

Evaluation of phenolic, flavonoid contents and antioxidant activity in leaves, stem and roots of *Solanum indicum* Linn.

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Received: 05.04.2019

Revised: 15.05.2019

Accepted: 04.06.2019

Abstract

The present investigation deals with the evaluation of phenolic, flavonoid contents and antioxidant activity in leaves, stem and roots of *Solanum indicum*. Powdered samples of leaves, stem and roots were subjected to successive extraction with solvents of increasing polarities i.e. Ethanol, Water: Ethanol (Aqua- alcoholic) (20: 80) and Water (aqueous) using soxhlet apparatus. The results showed the maximum phenolic content ($12.351\pm0.03\%$) in Aqua- alcoholic (80: 20) extract and maximum flavonoid content ($38.658\pm0.05\%$) in ethanolic extract of leaves. For stem, both the maximum phenolic ($11.750\pm0.02\%$) and flavonoid ($2.040\pm0.03\%$) contents was found in Aqua- alcoholic (80: 20) extract. Similarly, the maximum phenolic ($18.651\pm0.03\%$) and flavonoid ($2.701\pm0.05\%$) contents was found in ethanolic extract of roots. The Aqua- alcoholic (80: 20) extracts for leaves and stem and ethanolic extract for roots was found to contain the maximum antioxidant activity.

Key Words: Solanum indicum, leaves, stem, roots, phenolic and flavonoid contents, antioxidant activity

Introduction

Solanum indicum L. is commonly known as Birhata or Badikateri or Indian night shade and belongs to the family Solanaceae. It is an erect under shrub of 0.30 to 1.8 m in height and found throughout warmer parts of India, Asia and Africa upto an elevation of 1.5 m (Hasan et al., 2013). The national demand of S. indicum is 500-1000 MT per annum (Anonymous, 2018). All plant parts viz. berries, leaves, roots, seeds and stem of this species have been utilized in traditional system of medicine and are useful in various diseases such as bronchitis, asthma, dry cough, rhinitis, dysuria, leucoderma, sexual disorders, insomnia, cardiac weakness and pruritis (Anonymous, 1986, Bhakta, 1982, Bhattacharya, 1982 and Sharma et al., 2017). The species is among the ten medicinal plants whose roots are principally employed in preparation of Dashmoolarishta, a well-established ayurvedic drug used in the treatment of fatigue, oral sores and gynecological disorders (Yadav et al., 2009). Dashmool formulation is also utilized in the preparation of more than hundred ayurvedic drugs (Pathak et al., 2005). Phytochemicals are the natural compounds or products found in all plant

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parts and work together with nutrients and fibres to act as a defence system in plants and protect humans against diseases. These phytochemicals have specific pharmacological effects on human body such as anti-oxidants, anti-inflammatory, antiallergic, antimicrobial, hepato-protective, hypolipidemic, neuroprotective, hypotensive, antiaging. anti-diabetic. anticancer and cardioprotective, diuretic, CNS stimulant. analgesic, immuno-modulator etc (Prakash et al., 2012). Phenols and flavonoids are the most widely distributed phytochemicals in plants responsible for various pharmacological activities (Figure 1). The antioxidant activity of plants is of much concern in present time as antioxidants protect the human body from free radicals. Free radicals being the reactive oxygen and nitrogen species involved in the pathogenesis of several human diseases and disorders (Patel et al., 2011, Boynes, 1991). Phytochemicals such as phenolic diterpenes, flavonoids, tannins and phenolic acids of plant origin are useful sources of natural antioxidants (Lee et al., 2004). Flavonoids among polyphenols are known as the strongest antioxidant agents in plant extracts (Bernardi et al., 2008). Since the antioxidants provide immunity to the human body and protect us from a number of diseases, the search for novel natural antioxidants of plant origin



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is the major focus area of researchers these days. The scientific studies showed that antioxidant activity of plants play a major role in the protective effect of herbal medicine (Saeed *et al.*, 2012). *S. indicum* is one of the commercially important medicinal plants but the evaluation of the phenolic, flavonoid contents and antioxidant activity of leaves, stem and roots of *S. indicum* is still needed which are the objectives of this study.



Figure 1. Major groups of plant phytochemicals (Saxena *et al.*, 2013)

Material and Methods Chemicals and reagents:

All chemicals, reagents and solvents used in the study were of AR grade and distilled water was utilized wherever required.

