Lemon Catalyzed Fischer Indole Synthesis of 2-Aryl Indoles and Their Antioxidant Study
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ABSTRACT:
We have successfully achieved the synthesis of 2-(4'-substituted phenyl)-1H-indoles in the presence of lemon juice as Lewis acid catalyst and screened for their antioxidant study. The synthetic route is quite mild and efficient, and will be highly helpful in the development of naturally occurring indole skeletons and drug scaffolds containing indole moiety. The synthesized 2-arylindoles were characterized by FTIR, NMR, UV-Vis and mass spectral analyses and screened for antioxidant activity by DPPH assay method. All the compounds showed good to excellent antioxidant property. Thus, further pharmacological and phytochemical analyses are required to develop new bioactive molecules.

Keywords: Lemon catalyst, Fischer indole synthesis, 2-Aryl indoles, DPPH assay, Antioxidant Study

1. Introduction:
Indole is the most potent pharmacodynamic nucleus found in various natural products. The indole ring is also found in many natural products such as the indole alkaloids, fungal metabolites and marine natural products. Indole derivatives were reported to a wide range of biological activities (Sravanthi et al., 2016). Versatile indole alkaloids found in marine products have been reported for specific biological activities such as cytotoxicity, anti-inflammatory, antiviral, serotonin antagonism and so on (Waseem and Hamann, 2005). They are also found to have potential to become new drugs for the treatment of various psychiatric disorders (Kochanowska-Karamyan and Hamann, 2010).

Beyond biological importance, indoles also have significant role in diverse fields such as dyes, plastics, agrochemicals, flavor enhancers and perfumes (Barden, 2011). Later, Yamamoto et al have also demonstrated the ester and amide analogs of indomethacin as positron emission tomography (PET) imaging agents in the visualization of COX-2 enzymes in brain (Yamamoto et al., 2011). Recently, Shimizu et al developed 3,2'-silylene-bridged 2-phenylindoles exhibiting
blue photoluminescence in solid state with excellent fluorescence quantum yields (Shimizu et al., 2011).

Construction of indoles in more concise one-pot method is of considerable interest over a century due to their importance from a biological and economical perspective. Fischer indole synthesis is the most common method for indole cyclization in presence of acidic catalyst (Miller, 2004). The moderate-strength Lewis acid, zinc chloride (ZnCl₂) is widely used as acidic catalyst for Fischer indole synthesis. An environmentally facile procedure has been reported for the indolization using K-10 clay catalyst (Dhakshinamoorthy and Pitchumani, 2005). Although the various catalytic systems for Fischer indole cyclization have been reported in the literature, there is still continuing effort to identify the promoters to enhance the reaction rate under mild reaction conditions. The reported methods suffer from one or more disadvantages such as long reaction times, strong acid, high temperature and tedious work-up procedure to obtain the products of desired purity.

Recently, heterogeneous catalysis has emerged as an important tool in organic synthesis because of their ease of handling, enhanced reaction rates, greater selectivity, simple workup, and recovery of the catalyst. Mhaske and Narshinha have reported a facile zeolite-induced Fischer indole synthesis (Mhaske and Narshinha, 2004). In general, the use of homogenous acidic catalysts in the Fischer indole cyclization is associated with a large number of drawbacks; it has become desirable to find alternate environmentally friendly and recoverable catalysts. However, the above methods require expensive catalysts and liquid acids. We have reported an eco-friendly route for the synthesis of 2-substituted phenylindoles with good yields in the presence of clayzic catalyst and achieved better yields (Sravanthi et. al. 2015). Based on these considerations, we have utilized lemon as acid catalyst for Fischer indole cyclization to obtain 2-arylindoles from various substituted acetophenones and phenylhydrazine. These 2-aryl indoles were evaluated for their antioxidant activity.

