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In vitro evaluation of antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* Leaves

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ABSTRACT

Objective: To evaluate *in vitro* antimicrobial and antioxidant activities of methanolic extract of *Jasminum humile* (*J. humile*) leaves extract. **Method:** Methanolic extract of *J. humile* was evaluated for its antimicrobial activity by using agar well diffusion method & their possible antioxidant assay by two complementary test systems, namely DPPH and hydrogen peroxide scavenging activity. These various antioxidant activities were compared to standard antioxidants such as ascorbic acid for both the tests. **Results:** In the DPPH & hydrogen peroxide scavenging activity, the IC_{50} value of methanol extract was 70.43 μg/mL & 60.79 μg/mL respectively. Further, the extract showed inhibitory activity for Gram-positive and negative bacteria at different concentrations. The maximum antibacterial activity of extract was exhibited against *Staphylococcus aureus* (*S. aureus*) at concentration 50 mg/mL when compared with ciprofloxacin **Conclusions:** These results clearly indicate that *J. humile* is effective in scavenging free radicals and has the potential to be a powerful antioxidant. Thus, the results obtained in the present study indicate that *J. humile* leaves extract could be considered as a potential source of natural antioxidants and that could be used as an effective source against bacterial diseases.

1. Introduction

In last decade, there is an increasing interest in researches for production of biologically active compounds from natural sources. Bioactive compounds are remarkable due to prevention and/or treatment of diseases such as cardiovascular diseases and certain cancer types. In the recent years, the antimicrobial and antioxidant actions have received much attention. The antioxidant may be useful in retarding oxidative deterioration of food materials especially those with high lipid content. It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species[1-9]. Reactive oxygen species, causing damage to DNA, proteins and lipids, have been associated with carcinogenesis, coronary heart disease, and many other health problems[10]. Minimizing oxidative damage may well be one of the most important approaches to the primary prevention of these oxidative stress-related diseases and health problems, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions[11]. Plants are the primary sources of naturally occurring antioxidants for humans. The natural antimicrobial agents also protect living organisms from damages resulting in the prevention of various diseases. Although, much work has been done on the antimicrobial and antioxidant effects of different plants species but after that the current study is an attempt to determine the antioxidant and antibacterial activity of the leaves extract of *Jasminum humile* (*J. humile*) against the resistant clinical isolates of *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Streptococcus faecalis* (*S. faecalis*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).

J. humile (Yellow Jasmine, Peeli chameli) (Family: Oleaceae) is a small erect much-branched shrub, growing to 1 m or more tall, commonly found in the Himalayan region. It has green, angular branches. Leaves are pinnate with 3-7 ovate to lance like leathery leaflets. The last leaflet is somewhat larger. Inflorescences are lax clusters of yellow tubular flowers at the end of branches. Yellow

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jasmine contains indole alkaloids (including gelsemine and gelsedine), iridoids, coumarins, and tannins.

2. Materials and methods

2.1. Plant materials

Leaves of *J. humile* were collected from National Park, Yamunanagar and authenticated from NISCAIR, (Ref. NISCAIR/RHMD/Consult/2010-11/1679/277), New Delhi. They were air dried & pulverized (coarse power).

2.2. Preparation of the methanol extract

The dried leaf powdered (500 g) were extracted with 1 L of methanol using a Soxhlet extractor for 7 h at a temperature (64 ℃) not exceeding the boiling point of the solvent. The extract was filtrated using Whatman filter paper (No. 1) and then concentrated in vacuo at 40 ℃ using a rotary evaporator. The residues obtained were stored in freezer at −80 ℃ until further tests.

2.3. Chemicals & instruments

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, ascorbic acid and were obtained from Sigma- Aldrich, USA. All other chemicals and reagents used were of analytical grade. UV-Visible spectrophotometer (Shimadzu, Japan).

2.4. Antioxidant activity

2.4.1. DPPH(1,1-diphenyl-2-picryl-hydrazy) radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by Nickavar *et al*[12]. Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 10, 20, 40, 60, 80 and 100 μg/mL in methanol. 1 mL of a 0.3 mmol/L DPPH methanol solution was added to 2.5 mL solution of the extract or standard and allowed to react in dark at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity and the percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in μg/mL) of extracts that inhibits the formation of DPPH radicals by 50%. Solution of ascorbic acid served as positive control.

