Antioxidant potential of n-butanol fraction from extract of

Jasminum mesnyi Hance leaves

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Methanolic extract of Jasminum mesnyi Hance leaves having antidiabetic activity was subjected to fractionation to obtain antioxidant and antihyperglycemic rich fraction. Different concentrations of ethyl acetate and n-butanol fractions were subjected to antioxidant assay by DPPH method, nitric oxide scavenging activity and reducing power assay. The fractions showed dose dependent free radical scavenging property in all the models. IC_{50} values for ethyl acetate and n-butanol fractions were 153.45±6.65 and 6.22±0.25 µg/ml, respectively, as compared to L-ascorbic acid and rutin (as standards; IC_{50} values 6.54±0.24 and 5.43±0.21 µg/ml, respectively) in DPPH model. In nitric oxide scavenging activity, IC_{50} values were 141.54±9.95 µg/ml, 35.12±1.58 µg/ml, 21.06±0.95 µg/ml and 29.93±0.32 µg/ml for ethyl acetate, n-butanol fractions, L-ascorbic acid and rutin, respectively. n-Butanol fraction showed a good reducing potential and better free radical scavenging activity as compared to ethyl acetate fraction. Potent antioxidant n-butanol fraction showed better oral glucose tolerance test (antihyperglycemic) at par with metformin (standard drug). n-Butanol fraction contained secoiridoid glycosides which might be responsible for both antioxidant and antihyperglycemic activity.

Keywords: Antihyperglycemic, Antioxidant, Jasminum mesnyi, Secoiridoids

Jasminum mesnyi is a native of Himalayan region and is an evergreen rambling shrub with long and slender arching stems that climb like a sprawling vine. It is used in diabetic formulation under the name “Pahari Butti” particularly in Solan, India. Diabetes is associated with low level of antioxidants and many plants show antihyperglycemic property due to their antioxidant potential. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) have been widely applied in food processing, they have been reassessed for their possible toxic and carcinogenic components formed during degradation.

Antihyperglycemic potential of methanolic extract of the leaves of J. mesnyi has been reported from our laboratory by using the oral glucose tolerance test (OGTT) in normal rats and STZ induced diabetic rats (unpublished data). n-Butanol fraction of the methanol extract of leaves of J. mesnyi contains secoiridoids (jasmoside, jasmesoside, 9″-hydroxyjasmesoside, 9″-hydroxyjasmosidic acid, jasminin 10″-O-β-D-glucoside, 2″-hydroxyjasminin, isojasminin, jasmine, 4″-hydroxyisojasminin and jasmosidic acid).

Secoiridoids are monoterpenoids based on 7,8-seco-cyclopenta[c]-pyranoid skeleton. They are derived in plants from iridoid, loganin by oxidative cleavage with redox enzyme which undergo various secondary modifications of the basic skeleton. The study of biological and pharmacological activity has revealed that both iridoids and secoiridoids exhibit a wide range of bioactivities like antiallergic, antiarthritic, antibacterial, anticancer, anticoagulant, anticomplement, antifungal, antiinflammatory, antioxidantive, antiprotocoal, antispasmodic, antiviral, immunomodulatory, neuroprotective and wound healing activities.

In the present study methanolic extract of J. mesnyi leaves was fractionated to ethyl acetate and n-butanol fraction to assess antihyperglycemic and antioxidant potential.

Materials and Methods

Chemicals—All chemicals used were of analytical grade. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. Rutin, L- ascorbic acid, tri chloroacetic acid (TCA) and ferric chloride...
were procured from central drug house (CDH), New Delhi. Sulphanilic acid and naphthylethylendiammine dihydrochloride from Rankem (New Delhi). Sodium nitroprusside and potassium ferricyanide were obtained from Nice Chemicals. Metformin was procured from Zenlabs (Chandigarh). Solvents used during the experiment were purchased from Rankem (New Delhi).

Absorbance was noted using UV/Visible Spectrophotometer (UV 1700, Pharmaspec, SHIMADZU, Japan).

