHT were prepared by serially diluting stock dilution.

Normal male rats (250 g) were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared with homogenizer at 0-4°C with 0.15M KCl. The homogenate was centrifuged at 8,000 rpm for 15 min, and clear cell-free supernatant was used for the study with in vitro lipid peroxidation assay. Different concentrations (25-400 µg/ml) of extracts (1ml) were added in test tubes and 1 ml of 0.15 M KCl and 0.5 ml of rat liver homogenates were added. Peroxidation was initiated by adding 100 µl of 0.2 mM ferric chloride. After incubation at 37°C for 30 min, the reaction was stopped by adding 2 ml of ice-cold HCl (0.25 N) containing 15% TCA, 0.38% TBA, and 0.5% BHT. The reaction mixtures were heated at 80°C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm.

The percentage inhibition of lipid peroxidation is calculated by the formula:

Inhibition of lipid peroxidation (%) = 1 – (sample OD/blank OD) × 100

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations of J. mesnyi extracts, which is defined as the total antioxidant necessary to inhibit lipid peroxidation by 50 %. The measurements were triplicated and their scavenging effect was calculated based on the percentage of inhibition of lipid peroxidation [15].

Statistical analysis

Results were reported as Mean ± S.D. and data were tested by one-way analysis of variance. $p^* < 0.05$ regarded as significant.

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RESULTS

Total Phenolic Compounds

The total phenolic compounds estimation from leaves of J. mesnyi showed the absorbance of 2.335 at 765 nm wavelength and phenolic contents was found out to be 2.86 % w/w of the drug.

Total Flavonoids Content

The total flavonoids content estimation from 90% methanol extract of leaves of J. mesnyi showed the absorbance of 2.613 at 415 nm wavelength and flavonoids content was found out to be 8.118% w/w of the extract.

DPPH Radical Scavenging Activity

The 90% methanol and aqueous extracts of the J. mesnyi leaves showed promising free radical scavenging effect of DPPH in a concentration dependent manner. The 90% methanol extract showed better scavenging activity than the aqueous extract (table 1). Ascorbic acid and rutin were used as the reference standards.

Table 1: 50% inhibition (IC_{50}) for extracts of J. mesnyi by DPPH method

S. no.	Extract	IC_{50} (µg/ml)±S.D.
1.	Rutin	3.78±0.153
2.	Ascorbic Acid	8.84±0.05
3.	90% Methanol Extract	$25.27±0.6^{a,b}$
4.	Aqueous Extract	$71.84±0.06^{a,b,c}$
Values are means ± SD of three determinations. IC_{50} values were determined by interpolations. a = p< 0.05 vs rutin; b = p< 0.05 vs Ascorbic Acid; c = p< 0.05 vs 90% Methanol Extract		

Ferric reducing antioxidant power assay

The reducing power of 90% methanol and aqueous extracts of the J. mesnyi leaves were found to increase in concentration dependent manner up to 400 µg/ml the values were remained lower compared to the ascorbic acid. The 90% methanol extract showed higher reducing power than the aqueous extracts but less than ascorbic acid (standard) (table 2).

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Table 2: Total reducing power of extracts of J. mesnyi leaves

Conc. (µg/ml)	Absorbance		
	Ascorbic Acid	90% methanol extract	Aqueous extract
25	0.078	0.064	0.051
50	0.355	0.182	0.095
100	0.638	0.458	0.343
200	1.374	0.824	0.612
400	2.852	1.518	1.115

In-Vitro Lipid peroxidation method

The 90% methanol and aqueous extracts showed anti-lipid peroxidation activities, which are lower than BHT. The percentage antioxidant activity of 90% methanol, aqueous extracts and BHT increased in concentration dependant manner. These results were also expressed as the dose required to obtain 50% antioxidant index (table 3).

