

## Research Article



## Estimation of Total Phenolic, Flavonoid Content and Evaluation of DPPH Free Radical Scavenging Potential of *Abutilon indicum* L.

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### ABSTRACT

The present study was carried out to estimate Total Phenolic Content, Total Flavonoid Content and to evaluate DPPH free radical scavenging activity of the methanolic extract of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum*. The quantitative estimation of total phenolic and flavonoid content were determined by using Folin–Ciocalteu method and aluminum chloride colorimetric method. The free radical scavenging activities of the methanolic extracts of different plant parts (leaves, stems, fruits and roots) were assessed using DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) scavenging assay. The results showed that highest Total Phenolic Content was found in Roots ( $25.61 \pm 0.692$  mg Gallic Acid Equivalent/g dry weight of extract) while highest Total Flavonoid Content was observed in leaves ( $16.89 \pm 0.219$  mg Quercetin Equivalent/g dry weight of extract). The highest free radical scavenging activity was recorded in Roots with  $IC_{50}$  value ( $545.24 \pm 0.191$   $\mu$ g/ml). From the results of Present study it is concluded that methanolic extract of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum* is effective in scavenging free radicals therefore it may helpful to develop new drugs against free radical related diseases and could also serve as potential source of natural antioxidants.

**Keywords:** *Abutilon indicum*, Antioxidants, Phenolics, Flavonoids, DPPH Free radical scavenging activity.

### INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS<sup>1,2</sup>. Antioxidants are compounds that inhibit or delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions<sup>3</sup>. In the past few years, there is an increased importance for antioxidants from natural sources rather than from synthetic sources because of the health risks and toxicity of synthetic antioxidants<sup>4</sup>. Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential<sup>5</sup>. Flavonoids are capable of effectively scavenging the reactive O<sub>2</sub> species because of their phenolic hydroxyl groups and therefore they are potent antioxidants<sup>6</sup>.

*Abutilon indicum* Linn. (Malvaceae); commonly called as 'Kanghi' (Hindi) and 'Atibala' (Sanskrit). It is a perennial shrub, softly tomentose and up to 3 m in height. The plant is found in India, Sri Lanka, tropical regions of America and Malesia<sup>7</sup>. In Siddha system of medicine, it used as a remedy for jaundice, piles, ulcer and leprosy<sup>8</sup>. The plant contains mucilage, tannins, asparagines, gallic acid and sesquiterpenes<sup>9</sup>. The plant is also reported to possess analgesic activity<sup>10</sup>, larvicidal activity<sup>11</sup>,

hepatoprotective activity<sup>12</sup>, antifertility activity<sup>13</sup> and wound healing activity<sup>14</sup>.

Thus, the present study was undertaken with objective to estimate total phenolic content, total flavonoid content and to evaluate DPPH free radical scavenging activity of methanolic extracts of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum*.

### MATERIALS AND METHODS

#### Plant Material

The different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum* were collected from the University of Rajasthan Campus, Jaipur. The plant was identified and authenticated in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and a voucher specimen Number **RUBL21178** was deposited at the Herbarium, Department of Botany.

The plant parts were rinsed with tap water, dried under shade at room temperature. Then dried plant material was powdered by using electrical grinder and stored in air tight containers.

#### Chemicals and Reagents

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) and Quercetin were purchased from Sigma Chemical Co. (St., Louis, USA), Ascorbic acid, Folin Ciocatteu's (FC) phenol reagent and methanol were purchased from Thermo Fisher Scientific India Pvt. Ltd Powai, Mumbai.

Gallic acid, anhydrous sodium carbonate, aluminum chloride, and potassium acetate were purchased from



Sisco Research Laboratories (SRL) PVT. Ltd. (Mumbai, India). All chemicals used were of analytical grade.

### Preparation of Extracts

The dried powdered of leaves, stems, fruits and roots of *Abutilon indicum* (10 gram) was soxhlet extracted with methanol for 24 hours. The extract was filtered with Whatman filter paper no 1 and the filtrate was concentrated to dryness. The dry crude extracts were stored in air tight bottles and kept in refrigerator for further experiments.

### Determination of Total Phenolic Content

Total Phenolic Content were estimated using Folin-Ciocalteu reagent by the method of (McDonald et al. 2001) with modifications<sup>15</sup>. 1 ml of plant extract was mixed with 5ml Folin-Ciocalteu reagent (diluted tenfold) and Sodium carbonate solution in Distilled Water (4ml, 0.7 M). The mixtures were allowed to stand for 1 hour at room temperature. The absorption was measured at 765 nm using UV-visible spectrophotometer against a blank which was composed of the same reagents except test extract. The standard calibration curve was prepared using 50, 100, 150, 200, 250 µg/ml solutions of Gallic Acid in methanol ( $R^2 = 0.999$ ). Total Phenolic content in the plant extracts were expressed as Gallic Acid Equivalents (mg of GAE/g dry weight of extract) and were calculated by the formula:

$$T = (C \times V) / M$$

Where, T=Total content of phenolic compounds, milligram per gram dry weight of plant extract, in GAE; C= concentration of Gallic Acid established from the calibration curve, milligram per milliliter; V=the volume of extract, milliliter; M=the weight of methanolic plant extract, gram.