Plant material—Jasminum mesnyi Hance leaves were collected in the month of February from Solan, India and authenticated (Ref. NISCAIR/RHMD/Consult/-2008-09/1048/79) by the Department of Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. The leaves were separated, shade dried, powdered and stored at room temperature in an air tight container till further use.

Extraction and fractionation—Coarsely powdered leaves (100 g) were defatted with petroleum ether (60-80°C), dried and extracted with 90% methanol using Soxhlet apparatus. The methanol extract thus obtained was freed from solvent in a vacuum evaporator to obtain extract (55.5 g). This was further extracted with ethyl acetate and n-Butanol to obtain active fraction.

Phytochemical analysis—Ethyl acetate and n-butanol fractions were subjected to analysis for the presence of alkaloids, glycosides, tannins, flavonoids, iridoids and secoiridoids. n-butanol fraction was subjected to HPTLC analysis to identify secoiridoids.

Antihyperglycemic study

Animals—Wistar rats (either sex) weighing 180-250 g were procured from the animal house of I.S.F. College of Pharmacy, Moga (Reg. No. 816/04/c/CPCSEA). The animals were kept in polypropylene cages (3 in each cage) at 25±2°C and 55-65% RH. A 12 h light/dark schedule was maintained in the animal house. The rats had free access to water ad libitum and commercial feed.

Preparation of test drug material—Ethyl acetate, n-Butanol fraction and metformin were suspended in carboxymethylcellulose (1% w/v CMC).

Oral glucose tolerance test (OGTT) was performed according to the method of Blois. Glucose—Ethyl acetate fraction (300 mg/kg, po; Group 2); and metformin (0.5 g/kg, po; Group 3); and metformin (0.5 g/kg, po; Group 4). In vitro antioxidant studies

Ethyl acetate and n-Butanol fraction were tested in vitro for their free radical scavenging property using different models. L-ascorbic acid and rutin were used as standard control in each experiment. IC50 values were calculated. All experiments were performed in triplicate and values are represented as mean ± SD.

DPPH radical scavenging activity—DPPH radical scavenging activity was performed according to the method of Blois. Nitric oxide radical scavenging activity—Nitric oxide radical scavenging activity was performed according to the method of Garratt.

Reducing power assay—Reducing power assay was performed according to the method of Oyaizu.

Statistical analysis—All results are expressed as mean ± SD. One way ANOVA was used to evaluate differences between the groups. The differences among the means were analyzed by Tukey’s multiple comparison test using graphpad prism 5 software package. P<0.05 was considered to be statistically significant.

Results

Antidiabetic activity of methanolic extract—The diabetic rats treated with the methanol extract (400 mg/kg) of J. mesnyi showed significant reduction in fasting serum glucose after 7th, 14th and 21st days of treatment as compared to 0 day. Fall in serum glucose level was gradual and consistent by 22.42, 61.03 and 109.56%, respectively after 7th, 14th and 21st days of treatment. Diabetic rats treated with 0.5 mg/kg of glibenclamide showed consistent fall in serum glucose by 16.6, 72.35 and 120.28%, respectively, after 7th, 14th and 21st days of treatment (Table 1).
**Phytochemical analysis**—Flavonoids were found in traces in ethyl acetate fraction. HPTLC spectral analysis of n-butanol fraction showed absorbance peaks with $\lambda_{\text{max}}$ at 281, 236, 284, 237 and 234 nm which are characteristic absorbance of secoiridoids$^{21}$.  

**Antioxidant activity**—Several concentrations of ethyl acetate fraction and n-butanol fraction ranging from (5-400 µg/ml) were tested in vitro for their antioxidant activity in different models. It was observed that n-butanol fraction had higher antioxidant potential as compared to ethyl acetate fraction and its antioxidant activity was comparable to the standards rutin and L-ascorbic acid (Figs 1, 2). Antioxidant activity increased with increasing concentration in all the models in both fractions. IC$_{50}$ values of standards and fractions in DPPH model and nitric oxide scavenging assay are shown in Table 2. Reducing power of fractions increased with increasing concentration. n-Butanol fraction showed a significant reducing ability in comparison to rutin and L-ascorbic acid (Table 3).  