Collection of plant materials:

The plant materials (leaves, stem and roots) of *S. indicum* were collected by following the guidelines of good agricultural and collection practices (GACP) for medicinal plants (Anonymous, 2003) from the Chhindwada region of Madhya Pradesh.

Processing of plant materials:

Plant materials were washed thoroughly in running water to remove soil and other foreign particles. These were cut into small pieces and dried in shade. Shade dried plant samples were powdered using grinder mill and powdered plant materials were stored in air –tight polythene bags for further chemical analysis.

Preparation of plant extracts:

Powdered plant samples of leaves, stem and roots of selected species were subjected to successive extraction with solvents of increasing polarities i.e. Ethanol, Ethanol: Water (Aqua- alcoholic) (80: 20) and Water (aqueous) using soxhlet apparatus (Varghese *et al.*, 2013). A total of 20g of dried plant powder of concerned part was extracted in

250ml of each solvent in successive manner for 12 hrs. Solvents were evaporated to dryness to yield the respective extracts which were used for estimation of antioxidant activity.

Estimation of total phenols:

Total phenols were determined by Folin Ciocalteau method (McDonald *et al.*, 2001). 10 mg of the extract was dissolved in a known volume of distilled water. 0.2 ml of samples was then taken in test tube and volume made up to 3 ml with distilled water. 0.5 ml of Folin Ciocalteau reagent was then added. After 3 minutes, 2 ml of 20% Na₂CO₃ solution was added to each tube, mixed thoroughly, placed in boiling water for exactly 1 minute, cooled and absorbance was taken at 650 nm against blank. The phenolic content was calculated from the standard graph of catechol (figure 2).



Figure 2. Standard graph of Catechol

Estimation of Flavonoids: Total flavonoids were determined by aluminum chloride colorimetric technique (Chang *et al.*, 2002). 10 mg of the extract was dissolved to 25 ml with 80% ethanol. 0.5 ml of filtrate was then mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10% AlCl₃, 0.1 ml of potassium acetate and 2.8 ml distilled water. The tubes were than incubated at room temperature for 30 minutes. Observation was measured at 415 nm. The flavonoids content was calculated from the standard graph of quercetin (figure 3).

Antioxidant activity of plant extracts

DPPH radical scavenging activity assay: The free radical scavenging activity of the fractions was measured in-vitro by 1, 1 - diphenyl-2-picrylhydrazyl (DPPH) assay according to the method described earlier (Pandeya *et al.*, 2016) with some modification. The stock solution was prepared by dissolving 0.006g DPPH with 100 ml



methanol and stored at 20°C until required. 3 ml aliquot of this solution was mixed with 100 μ l of the sample at various concentrations (10 - 50 μ g/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. The percentage inhibition was estimated based on the percentage of DPPH radical scavenged as the following equation:



Figure 3. Standard graph of Quercetin

% Radical Scavenging Activity (RSA) = {(Abs control – Abs sample)}/ (Abs control) × 100

Where Abs control is the absorbance of the DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract.

Calculation of IC₅₀: IC₅₀ value is the concentration of the sample required to inhibit 50% of radical. IC₅₀ values of the sample extracts were calculated.

Statistical analysis: Each experiment was carried out in triplicate and results are expressed as Mean \pm SD (n=3).

Results and Discussion

Total phenolic and flavonoid contents in leaf, stem and root extracts of *S. indicum* prepared in different solvents are given in Table 1. It can be seen from Table 1 that Ethanol: Water (80: 20) extract of leaves contained the maximum phenolic content (12.351 \pm 0.03%) and ethanolic extract contained the maximum flavonoid content (38.658 \pm 0.05%). The maximum phenolic (11.750 \pm 0.02%) and flavonoid (2.040 \pm 0.03%) contents was found in Ethanol: Water (80: 20) extract of stem. Similarly, the maximum phenolic (18.651 \pm 0.03%) and flavonoid (2.701 \pm 0.05%) contents was found in ethanolic extract of roots.

