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Synthesis, Characterization and Antioxidant Activities of Synthetic Chalcones and Flavones

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Abstract

In the present study, newer chalcones and flavones were synthesized by Algar-Flynn-Oyamada method, purified and characterized by spectral methods. The pure test compounds were evaluated for in vitro antioxidant activity using four models namely 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•), 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•), Nitric oxide (NO) and Lipid Peroxidation scavenging methods. This study indicate that, amongst the ten evaluated chalcones and flavones, five test compounds showed activity with IC50 value lesser than that of the standard ascorbic acid at 91.21 μM for 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging.

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1. Introduction

In the present study we have undertaken the synthesise, characterization and antioxidant activity of novel flavones obtained from various chalcones which are well known for their antioxidant activity. Antioxidants are compounds capable of preventing the damage caused in human tissue by the normal effects of physiological oxidation. Research has shown that antioxidants can play a role in preventing the development of chronic diseases such as cancer, diabetes and cardiovascular diseases.

Chalcones basic structure includes two aromatic ring bound by an α, β-unsaturated carbonyl group, a
unique template associated with very diverse application [1]. Due to the presence of the reactive keto, vinylenic group, chalcones and their analogues have been reported to be antioxidant [2]. Hydroxyl and phenyl substituents are associated with antioxidant properties. When chalcones get cyclised they form flavones. Flavones are also prominent plant secondary metabolites that have been found in dietary components, including tea and red wine. They express various biological activities such as anticancer, antitumour, antiprotozoal and antioxidant [3].

2. Materials and Methods

2.1. Synthesis

The synthesis of the test compounds were undertaken by Algar-Flynn-Oyamada method.

Aryl aldehyde = 3,4-(methylenedioxy)benzaldehyde (JVC1, JVF1)
4-benzyloxybenzaldehyde (JVC2, JVF2)
4-[(2-cyanoethyl)methylamino]benzaldehyde (JVC3, JVF3)
4-[(2-pyridyl)benzaldehyde (JVC4, JVF4)
4-(methylthio)benzaldehyde (JVC5, JVF5)

The synthesized test compounds were purified by recrystalization and characterized by UV-Visible, Infra Red, NMR and Mass spectroscopy.

2.2. Antioxidant activity

The following antioxidant methods were used to evaluate the antioxidant properties of our test compounds.

2.2.1. DPPH• Scavenging Activity

Procedure: Equal volumes of 100 µM 2,2'-diphenyl-1-picrylhydrazyl (DPPH) in methanol was added to different concentrations of test compounds (0 – 200 µM/ml) in methanol, mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured using the spectrophotometer UV-1650, Shimadzu [4]. Plotting the percentage DPPH• scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation

\[
\% \text{ scavenging} = \left(\frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}}\right) \times 100
\]

IC50 was obtained from a plot between concentration of test compounds and % scavenging. Ascorbic acid was used as standard for comparison.

2.2.2. ABTS radical scavenging assay
ABTS is chemically 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid). To the reaction mixture containing 0.5 ml of different concentration (2-200 μM/ml) of compounds, 1.7 ml of phosphate buffer (20 mM) and 0.3 ml of 100 μM ABTS•– (prepared by mixing 2 mM (ABTS2–) with 0.17 mM potassium persulphate in 20 mM phosphate buffer pH 7.4; kept overnight before use) was added. Immediately, the decrease in absorbance was measured at 734 nm [4]. Ascorbic acid was used as standard for comparison. Plotting the percentage ABTS•– scavenging against concentration produced the standard curve. The % scavenging and the IC₅₀ values were calculated as mentioned in the DPPH assay.

2.2.3. Nitric Oxide Scavenging Activity

To a reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, 1.0 ml) and 1.0 ml of different concentration of test compounds/standard were incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed and 1.0 ml of sulphanilic acid was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then 1.0 ml of naphthylethylenediaminedihydrochloride was added, mixed and allowed to stand for 30 min in dark at room temperature. The absorbance of these solutions was measured at 540 nm against corresponding blank solution without sodium nitroprusside [5]. The % scavenging and IC₅₀ values were determined as explained in DPPH assay.

2.2.4. Lipid peroxidation assay

One Wistar rat was sacrificed by cervical dislocation and the whole brain was dissected out and placed in ice cold 0.154 M potassium chloride immediately. The brain was blotted dry, weighed and added to nine times its weight of 0.154 M potassium chloride to prepare 10% homogenate. The tissue was then homogenised carefully in a homogeniser tube placed in an ice bucket to form a smooth homogenate without frothing.