**Oral glucose tolerance test (OGTT)**—Animals of vehicle control and metformin pretreated group showed 35.62 and 18.59% increase in serum glucose level, respectively after 1 h of glucose administration in comparison to initial 0 h serum glucose level. The group of animals pretreated with the ethylacetate fraction of methanolic extract (300 mg/kg body wt) showed increase (29.32%) in serum glucose level. Similarly, n-butanol fraction of methanolic extract (300 mg/kg body wt) pretreated group showed increase (16.97%) in serum glucose level after 1 h of glucose administration in comparison to 0 h serum glucose level. However control, metformin and test drug pretreated groups of animals normalize the serum glucose level within 2 h (Table 4).

![Fig. 1](image1.png)—Free radical scavenging effect of n-butanol fraction of extract of *J. mesnyi*, ascorbic acid and rutin by DPPH

![Fig. 2](image2.png)—Nitric oxide scavenging effect of fractions of methanol extract of leaves of *J. mesnyi*, ascorbic acid and rutin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>93.90 ± 4.70</td>
<td>94.70 ± 3.34</td>
<td>94.78 ± 3.80</td>
<td>94.11 ± 3.72</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>268.18 ± 9.91*</td>
<td>271.83 ± 10.86*</td>
<td>275.05 ± 10.29*</td>
<td>280.59 ± 12.75*</td>
</tr>
<tr>
<td>Standard (GLB 0.5 mg/kg)</td>
<td>242.93 ± 10.51</td>
<td>208.33 ± 8.01b</td>
<td>140.95 ± 8.01b</td>
<td>110.28 ± 8.49b</td>
</tr>
<tr>
<td>Test (ME 400 mg/kg)</td>
<td>272.23 ± 7.52</td>
<td>222.37 ± 8.11b</td>
<td>169.05 ± 6.11b</td>
<td>129.90 ± 6.81b</td>
</tr>
</tbody>
</table>

*P < 0.05 vs normal group; bP < 0.05 vs diabetic control group

Table 1—Effect of methanolic extract of *J. mesnyi* (400 mg/kg body wt) on serum glucose in STZ induced diabetic rats
[values are mean ±SD of 3 replicates]
Discussion

Earlier studies in our laboratory has shown that the methanolic extract of the leaves of *J. mesyni* (400 mg/kg body wt.) causes a significant fall in serum glucose of diabetic rats in comparison to 0 day and diabetic control group. Present study was designed to fractionate the methanolic extract into ethyl acetate and n-butanol fractions to investigate the active fraction. Chemical and HPTLC analysis confirmed the presence of flavonoids in ethyl acetate and secoiridoid in n-butanol fraction. Both flavonoids and secoiridoids, are reported to have antioxidant potential\(^{22-23}\). Numerous studies have demonstrated that hyperglycemia induced generation of free radicals and consequent development of oxidative stress mainly contributes to the development and progression of diabetes and related complications. Thus, the ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications\(^{24-26}\).

Hence, it was planned to study the antioxidant potential of ethyl acetate and n-butanol fraction of methanolic extract of *J. mesnyi* leaves.

The ethyl acetate and n-butanol fractions showed significant DPPH scavenging activity \((\text{IC}_{50}=153.45\pm6.65 \text{ µg/ml} \text{ and } \text{IC}_{50}=6.22\pm0.25 \text{ µg/ml}, \text{ respectively})\) when compared with the \(\text{IC}_{50}\) values of the standards L-ascorbic acid and rutin \((\text{IC}_{50}=6.54\pm0.24 \text{ and } 5.43\pm0.21 \text{ µg/ml}, \text{ respectively})\). *J. mesnyi* fractions significantly inhibited nitric oxide in a dose dependent manner with \(\text{IC}_{50}\) being 141.54±9.95 and 35.12±1.58 µg/ml for ethyl acetate and n-butanol fractions, respectively as compared with standards, L-ascorbic acid and rutin, having \(\text{IC}_{50}\) values of 21.06±0.95 and 29.93±0.32 µg/ml, respectively.