2.4.2. Scavenging of hydrogen peroxide

Different concentrations of plant extract and standard ascorbic acid solution *viz*. 10, 20, 40, 60, 80 and 100 μg/mL in methanol (1 mL) where added to hydrogen peroxide solution (2 mL). A solution of hydrogen peroxide (20 mmol/L) was prepared in phosphate buffer saline (pH 7.4). The percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard. The antioxidant activity of the extract was expressed as IC_{50}[13]. Solution of ascorbic acid served as positive control

2.5. Assay for antibacterial activity using agar well diffusion method

The screening of antibacterial activity of plant extracts was carried out using the agar well diffusion method as described by Lino and Deogracious[14].

2.5.1. Prepration of working stock

The Working stock was prepared as 1ml of each bacterial strain was initially inoculated in 100 mL of sterile nutrient broth and incubated for (37±1) ℃ for 24 h respectively. The 0.5 mL of the each test organisms from the working stock were seeded onto Mueller Hinton agar (HiMedia) plate and uniformly spread with a spreader and cooled to 48 ℃ to 50 ℃. When the nutrient agar medium solidifies, wells (holes) of uniform diameter (7 mm) were made using sterile sterile cork borer.

2.5.2. Agar-well diffusion method

Agar well bioassay was employed for testing antibacterial activity of *J. humile* leaves. The final concentration of leaves extract (10, 20, 30, 40, & 50 mg/mL) were dissolved in 0.25% dimethyl sulphoxide (DMSO, Merck). The different concentration of leaves extracts, ciprofloxacin (standard drug), DMSO (vehicle control) & 75% methanol (negative control) were introduced (0.2 mL) into the wells with the help of micropipettes separately under aseptic condition. The plates were then maintained at room temperature for 1 h to allow the diffusion of the solution into the medium. All the bacterial plates were then incubated in upright position at (37±1) ℃ for 24 h. After 24 h, the antibacterial activity was determined by measuring the diameter of the inhibition zone [cm, including the diameter of the bore (7 mm). The antibacterial assay for each of the extracts against all microorganisms tested was performed in triplicates.

2.5.3. Determinatiion of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth[15,16]. The MIC was determined by agar well dilution methods. The leaves extract concentration of 10 mg/mL was further serially diluted (0.5, 1, 2, 4, 6, and 8 mg/mL) were assayed against the test bacteria. MIC was taken as the highest dilution (least concentration) of extract or drug showing no detectable growth.

2.6. Statistical analysis

Data were expressed as mean±SEM. Linear regression analysis was used to calculate the IC_{50} value. Student's *t*-test was used for the comparison between two means for

the possible significant interrelation. Data were considered statistically significant only when P value < 0.05.

3. Result

3.1. DPPH radical scavenging activity

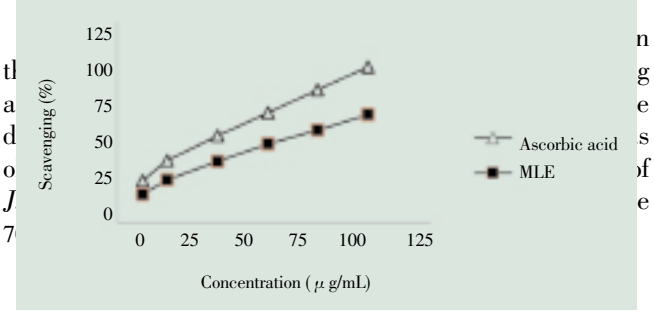

Figure 1. DPPH radical scavenging activity of *J. humile*.

3.2. Scavenging of hydrogen peroxide

Figure 2 reveals that a significant (P<0.05) dose dependent response was found in the hydrogen peroxide scavenging activity in *J. humile* leaves extract. Maximum scavenging activity (67.01%) was observed at 100 μg/mL concentration and the IC_{50} value of *J. humile* leaves extract and ascorbic acid were found to be 60.79 μg/mL and 38.84 μg/mL respectively (Table 1).

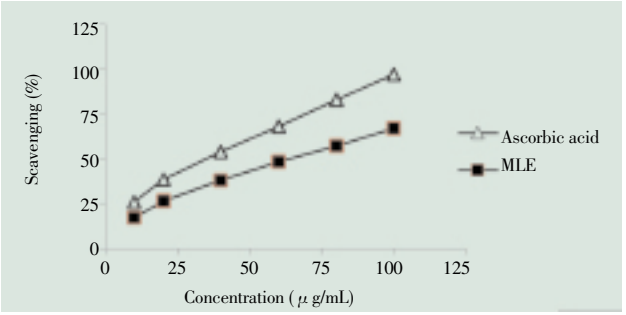

Figure 2. Hydrogen peroxide radical scavenging activity of *J. humile*.