### Determination of Total Flavonoid Content

Total Flavonoid Content was estimated using Aluminum Chloride Colorimetric method of (Chang et al. 2002) with some modifications<sup>16</sup>. 1 ml of Plant extract was mixed with 3 ml methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml potassium acetate (1M) and 5.6 ml of distilled water. Then the solution was incubated for 30 minutes at room temperature. The absorbance was measured at 415 nm using UV-visible spectrophotometer against a blank. The Standard calibration curve was prepared using 25, 50, 100, 200 µg/ml solutions of Quercetin in methanol ( $R^2 = 0.996$ ). The Total Flavonoid Content of plant methanolic extracts was expressed in Quercetin Equivalent (mg of QE/g dry weight of extract) and was calculated by the following Formula:

$$T = (C \times V) / M$$

Where; T = total content of flavonoid compounds, mg per gram dry weight of plant extract, in Quercetin Equivalent, C = concentration of Quercetin established from the calibration curve in mg/ml, V = volume of extract in ml and M = weight of methanolic plant extract in gram.

### Determination of DPPH free radical scavenging activity

The free radical scavenging activities of the methanolic extracts of different plant parts were evaluated by using the modified method of (Blois 1958)<sup>17</sup>. 1 ml each of the different concentrations of extracts or standard (Ascorbic acid) was added to 1 ml of 0.3 mM DPPH (in methanol). The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. The Percentage scavenging activity was expressed as percentage inhibition of DPPH free radical by the sample and was calculated by using the following expression<sup>18</sup>.

$$\% \text{ Inhibition of DPPH} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The IC<sub>50</sub> value is the concentration (in µg/mL) of sample (extract/standard) that provided 50% inhibition of the DPPH radical. Linear graph of concentration Vs percentage inhibition was prepared and IC<sub>50</sub> values were calculated. The lower the IC<sub>50</sub> value indicates high antioxidant capacity.

### Statistical Analysis

All the experiments done in triplicates and results were shown as mean ± Standard Deviation (S.D.). Linear regression analysis was used to calculate the IC<sub>50</sub> values. Microsoft Excel 2007 software was used for all statistical analysis.

## RESULTS AND DISCUSSION

### Total Phenolic Content

The high potential of phenolics to scavenge free radicals may be due to presence of many phenolic hydroxyl groups<sup>19</sup>.

Total phenolic content were determined by using the Folin-Ciocalteu reagent in methanolic extracts of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum*. Total Phenolic Contents were calculated using the standard curve of gallic acid and were expressed as Gallic Acid Equivalent per gram dry weight of plant extract. The highest Total Phenolic Content was found in roots (25.61 ± 0.692 mg GAE/g dry weight of extract). The results of Total phenolic content of different plant parts of *Abutilon indicum* are presented in Table 1.

### Total Flavonoid Content

The compounds such as flavonoids, which hold hydroxyls groups, are responsible for the radical scavenging activity in the plants.<sup>20,21</sup> Aluminium chloride colorimetric method was used to determine the total flavonoid contents of methanolic extracts of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum*. Total flavonoid content was calculated using the standard curve of quercetin and was expressed as Quercetin Equivalent per gram dry weight of extract. The highest Total Flavonoid content was found in leaves (16.89 ± 0.219 mg QE/g dry weight of extract). The results of Total



Flavonoid content of different plant parts of *Abutilon indicum* are presented in Table 1.

### DPPH Free Radical Scavenging Assay

Free radical scavenging activity of the methanolic extract of different plant parts (leaves, stems, fruits and roots) of *Abutilon indicum* was measured by DPPH assay. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule<sup>22</sup>. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidative activity of antioxidants<sup>23</sup>. According to the results obtained, the methanolic roots extract showed highest Free radical scavenging activity ( $IC_{50} = 545.24 \pm 0.191 \mu\text{g/ml}$ ). Lower  $IC_{50}$  value indicates higher antioxidant potential. It was also found that the Free radical-scavenging activities of all the extracts increased with increasing concentration. The  $IC_{50}$  values of different plants parts of *Abutilon indicum* of DPPH radical scavenging assay are reported in Table 2 and graphs of DPPH radical-scavenging activities of

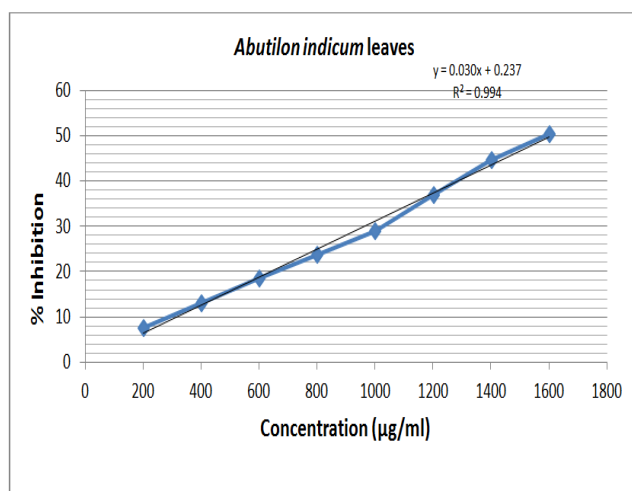
different plant parts extracts are presented in Figure 1-4.