2. Experimental:
2.1 General synthesis of 2-arylindoles (3a-3m) with lemon catalyst:

Acetophenone (1a, 0.5 mmol) and phenylhydrazine (2, 0.5 mmol) were dissolved in methanol (10 mL). The mixture was then warmed on a water bath and glacial acetic acid was added till the solution became clear. 2 mL of Lemon juice was added to the mixture and
refluxed on the water bath. The reaction was monitored by TLC. Upon completion of the reaction (40 min), the reaction mixture was cooled to room temperature and filtered into crushed ice. The product formed was then extracted with dichloromethane and evaporated to get the crude product (3a). The crude product was purified by column chromatography using petroleum ether and ethyl acetate mixture (90:10 v/v), yield (89%). The other substituted acetophenones (1b-1g) were also subjected to the same procedure and obtained the cyclized compounds (3b-3g) in about 40-90% yield. The formation of the targets were confirmed by finding their melting points and compared with the reported results. We observed here that a better yield of the desired products was obtained in an ecofriendly way compared to the reported methods. The column purified products were characterized by $^1$H NMR, $^{13}$C NMR, FTIR and GC-MS analyses.

Figure 1: Lemon catalyzed Fischer indole synthesis

2.2 Antioxidant study:

The DPPH radical scavenging method is extensively used to evaluate the antioxidant activity in shorter time duration. DPPH, a stable free radical capable of accepting hydrogen radical or an electron, shows strong absorption band at 517 nm. This assay measures the electron donor capability of the compounds. The color of DPPH changes from violet to yellow on reduction by the compounds.

The radical scavenging activity of synthesized compounds against 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined by using Brand-Williams et al. (1995) method (Brand-Williams et al. 1995). Ascorbic acid was used as a standard. The reaction mixture contains 0.4 ml of 1 mmol freshly prepared DPPH, different volume (80, 160, 240, 320 and 400 µl) of 1mg/ml solution of the compounds and the required volume of ethanol to make the whole mixture to 4 ml. A blank was prepared without the addition of the samples. After additions, the
reaction mixtures were kept in the dark at room temperature for 30 min. The change in color (from violet to yellow) was observed, and their absorbance was measured at 517nm by using UV-Vis spectrophotometer. Lower the absorbance of the mixture indicates the higher radical scavenging activity. The experiment was done in triplicate to find the mean and standard deviation. % Radical scavenging activity was calculated by using the following equation:

\[
\% \text{ Inhibition} = \left( \frac{(AB - AS)}{AB} \right) \times 100
\]

Where, AB – Absorbance of blank sample (t = 0 min); AS – Absorbance of test samples (t = 30 min).

The antioxidant results are tabulated and shows that compounds (3a, 3c, 3d & 3g) possessing better radical scavenging activity (85, 88, 71, 86 and 76%) at 100 μg/ml and the remaining compounds shown moderate. The IC\textsubscript{50} values of all the screened compounds have been calculated and tabulated.

3. Results and discussion:
3.1 Chemistry:

Table 1: Reaction time and product yield (%) in presence of clayzic and lemon catalysts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Clayzic catalyst</th>
<th>Lemon catalyst</th>
<th>Color and appearance</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>time (min)</td>
<td>Product Yield (%)</td>
<td>time (min)</td>
<td>Product Yield (%)</td>
</tr>
<tr>
<td>3a</td>
<td>20</td>
<td>92</td>
<td>40</td>
<td>89</td>
</tr>
<tr>
<td>3b</td>
<td>30</td>
<td>62</td>
<td>120</td>
<td>40</td>
</tr>
<tr>
<td>3c</td>
<td>25</td>
<td>86</td>
<td>90</td>
<td>85</td>
</tr>
<tr>
<td>3d</td>
<td>30</td>
<td>70</td>
<td>90</td>
<td>58</td>
</tr>
<tr>
<td>3e</td>
<td>30</td>
<td>65</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>3f</td>
<td>35</td>
<td>89</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>3g</td>
<td>40</td>
<td>60</td>
<td>90</td>
<td>50</td>
</tr>
</tbody>
</table>