3.3. Antimicrobial activity

Antibacterial activity of the extracts was recorded when the zone of inhibition was greater than 6 mm[15]. Results of the antibacterial screening of methanol extracts of *J. humile* revealed significant antibacterial activity against all tested bacterial strains (3 Gram-positive and 2 Gram-negative bacteria) at different concentrations (Table 2). But the maximum antibacterial activity (3.7 cm zone of inhibition) of extract was exhibited against *S. aureus* at concentration 50 mg/mL when compared with ciprofloxacin. Ciprofloxacin, which was used as a positive experimental control against all bacterial strains assayed, produced a zone of inhibition between 2.8 to 4.3 cm, while no inhibitory effect could be observed for DMSO used as negative control. The methanol extract of *J. humile* leaves also showed significant antimicrobial activity against all tested bacterial organisms but dose dependent.

Table 1
Antioxidant activity of methanolic extract of *J. humile* leaves.

Concentration (μ g/mL)	DPPH radical scavenging activity (% inhibition)		Hydrogen peroxide scavenging activity (% inhibition)	
	Ascorbic acid	MLEJ	Ascorbic acid	MLEJ
10	29.43	18.37	26.22	17.39
20	39.78	25.84	38.73	26.49
40	49.09	33.98	53.71	37.41
60	63.46	45.36	68.34	48.49
80	76.30	54.52	76.60	57.24
100	85.93	63.72	82.75	67.01
IC_{50}	40.24	70.43	38.84	60.79

MLEJ = Methanolic leaves extract of *J. humile*.

Table 2
Evaluation of antibacterial activity of the methanolic leaf extract of *J. humile*.

	Concentration (mg/mL)	Zone of inhibition (cm, diameter)				
		S. aureus (G^+)	*B. subtilis* (G^+)	*S. faecalis* (G^+)	*E. coli* (G^-)	*P. aeruginosa* (G^-)
MLEJ	10	2.1	1.9	2.0	1.7	1.9
	20	2.5	2.2	2.2	2.1	2.1
	30	2.9	2.6	2.7	2.2	2.2
	40	3.3	2.9	3.0	2.3	2.5
	50	3.7	3.1	3.3	2.6	2.7
Ciprofloxacin	10	3.7	3.0	3.2	3.1	2.8
	20	3.9	3.1	3.3	3.2	3.1
	30	4.1	3.3	3.5	3.4	3.2
	40	4.2	3.5	3.7	3.5	3.4
	50	4.3	3.7	4.0	3.6	3.7

MLEJ = Methanolic leaves extract of *J. humile*. The minimum inhibitory concentration (MIC) of the methanol extract of *J. humile* leaves for different organisms is 4 to 6 mg/mL. (G^+) gram positive bacteria, (G^-) gram negative bacteria.

4. Discussion

Antioxidant compounds act by several mechanisms such as, inhibition of generation and scavenging activity against reactive oxygen species (ROS); reducing power; metal chelation; activity as antioxidative enzymes; inhibition of oxidative enzymes. Oxidative damage caused by ROS leads to DNA lesions, loss of functions of enzymes, increased cell permeability, disturbed signaling over the cell and eventually necrotic cell death or apoptosis[17-21]. The stable DPPH is a widely used method to evaluate antioxidant activities in a relatively short time compare to other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effect of MLEJ on DPPH scavenging is thought to be due to their hydrogen donating ability. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. Hydrogen peroxide is a non-radical form of ROS that is formed in living organisms by superoxide dismutase. Hydrogen peroxide is not by itself very active but it can cross biological membranes and generates hydroxyl radicals which are toxic to cells and can damage a number of biomolecules[19]. Thus, removing of H_2O_2 is very important for protection of living organism. The encouraging results of *J. humile* leaves with the various in vitro antioxidant tests proved the plant as a reducing agent and effectiveness as scavengers of free radicals. The result of this study showed that MLEJ have antibacterial activities against the tested organisms. This suggests that the extracts of these plants are broad spectrum in their activities. The antimicrobial effect of methanol extract against these organisms may be due to presence of the bioactive compounds of these plants like flavanoids, alkaloids, saponin and tannins. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity. In conclusion, the study has shown that methanol extract from *J. humile* have in vitro antimicrobial and antioxidant activities which could support the use of the plant by traditional healers to treat various infective diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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