EVALUATION OF ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS ISOLATED FROM *RHYNCHOSIA SUAVEOLENS* (FABACEAE)*

One of the areas which has attracted a great deal of attention is antioxidants in the control of degenerative diseases in which oxidative damage has been implicated. Antioxidants play an important role to protect the human body against damage by free radicals or reactive oxygen species (ROS). Reactive oxygen species such as hydroxyl, superoxide and peroxy radicals are formed in human tissue cells which cause extensive oxidative damage that leads to age related degenerative conditions, hypertension, anti-inflammatory cancer and a wide range of other human diseases such as rheumatoid arthritis, diabetics, vascular diseases and hyperplasic disease.

Consumption of foods rich in natural antioxidants has been reported as being protective against certain types of cancer and coronary disease and may also reduce the risk of cardiovascular events. Natural antioxidants prevent oxidative damage of cellular biomolecules such as lipids, proteins and nucleic acids mainly by breaking chain reactions, reducing the concentration of ROS, scavenging free radical initiators and chelating the metal ions, which catalyze the formation of free radicals.

Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity. Many of these antioxidants possess anticancer, antiinflammatory, anticarcinogenic, atherosclerotic and antimicrobial activities. The search for newer natural antioxidants, especially of plant origin is getting popularized due to their cost effectiveness, no side effects and multifaceted activities.

Natural antioxidant constitutes a broad range of compounds including phenolic compounds (Flavonoids, stilbenes, hydrolysable tannins, proanthocyanidins, caffeates and lignans), nitrogen compounds and carotenoids besides vitamin E and ascorbic acid. Flavonoids are polyphenolic compounds isolated from a wide range of vascular plants recognized as a major class of secondary metabolites with antioxidant, antiviral, anti-inflammatory, antioxidants, anticancer, antiallergic,
antibacterial activities\textsuperscript{61}. The basic flavonoid structure is the flavan (2-phenyl benzopyran or chroman). The various classes of flavonoids differ in the level of oxidation and pattern of substitution in the hetero cyclic ring while individual compounds within a class differ in the pattern of substitution in the two aromatic rings. Among the many classes of flavonoids, flavones, flavonols, dihydroflavones, dihydroflavonols, chalcones, isoﬂavones, biflavonoids and flavan-3-ols are very interesting from the point of view of their antioxidant activity. The antioxidant activity of flavonoids was suggested to be related to number and position of hydroxyl groups\textsuperscript{62}.

Medicinal plants rich in phenolic compounds possess nutraceutical importance because they inhibit the formation of free radicals by retarding oxidative degradation of lipids and can enhance the quality and nutritional value of food\textsuperscript{63}. Flavonoids are the major phenolic compounds that possess potential antioxidant activity against most oxidizing molecules and various free radicals implicated in several dreadful diseases\textsuperscript{64}.

In the present study, different extracts of the flowers of \textit{R. suaveolens} as well as the isolated compounds (1-4) were evaluated for their total phenolic content (TPC) total flavonoid content (TFC) and DPPH radical scavenging activity.
MATERIALS AND METHODS

1. Estimation of Total Phenolic Content (TPC)

TPC of the plant extracts was determined by the spectrophotometric method using Folin-Ciocalteu’s reagent\textsuperscript{65}. To one mL of plant extract (1mg/mL), 1 mL of Folin-Ciocalteu’s reagent was added followed by addition of 10 mL of 7% Na\textsubscript{2}CO\textsubscript{3} and 13 mL of sterilized double distilled water. The mixture was incubated at 25°C in the dark for about 90 mins. After incubation, the absorbance was measured at 760 nm. The TPC was determined using standard curve with gallic acid as standard and TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample. Absorbance of all the samples was analyzed in three replications.

2. Estimation of Total Flavonoid Content (TFC)

TFC of the plant extracts was determined using slightly modified colorimetric method\textsuperscript{66}. In this method, 1 mL of plant extract (1mg/mL) was added to a 10 mL volumetric flask containing 4 mL of distilled water. At zero time, 0.3 mL of 5% NaNO\textsubscript{2} and 0.3 mL of 10% AlCl\textsubscript{3}.6H\textsubscript{2}O was added. After 5 min, 2mL of 1 M NaOH was added to the mixture and the final volume was made up to 10 mL with sterilized double distilled water. The solution was mixed well and the absorbance was measured at 510 nm. The TFC was determined using standard curve with quercetin as standard. TFC was expressed as milligrams of quercetin equivalents (QE) per gram of dried sample. Absorbance of all the samples was analyzed in three replications.

3. DPPH radical scavenging assay

The radical scavenging activity of the plant extracts and the isolated compounds was carried out \textit{in vitro} by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay\textsuperscript{67}. 1 mL of various concentrations (20, 40, 60, 80 & 100 µg/mL) of test samples prepared in methanol was added to 2.0 mL of methanolic solution containing DPPH radical (1.0 mM/L). The mixture was vortexed for 1 min and then incubated for 30 min at room temperature. After incubation, the absorbance was measured at 517 nm with ascorbic acid as the standard. The radical scavenging activity (RSA) was calculated using the equation

\[
\% \text{RSA} = \left[ \frac{(A_c - A_s)}{A_c} \right] \times 100
\]

Where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the sample.
RESULTS AND DISCUSSION

TPC of hexane, acetone and methanol extracts of the flowers of *R. suaveolens* were determined spectrophotometrically according to the Folin–Ciocalteu method\(^6\). The results revealed that the methanolic extract (RSM) showed highest TPC (121.96±0.56 µg/mL of GAEs) followed by acetone extract (RSA) (98.88±0.62 µg/mL of GAEs) and hexane extract (RSH) (23.29±0.52 µg/mL of GAEs). The TFC of hexane, acetone and methanol extracts of the flowers of *R. suaveolens* is presented in Table 1 and Fig. 1. Highest flavonoid content was found in methanol extract (68.66±0.61 µg/mL of QEs) and acetone extract (56.93±0.3 µg/mL of QEs) whereas lowest content (13.06 ±0.41 µg/mL of QEs) was found in hexane extract. Comparison of TPC and TFC results of plant extracts presented in Fig. 1, revealed that there is a linear correlation between the total phenolic and flavonoid contents.