Initially, the cyclization between various acetophenones and phenylhydrazine has been carried out thermally in the presence of clayzic catalyst as per our previous work. The reaction
was completed much earlier also in better yields compared to that obtained with other catalysts (Table 3.1). It is expected that the Lewis acidity of the cations on the edge sites on the K-10 clay catalyst was accelerated by the addition of the ZnCl₂. In order to carry out the synthesis by using some other economical ecofriendly catalysts, we have utilized lemon juice as acid catalyst for the indole cyclization. The successful results prompted us to go for one pot synthesis of the series of 2-arylindoles in the methanol by thermal method.

3.2 Spectral characterization of compounds (3a-3g):

2-Phenylindole (3a): FTIR (KBr, cm⁻¹) v_max: 3442.94 (-NH); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 6.835 (s, 1H_Arom), 7.106-7.181 (m, 4H_Arom), 7.200-7.219 (t, 1H_Arom, J = 7.60 Hz), 7.312-7.683 (m, 4H_Arom) 8.348 (s, 1H, -NH); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 100.01, 110.89, 120.29, 120.69, 122.38, 125.17, 127.74, 129.05, 129.28, 136.82, 137.89; GC-MS m/z: 193.3195 (MH⁺).

2-(4'-Aminophenyl)indole (3b): FTIR (KBr, cm⁻¹) v_max: 3527.80 (-NH); 3257.77 (-NH₂); ¹H NMR (400 MHz, DMSO-d₆, ppm) δ: 5.286 (s, 2H, -NH₂), 6.583-6.603 (d, 4H_Arom, J = 8 Hz), 6.728 (s, 1H_Arom), 6.72 (s, 1H_Arom), 7.202-7.536 (m, 4H_Arom), 8.946 (s, 1H, -NH); ¹³C NMR (100 MHz, DMSO-d₆, ppm) δ: 112.48, 113.42, 118.03, 126.21, 128.75, 130.53, 141.92, 146.62; GC-MS m/z: 208.2850 (MH⁺).

2-(4'-Chlorophenyl)indole (3c): FTIR (KBr, cm⁻¹) v_max: 3442.87 (-NH); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 6.814 (s, 1H_Arom), 7.117-7.912 (m, 8H_Arom), 8.287 (s, 1H, -NH); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 100.62, 111.08, 120.61, 120.90, 122.83, 126.46, 129.31, 129.37, 131.04, 133.59, 136.82, 137.05; GC-MS m/z: 207.1427 (MH⁺).

2-(4'-Methylphenyl)indole (3d): FTIR (KBr, cm⁻¹) v_max: 3433.29 (-NH); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 2.208 (s, 3H, -CH₃), 6.844-6.880 (t, 1H_Arom, J = 14.4 Hz), 7.165-7.183 (d, 4H_Arom, J = 7.2 Hz), 7.238-7.293 (t, 1H_Arom, J = 22 Hz), 7.289 (s, 1H_Arom), 7.673-7.693 (d, 2H_Arom, J = 8 Hz); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 26.85, 122.39, 122.99, 126.43, 126.88, 128.44, 129.07, 129.24, 130.39, 131.13, 145.91, 152.35; GC-MS m/z: 207.1427 (MH⁺).