The homogenate thus obtained was immediately centrifuged at 10000 RPM for 10 minutes at 4°C. The supernatant was used for the subsequent steps of the study. To 0.5 ml of rat brain homogenate, 1.0 ml solution of test compounds was added to yield a final concentration of 10 to 400 μM. Lipid peroxidation was stimulated by adding 0.5 ml of 400 μM ferrous sulphate (final concentration 100 μM). The reaction was stopped after 30 min by addition of 2 ml ice-cold TCA-TBA-HCl reagent [containing thiobarbituric acid (0.375% w/v) + trichloroaceticacid(15% w/v) + hydrochloric acid (0.25 N)]. The test tubes were heated in a water bath at 80°C for 15 min and then further centrifuged at 10000 RPM for 10 min. The absorbance of the supernatant was measured at 535nm [6]. Simultaneously, a blank absorbance was determined without the compounds. The % inhibition and IC₅₀ were calculated as explained in DPPH assay method.

3. Result and Discussion

The results of antioxidant values expressed as IC₅₀ with different antioxidant markers used are shown in Table 1. Out of 10 compounds tested using DPPH• scavenging method, JVF3 showed IC₅₀ at 61.4 μM, when compared to that of the standard ascorbic acid at 54.08 μM. However, JVC1-JVC5 did not show any significant activity.

Further, from the antioxidant studies carried out using ABTS• scavenging assay for the 10 test compounds, JVC1, JVC3, JVC4, JVC5, and JVF2 showed IC₅₀ values at 85.3, 53.76, 50.34, 83.15 and 89.12 μM respectively when compared to that of the standard ascorbic acid at 91.21 μM. However, JVF1 and JVF3 showed comparable IC₅₀ value with that of the standard as shown in the table 1.

The antioxidant studies by NO scavenging method showed the IC₅₀ values of all the test compounds and were greater than 300 μM except for JVC3 which showed its IC₅₀ at 250.51 μM when compared to standard
ascorbic acid at 89.33 μM.

Antioxidant studies by Lipid peroxidation method was undertaken for all 10 test compounds, JVC2 showed maximum inhibition of lipid peroxidation with IC₅₀ value of 33.64 μM and JVF3 had IC₅₀ at 358.47 μM when compared to that of the standard quercetin with IC₅₀ at 320.36 μM.

Table 1. Comparison of the IC₅₀ of test compounds against various free-radicals

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DPPH• Scavenging(μM)</th>
<th>ABTS•– Scavenging(μM)</th>
<th>NO Scavenging Activity(μM)</th>
<th>Inhibition of Lipid Peroxidation(μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JVC1</td>
<td>NS</td>
<td>85.3</td>
<td>736.25</td>
<td>530.26</td>
</tr>
<tr>
<td>JVC2</td>
<td>NS</td>
<td>447.5</td>
<td>790.95</td>
<td>330.64</td>
</tr>
<tr>
<td>JVC3</td>
<td>NS</td>
<td>53.76</td>
<td>250.51</td>
<td>457.52</td>
</tr>
<tr>
<td>JVC4</td>
<td>NS</td>
<td>50.34</td>
<td>335.58</td>
<td>NS</td>
</tr>
<tr>
<td>JVC5</td>
<td>NS</td>
<td>83.14</td>
<td>315.93</td>
<td>NS</td>
</tr>
<tr>
<td>JVF1</td>
<td>159.07</td>
<td>109.89</td>
<td>305.71</td>
<td>NS</td>
</tr>
<tr>
<td>JVF2</td>
<td>123.28</td>
<td>89.12</td>
<td>NS</td>
<td>408.78</td>
</tr>
<tr>
<td>JVF3</td>
<td>61.4</td>
<td>100.5</td>
<td>639.94</td>
<td>358.47</td>
</tr>
<tr>
<td>JVF4</td>
<td>457.1</td>
<td>186.3</td>
<td>783.19</td>
<td>NS</td>
</tr>
<tr>
<td>JVF5</td>
<td>143.4</td>
<td>56.68</td>
<td>618.13</td>
<td>425.79</td>
</tr>
<tr>
<td>Standard</td>
<td>*54.08</td>
<td>*91.21</td>
<td>*89.33</td>
<td>#320.36</td>
</tr>
</tbody>
</table>

NS – Not Significant, *Ascorbic acid, #Quercetin

4. Conclusion

The antioxidant properties of the novel synthesised test compounds of chalcones and flavones when tested using 4 different antioxidant evaluating methods it was found that, the ABTS method gave better antioxidant activity for both flavones and chalcones whereas, the DPPH method gave antioxidant profile only for flavones. However, all the four evaluating in vitro methods showed that the test compounds such as JVC1, JVC2, JVC3, TVC4, JVC5, JVF2 and JVF3 had the maximum antioxidant properties. These methods have given us insight and the antioxidant profiles on few of the test compounds tested which will further provide scope for screening other related activities such as antimicrobial, anti-inflammatory and anticancer activities.

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References