 Table 1. Total phenolic, flavonoid contents in leaf, stem and root extracts of S. indicum

Plant name	Parts used	Extracts	Phenols (%) (Mean ± SD)	Flavonoids (%) Mean ± SD)	
	Leaves	Ethanol	12.059±0.03	38.658±0.05	
S. indicum		Aqueous	6.276±0.05	27.643±0.02	
		Aqua- alcoholic (80:20)	12.351±0.03	9.5±0.04	
	Stem	Ethanol	8.122±0.04	1.164±0.09	
		Aqueous	3.885±0.01	0.493±0.04	
		Aqua- alcoholic (80:20)	11.75±0.02	2.040±0.03	
	Roots	Ethanol	18.651±0.03	2.701±0.05	
		Aqueous	12.702±0.02	2.354±0.03	
		Aqua- alcoholic (80:20)	8.812±0.03	0.555±0.06	

Table 2. Antioxidant activity of leaves, roots and stem of S. indicur	т
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Plant name	Parts used	Extracts	IC ₅₀ (μg/ ml)
	Leaves	Ethanol	28.40±0.0122
		Aqueous	23.30±0.007
		Aqua- alcoholic (80:20)	10±0.006
	Stem	Ethanol	ND*
S. indicum		Aqueous	37.55±0.154
		Aqua- alcoholic (80:20)	16.16±0.005
	Roots	Ethanol	12.89±0.004
		Aqueous	15.88±0.013
		Aqua- alcoholic (80:20)	36.49±0.008
Ascorbic acid s	tandard (Vitamin C)	42.27±0.399	
			*Not detected



 IC_{50} values (µg/ ml) for antioxidant activity of ascorbic acid standard and leaf, stem and root extracts of S. indicum are presented in Table 2 and Figure 4, 5, 7, 8, 9, 10, 11, 12, 13 and 13 respectively. Fig. 5-13 shows that inhibition pattern of DPPH free radical is dose dependent. The IC_{50} values vary from part to part and extract to extract due to presence of different number of antioxidants.



Figure 4. DPPH radical scavenging activity of Ascorbic acid



ethanolic extract of S. indicum leaves



aqueous extract of S. indicum leaves



Figure 7. DPPH radical scavenging activity of Aquaalcoholic (20:80) of S. indicum leaves



Figure 8. DPPH radical scavenging activity of ethanolic extract of S. indicum stem



Figure 5. DPPH radical scavenging activity of Figure 9. DPPH radical scavenging activity of aqueous extract of S. indicum stem



Figure 6. DPPH radical scavenging activity of Figure 10. DPPH radical scavenging activity of Aquaalcoholic (20:80) of S. indicum stem





Figure 11. DPPH radical scavenging activity of ethanolic extract of *S. indicum* roots



Figure 12. DPPH radical scavenging activity of aqueous extract of *S. indicum* roots



Figure 13. DPPH radical scavenging activity of Aquaalcoholic (20:80) of *S. indicum* roots

Table 2 showed that among all the three extracts of leaves and stem, Aqua- alcoholic (80:20) extract was found to have the lowest IC_{50} value in both the cases and hence the highest radical scavenging activity. In case of roots, the ethanolic extract was found to contain the lowest IC_{50} value and the highest radical scavenging activity. It can also be seen that all the extracts except the ethanolic extract of stem contained the better radical scavenging activity than ascorbic acid.

Scientific studies have proved the neutralization / scavenging effect of free radicals produced in the body with the help of antioxidants (Oboh *et al.*, 2007). The formation of free radicals is linked with the normal metabolism of aerobic cells. The phenolics have the capability to scavenge free radicals, activate antioxidant enzymes, chelate metal catalysts, reduce α - tocophenol radicals and inhibit oxidases (Amic *et al.*, 2003). Plants are the rich sources of flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin which have been reported to bestow with tremendous antioxidant potential apart from vitamins C, E and β - carotene (Marin *et al.*, 2004; Odubanjo, 2013).

Leaf, stem and root extracts of *S. indicum* showed antioxidant activity which is in agreement with the phenolic and flavonoid contents. Investigations have established the positive correlations between phenolics and antioxidant ability of some plant origin foods (Amic *et al.*, 2003, Patel *et al.*, 2011; Saxena *et al.* 2013).

Conclusion

In the present findings, it can be concluded that aqua-alcoholic extracts of leaves, stem and ethanolic extract roots of *S. indicum* exhibited significant radical scavenging ability due to the presence of phenolic and flavonoid compounds in the extracts. Further research work can also be planned for isolation and structure elucidation of responsible antioxidant components of this plant.

Aknowledgement

Author is highly grateful to the Principal of Govt. Science College, Jabalpur and Director of Tropical Forest Research Institute, Jabalpur for giving permission and providing necessary facilities to conduct the research work.

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