**Table 1: Total Phenolic content (TPC) and Total Flavonoid content (TFC) of the extracts of the flowers of *R. suaveolens***

<table>
<thead>
<tr>
<th>Plant extracts (crude)</th>
<th>TPC (µg/ml) as gallic acid equivalents</th>
<th>TFC (µg/ml) as catechin equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extract (RSH)</td>
<td>23.29±0.52</td>
<td>13.06±0.41</td>
</tr>
<tr>
<td>Acetone extract (RSA)</td>
<td>98.88±0.62</td>
<td>56.93±0.30</td>
</tr>
<tr>
<td>Methanol extract (RSM)</td>
<td>121.96±0.56</td>
<td>68.66±0.61</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± standard deviation (n = 3).
The radical scavenging activities of the plant extracts and the isolates (1-4) of the flowers of *R. suaveolens* were estimated by comparing the percentage inhibition of DPPH radicals with that of ascorbic acid. The results showed (Table 2 and Fig. 3) that the DPPH radical scavenging activity of the extracts and the isolates (1-4) were followed in the order 2 > 4 > 3 > 1 > RSA > RSM > RSH. The results revealed that compounds 2 and 4 exhibited highest radical scavenging activity (IC$_{50}$ 51.74 and 57.72 µg/mL) comparable to the positive control, ascorbic acid (IC$_{50}$ 34.15 µg/mL). The acetone and methanolic extracts (RSA and RSM), and compounds 1 and 3 showed moderate antioxidant activities (IC$_{50}$ 118.49, 103.13, 91.00 and 87.35 µg/mL), while the hexane extract (RSH) showed weaker antioxidant activity (IC$_{50}$ 325.28 µg/mL).
Table 2: DPPH radical scavenging activities of the extracts and isolates of the flowers of *R. suaveolens*

<table>
<thead>
<tr>
<th>Extracts / Isolates</th>
<th>20 (μg/mL)</th>
<th>40 (μg/mL)</th>
<th>60 (μg/mL)</th>
<th>80 (μg/mL)</th>
<th>100 (μg/mL)</th>
<th>IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSH</td>
<td>28.22±0.44</td>
<td>29.10±0.34</td>
<td>30.91±0.42</td>
<td>32.78±0.34</td>
<td>33.61±0.25</td>
<td>325.28±0.36</td>
</tr>
<tr>
<td>RSA</td>
<td>43.51±0.67</td>
<td>44.50±0.58</td>
<td>46.81±0.50</td>
<td>47.19±0.60</td>
<td>48.79±0.42</td>
<td>118.49±0.55</td>
</tr>
<tr>
<td>RSM</td>
<td>44.67±0.35</td>
<td>46.43±0.19</td>
<td>46.92±0.58</td>
<td>47.97±0.34</td>
<td>50.33±0.33</td>
<td>103.13±0.36</td>
</tr>
<tr>
<td>1</td>
<td>43.23±0.33</td>
<td>44.72±0.33</td>
<td>46.76±0.42</td>
<td>48.68±0.29</td>
<td>51.32±0.87</td>
<td>91.00±0.45</td>
</tr>
<tr>
<td>2</td>
<td>45.43±0.58</td>
<td>47.91±0.10</td>
<td>51.87±0.25</td>
<td>54.79±0.60</td>
<td>55.83±0.58</td>
<td>51.74±0.42</td>
</tr>
<tr>
<td>3</td>
<td>43.29±0.34</td>
<td>45.99±0.50</td>
<td>46.65±0.50</td>
<td>49.56±0.25</td>
<td>51.38±0.19</td>
<td>87.35±0.36</td>
</tr>
<tr>
<td>4</td>
<td>43.67±0.25</td>
<td>47.47±0.19</td>
<td>51.76±0.19</td>
<td>53.36±0.25</td>
<td>55.72±0.10</td>
<td>57.72±0.20</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>47.86±0.16</td>
<td>50.66±1.35</td>
<td>54.13±0.76</td>
<td>57.15±0.41</td>
<td>59.35±0.53</td>
<td>34.15±0.64</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± standard deviation (n = 3). RSH, RSA, RSM and 1-4, stands for hexane, acetone, methanol extracts and isolates (1-4), respectively of *Rhynchosia suaveolens*.

Fig.3: DPPH radical scavenging activities of the extracts and isolates of the flowers of *R. suaveolens*

SH, RSA, RSM and 1-4, stands for hexane, acetone, methanol extracts and isolates (1-4), respectively of *Rhynchosia suaveolens*.
Conclusions

In the present study, phytochemical investigation of the flowers of *R. suaveolens* resulted in the isolation and characterization of a new benzophenone (1) and three known C-glycosyl phenolic compounds (2-4). Their structures were elucidated by chemical and spectroscopic analysis, including 1D, 2D NMR and ESITOFMS. Among the isolated compounds and plant extracts of the flowers of *R. suaveolens*, mangiferin (2) and isoorientin (4) showed significant DPPH radical scavenging activity which confirmed that the potent antioxidant activity of the plant was due to abundant presence of phenolic compounds, and hence this shrub along with other *Rhynchosia* species which are rich in similar type of compounds may be exploited for their antioxidant potential.
REFERENCES