**Table 1:** Total Phenolic Content and Total Flavonoid Content in different plant parts of *Abutilon indicum*.

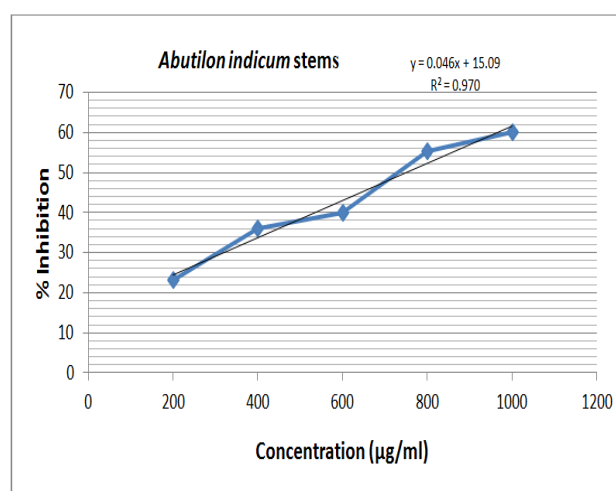
Plant parts	Total Phenolic Content (mg GAE /g dry weight of extract)	Total Flavonoid Content (mg QE /g dry weight of extract)
Leaves	14.88 ± 0.281	16.89 ± 0.219
Stems	22.98 ± 0.789	3.10 ± 0.122
Fruits	24.58 ± 0.761	1.98 ± 0.601
Roots	25.61 ± 0.692	2.86 ± 0.392

**Table 2:**  $IC_{50}$  Values of different plants parts of *Abutilon indicum* of DPPH Radical Scavenging Assay

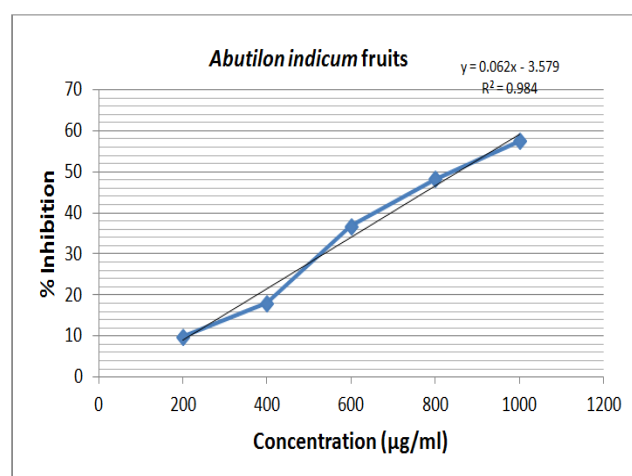
Plant Parts	$IC_{50}$ values ( $\mu\text{g/ml}$ )
Leaves	1658.76 ± 0.535
Stems	758.91 ± 0.220
Fruits	864.17 ± 0.306
Roots	545.24 ± 0.191
Ascorbic acid	16.33 ± 0.325



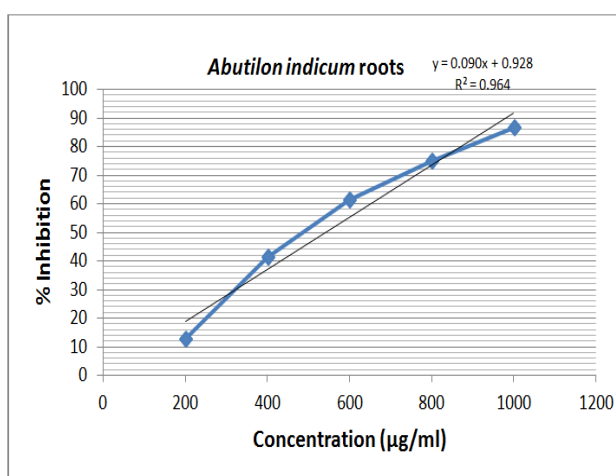
**Figure 1:** DPPH Radical Scavenging activity of methanolic extract of *Abutilon indicum* leaves.



**Figure 2:** DPPH Radical Scavenging activity of methanolic extract of *Abutilon indicum* stems.



**Figure 3:** DPPH Radical Scavenging activity of methanolic extract of *Abutilon indicum* Fruits.



**Figure 4:** DPPH Radical Scavenging activity of methanolic extract of *Abutilon indicum* roots.

**CONCLUSION**

From the results of present study it was concluded that the methanolic extracts of different plant parts of *Abutilon indicum* has showed significant antioxidant activity.

The antioxidant potential of plant parts extracts may be due to presence of good amount of phenolic and flavonoid content.

Thus, the methanolic extracts of different plant parts of *Abutilon indicum* could serve as the Potential source of natural antioxidants and may help to develop new natural drugs against various diseases caused by free radicals.

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