Table 3: 50% inhibition (IC_{50}) for extract of J. mesnyi by TBARS method

S. No.	Extract	IC50 (µg/ml)±S.D.
1.	BHT	48.89±0.01
2.	90% Methanolic Extract	84.69±0.008[a]
3.	Aqueous Extract	145.62±0.007[a,b]
Values are means ± SD of three determinations. IC_{50} values were determined by interpolations. a = p< 0.05 vs BHT; b = p< 0.05 vs 90% Methanol Extract		

DISCUSSION

Total Phenolic Compounds

The total phenolic contents in the leaves of J. mesnyi were determined by using Folin Ciocalteu's method. The sample extract dilution was oxidized with Folin Ciocalteu reagent and the absorbance of the resulting blue colour was measured at 765 nm after 30 min [16]. The result of Folin Ciocalteu's method revealed the presence of total phenolic contents 2.86% w/w which was not reported earlier. Phenolic constituents are very important in the plant because of their scavenging ability due to their hydroxyl groups. Phenolic compounds are famous powerful chain breaking antioxidants and has been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation [17].

Total Flavonoids Content

The aluminium chloride colorimetric method uses wavelength scan of the complexes of the sample and standard with aluminum chloride showed that the complexes formed by flavonoids (rutin) with C-3 or C-5 hydroxyl group [12] revealing total flavonoids content in 90% methanol extract 8.118% w/w which was not reported earlier.

DPPH Radical Scavenging Activity

DPPH is stable nitrogen centered free radical which can be effectively scavenged by antioxidants and shows strong absorbance at 517 nm. The change in absorbance of DPPH radical caused by the extracts was due to the reaction between the antioxidant molecules and the extracts, which resulted in the scavenging of the radical by hydrogen donation. It was visually noticeable as a discoloration from purple to yellow. Extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} values [18]. The 90% methanol and the aqueous extract showed significant DPPH scavenging activity (IC_{50}=25.27±0.6 µg/ml and 71.84±0.06 µg/ml) respectively when compared with the IC_{50} values of the standards ascorbic acid and rutin (IC_{50}=8.84±0.05 and 3.78±0.153 µg/ml respectively). Our observation revealed the 90% methanol extract has higher DPPH scavenging activity in comparison to aqueous extract.

Ferric Reducing Antioxidant Power Assay

The reductive capability of the 90% methanol extract is more prominent than aqueous extract with reference to ascorbic acid. For the measurement of the reductive ability, we investigated the ferric (Fe^{3+}) - ferrous (Fe^{2+}) transformation in the presence of the extracts was investigated [10]. In this method, the extracts form a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride that was measured at 700 nm. The reducing capacity of the extracts may serve as the antioxidant activity, the reducing power of the extracts increased with increasing the concentration. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. The antioxidant activity can be attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and free radical scavenging [17].

In-Vitro Lipid Peroxidation Method

The methanol and aqueous extracts were tested to be effective in reducing the production of thiobarbituric acid reducing substances (TBARS) in a dose-dependent manner, thus allowing calculation of the concentration that would inhibit the TBARS production by 50% (i.e. the IC_{50}). Thus the decrease in the MDA levels in the presence of increased concentration of extracts indicates their role as antioxidants. TBARS assay was used to determine the anti lipid peroxidation properties of the methanol and the aqueous extract. Thus the methanol and the aqueous extract inhibit the initiation of lipid peroxidation by scavenging the free radicals that

form alkyl peroxyl and alkoxyl radicals or can donate hydrogen atom to alkyl peroxyl and alkoxyl radicals and thus stop chain propagation [19]. These observations also support our finding that 90% methanol extract of J. mesnyi leaves have more antioxidant potential in comparison to the aqueous extract and are at par with standard antioxidants.

CONCLUSION

The results of the study clearly indicate that 90% methanol extract of J. mesnyi leaves possess in vitro antioxidant activity. The encouraging results of J. mesnyi leaves with the various in vitro antioxidant tests proved the plant as a reducing agent and effectiveness as scavengers of free radicals. Hence, it is worthwhile to isolate and elucidate the bioactive principles that are responsible for the antioxidant activity that is underway.

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ABBREVIATIONS

BHT - Butylhydroxytoluene, DPPH - 1, 1-Diphenyl-2-picrylhydrazyl, FRAP - Ferric Reducing Antioxidant Power, IC_{50} - 50% Inhibitory Concentration, MDA- Malondialdehyde, ROS - reactive oxygen species, rpm - Rotation per minute, TBA - Thiobarbituric acid, TBARS - Thiobarbituric Acid Reducing Substance, TCA - trichloroacetic acid, UV – Ultraviolet

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