In addition, reductive capability of n-butanol fraction was more prominent than ethyl acetate fraction with reference to ascorbic acid and rutin (standards). Reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing activities are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions and by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation\(^{27}\). The rats pretreated with n-butanol fraction had shown significant check on rise of serum glucose in OGTT as compared to normal control animals and it was at par with metformin. The observed hypoglycemic effect may be attributed to

<table>
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<tr>
<th>Fraction</th>
<th>(\text{IC}_{50}) (µg/ml) (Free radical scavenging effect by DPPH assay)</th>
<th>(\text{IC}_{50}) (µg/ml) (Nitric oxide scavenging activity)</th>
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<tbody>
<tr>
<td>Rutin</td>
<td>5.44±0.21</td>
<td>29.93±0.32</td>
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<tr>
<td>Ascorbic acid</td>
<td>6.54±0.24</td>
<td>21.06±0.95</td>
</tr>
<tr>
<td>n-butanol fraction</td>
<td>6.22±0.25</td>
<td>35.12±1.58</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>153.45±6.65</td>
<td>141.54±9.95</td>
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<table>
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<tr>
<th>Conc. (µg/ml)</th>
<th>Ascorbic acid</th>
<th>Rutin</th>
<th>n-butanol</th>
<th>Ethyl acetate</th>
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<tr>
<td>25</td>
<td>0.07 ± 0.003</td>
<td>0.06 ± 0.062</td>
<td>0.07 ± 0.001</td>
<td>0.05 ± 0.001</td>
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<tr>
<td>50</td>
<td>0.35 ± 0.004</td>
<td>0.31 ± 0.004</td>
<td>0.33 ± 0.007</td>
<td>0.09 ± 0.004</td>
</tr>
<tr>
<td>100</td>
<td>0.61 ± 0.002</td>
<td>0.59 ± 0.004</td>
<td>0.60 ± 0.004</td>
<td>0.32 ± 0.005</td>
</tr>
<tr>
<td>200</td>
<td>1.39 ± 0.004</td>
<td>1.29 ± 0.005</td>
<td>1.32 ± 0.004</td>
<td>0.61 ± 0.004</td>
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<tr>
<td>400</td>
<td>2.82 ± 0.005</td>
<td>2.76 ± 0.005</td>
<td>2.76 ± 0.006</td>
<td>1.11 ± 0.003</td>
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<table>
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<th>Groups</th>
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<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
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<tr>
<td>Normal Control</td>
<td>94.55 ± 2.57</td>
<td>92.64 ± 2.25</td>
<td>143.91 ± 2.96 (↑ 35.62%)</td>
<td>89.79 ± 6.74</td>
</tr>
<tr>
<td>Test IG (EAF 300 mg/kg)</td>
<td>93.29 ± 6.13</td>
<td>89.62 ± 6.30</td>
<td>126.80 ± 7.31 (↑ 29.32%) (^a)</td>
<td>101.42 ± 5.84</td>
</tr>
<tr>
<td>Test IIG (NBF 300 mg/kg)</td>
<td>87.52 ± 6.87</td>
<td>71.59 ± 6.24</td>
<td>86.23 ± 5.28 (↑ 16.97%) (^a)</td>
<td>83.44 ± 3.89</td>
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<tr>
<td>Test IIIG (MF 0.5 g/kg)</td>
<td>90.44 ± 6.04</td>
<td>78.01 ± 4.74</td>
<td>95.83 ± 5.52 (↑ 18.59%) (^a)</td>
<td>91.36 ± 4.91</td>
</tr>
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</table>

\(^a \equiv P<0.05 \text{ vs normal groups}\)
stimulation of the pancreas for the release of more insulin in response to hyperglycemia.

Hence, it may be concluded that n-butanol fraction being more potent antioxidant may also be responsible for antidiabetic activity of methanolic extract of leaves of *J. mesnyi*.

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**References**