Estimation of total phenolic and flavanoid content of ethenolic extract of seeds Solanum Nigrum

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ABSTRACT
Solanum nigrum Linn. (Solanaceae) has been extensively used in traditional medicine and other parts of the world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhea, eye diseases etc. Present study showed that the ethanolic extract of Solanum nigrum which contain highest amount of flavonoids and phenolic compounds so can exhibit the greatest antioxidant activity and among other extract used for the study. Solanum nigrum species contain the highest phenol and flavonoids, thus can be used to explore new drugs. The results revealed that there is relation between phenol and flavonoids content with antioxidant activities.

Introduction
The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures (Agarwal, 2005).

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity.

The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.

In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2002).

Herbal practitioners use different diagnostic principles from conventional practitioners. For example, when treating arthritis, they might observe, “Under functioning of a patient’s symptoms of elimination” and decide that the arthritis results from “an accumulation of metabolic waste products”.

A diuretic, choleric or laxative combination of herbs might then be prescribed alongside herbs with anti-inflammatory properties. (Vickers and Zollman, 1999)

Material method
Estimation of Total Phenolic and Flavanoid Content
a) Total Phenolic content estimation:
Principal.
The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Olajuyigbe et al., 2011).

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 25-125\mu g/ml was prepared in methanol.

Preparation of Extract
1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development.

The absorbance was measured at 765 nm using a spectrophotometer.

b) Total flavanoid content estimation:
Principal.
Determination of total flavonoids content was based on aluminium chloride method (Olajuyigbe et al., 2011).

Preparation of standard.
50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25-125\mu g/ml were prepared in methanol.

Preparation of extract
1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure.
1 ml of 2\% AlCl3 methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

Result and discussion
Results of total Phenolic and Flavanoids Content
Total Phenolic content estimation
Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals. The amount of total phenol was determined with the Folin-Ciocalteu reagent.
The content of total phenolic compounds (TPC) was expressed as mg/g of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: 
$$Y = 0.007X + 0.218, \quad R^2 = 0.999, $$
where X is the absorbance and Y is the gallic acid equivalent (GAE).

Where X is absorbance at 760 nm and Y is total phenolic content in the extracts of Solanum nigrum expressed in mg/gm. Table 1 shows the variation of mean absorbance with concentration of Gallic acid and graph 1 shows the calibration curve of standard gallic acid.

The contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent.

Table 1. Preparation of calibration curve of Gallic acid.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance (Mean) λmax=760 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.740</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.855</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>0.954</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1.050</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>1.151</td>
</tr>
</tbody>
</table>

Graph 1. Calibration curve of Gallic acid

5.4.1.2 Total flavonoids content estimation

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: 
$$Y = 0.008X + 0.478, \quad R^2 = 0.999, $$
where X is the absorbance and Y is the quercetin equivalent (QE). Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phytoconstituent. The amount of total flavonoids was determined with the Quercetin.

Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g Quercetin equivalent using the standard curve equation: 
$$Y = 0.008X + 0.478, \quad R^2 = 0.999, $$
Where Y is absorbance at 420 nm and X is total flavonoids content in the extracts of Solanum nigrum expressed in mg/gm. Table 2 shows the variation of mean absorbance value with different concentration of Quercetin reagent and graph 2 shows the calibration curve of Quercetin.

Table 2. Preparation of calibration curve of Quercetin.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance (Mean) λmax=420 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.740</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>0.855</td>
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</tr>
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<td>5</td>
<td>125</td>
<td>1.151</td>
</tr>
</tbody>
</table>

Graph 2. Calibration curve of Quercetin

The contents of total flavonoids were measured by AlCl₃ reagent in terms of Quercetin equivalent.

Table 3. Total Phenolic and flavonoids content in different species of curcuma.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Different plant Extracts</th>
<th>Total Phenol (mg/gm)</th>
<th>Total Flavonoids (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanolic</td>
<td>3.56±0.078</td>
<td>15.10±0.012</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous</td>
<td>3.04±0.095</td>
<td>13.93±0.045</td>
</tr>
</tbody>
</table>

Conclusion

The results obtained from present study showed that the Ethanolic extract of Solanum nigrum which contain highest amount of flavonoids and phenolic compounds so can exhibit the greatest antioxidant activity and among other extract used for the study. Solanum nigrum species contain the highest phenol and Flavonoids, thus can be used to explore new drugs. The results revealed that there is relation between phenol and flavonoids content with antioxidant activities. Funding; non funding

References