2-(4'-Methoxyphenyl)indole (3e): FTIR (KBr, cm⁻¹) v_max: 3429.43 (-NH); ¹H NMR (400 MHz, CDCl₃, ppm) δ: 3.833(s, 3H, -OCH₃), 6.842-6.904 (t, 2H_Arom, J = 824.8 Hz), 6.921 (s, 1H_Arom), 7.257-7.293 (d, 4H_Arom, J = 14.4 Hz), 7.729-7.750 (d, 2H_Arom, J = 8.4 Hz); ¹³C NMR (100 MHz, CDCl₃, ppm) δ: 55.35, 113.17, 113.70, 113.74, 119.97, 126.88, 129.24, 130.62, 145.46, 159.67; GC-MS m/z: 224.2504 (MH⁺).
2-(4'-Nitrophenyl)indole (3f): FTIR (KBr, cm\(^{-1}\)) \( \nu_{\text{max}} \): 3429.43(-NH); \(^1\)H NMR (400 MHz, CDCl\(_3\), ppm) \( \delta \): 7.032 (s, 1H \text{Arom}), 7.250-7.296 (t, 2H \text{Arom}, J = 18.4 Hz), 7.322-8.345 (d, 6H \text{Arom}, J = 9.2 Hz), 8.453 (s, 1H, -NH); \(^1^3\)C NMR (100 MHz, CDCl\(_3\), ppm) \( \delta \): 103.48, 111.27, 120.99, 121.39, 123.90, 124.00, 124.59, 125.17, 128.96, 196.35; LC-MS m/z: 238.10 (MH\(^+\)).

2-(4'-Fluorophenyl)indole (3g): FTIR (KBr, cm\(^{-1}\)) \( \nu_{\text{max}} \): 3395.12(-NH); \(^1\)H NMR (400 MHz, DMSO-d\(_6\), ppm) \( \delta \): 6.710-6.729 (d, 4H \text{Arom}, J = 7.6 Hz), 7.123-7.141 (d, 4H \text{Arom}, J = 7.2 Hz), 7.992 (s, 1H \text{Arom}); GC-MS m/z: 213.2732 (MH\(^+\)+2).

3.3 Antioxidant study:

\[% \text{Radical scavenging activity} = \left( \frac{(AB - AS)}{AB} \right) \times 100\]

Where, AB – Absorbance of blank sample (t = 0 min); AS – Absorbance of test samples (t = 30 min).

Table 2: Antioxidant (DPPH radical scavenging) assay results of compounds (3a-3m)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Radical scavenging activity (Mean ± SD)</th>
<th>(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>52.30±0.01</td>
<td>88.27±0.04</td>
</tr>
<tr>
<td>3a</td>
<td>9.18±0.03</td>
<td>32.40±0.04</td>
</tr>
<tr>
<td>3b</td>
<td>1.53±0.01</td>
<td>34.95±0.05</td>
</tr>
<tr>
<td>3c</td>
<td>25.17±0.03</td>
<td>31.72±0.11</td>
</tr>
<tr>
<td>3d</td>
<td>17.18±0.05</td>
<td>31.89±0.09</td>
</tr>
<tr>
<td>3e</td>
<td>10.03±0.03</td>
<td>17.18±0.05</td>
</tr>
<tr>
<td>3f</td>
<td>5.10±0.01</td>
<td>26.87±0.11</td>
</tr>
<tr>
<td>3g</td>
<td>7.91±0.02</td>
<td>11.82±0.02</td>
</tr>
</tbody>
</table>

The antioxidant results are tabulated in Table 3.3 shows that compounds (3a, 3c, 3d & 3g) possessing better radical scavenging activity (85, 88, 71, 86 and 76%) at 100 µg/ml and the
remaining compounds shown moderate. The IC$_{50}$ values of all the screened compounds have been calculated and tabulated.

**5. CONCLUSION**

In conclusion, lemon juice is found to be an economical and ecofriendly catalyst for the synthesis of 2-arylimidoles in short time. The targets were obtained in high yields under mild reaction conditions via a simple working procedure. Also, this method is quite mild and efficient, and will be highly helpful in the development of naturally occurring indole skeletons and drug scaffolds containing indole moiety. The synthesized 2-arylimidoles were characterized by FTIR, NMR, UV-Vis and mass spectral analyses and screened for antioxidant activity by DPPH assay method. All the compounds showed good to excellent antioxidant property. Thus, further pharmacological and phytochemical analyses are required to develop new bioactive molecules.

**6. References:**


