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Anti-Inflammatory and Antioxidant Effect of Various Extracts of *Coleus forskohlii* Root

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Abstract: Currently, medicinal plants are attracting a great deal of interest as sources of chemicals with antioxidant and anti-inflammatory characteristics. Concerning inflammatory processes and oxidative stress, we evaluated the possible therapeutic benefit of several extracts (methanolic, ethanolic, aqueous, and chloroform) of *Coleus forskohlii* root. This research used the *in vitro* antioxidant model as DPPH radical scavenging and anti-inflammatory action as protein denaturation and HRBC membrane lysis. For this objective, a rather novel strategy based on the measurement of anti-inflammatory and antioxidant characteristics in combination was used. Using *in vitro* models, the methanolic extract of *Coleus forskohlii* root has the highest antioxidant and anti-inflammatory activity, followed by ethanol, aqueous, and chloroform extracts. The root of *Coleus forskohlii* might serve as a promising material for the creation of innovative and safer plant-based anti-inflammatory and antioxidant medicines.

Keywords: Anti-inflammatory, Antioxidant, *Coleus forskohlii* root, Medicinal plants, Oxidative stress


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Introduction

Globally, musculoskeletal conditions (such osteoarthritis and rheumatoid arthritis) account for a disproportionate share of the disabled and deceased (Connelly *et al.*, 2006). Medicines having anti-inflammatory effect are the agents of choice in the pharmacological management of various degenerative inflammatory joint diseases (Warner *et al.*, 1999). The cyclooxygenase (COX-1 and COX-2) and lipoxygenase (5-LOX) pathways are well-known to be the primary enzymatic pathways linked to inflammatory processes in mammalian cells, making them of particular therapeutic interest (González-Périz and Clària, 2007). Non-steroidal anti-inflammatory drugs (NSAIDs; e.g. aspirin, ibuprofen, naproxen, and indomethacin) and selective COX-2 inhibitors (e.g. rofecoxib) have well-documented efficacy, but they also have serious side effects, such as the collapse of the stomach wall leading to internal bleeding in the case of NSAIDs (Wolfe *et al.*, 1999; Maxwell and
Webb, 2005). For these reasons, the use of medications that specifically inhibit COX-2 and 5-LOX is currently recognised as one of the most promising strategies for the safer treatment of inflammatory disorders (Fiorucci et al., 2001; Charlier and Michaux, 2003). As a result, research and development into novel drugs that inhibit both COX-2 and 5-LOX remains a major area of focus (Altavilla et al., 2012).

Previous research has revealed a close relationship between oxidative stress and pathogenic inflammatory processes (Halliwell and Gutteridge, 2007, Hemalatha et al., 2017). The generation of proinflammatory or growth-stimulating signals may be further boosted by reactive oxygen species (ROS), which are thought to operate as secondary messengers (Hensley et al., 2000). In addition, elevated levels of ROS may cause DNA damage (such as oxidised DNA bases), which can further progress to carcinogenesis and tumour growth (Gerhauser et al., 2003). Today, the development of anti-inflammatory medications is commonly linked with the investigation of their antioxidant activity in order to assess their broader potential value as prophylactic or therapeutic agents in the treatment of illnesses directly or indirectly associated to inflammation (Poonkodi et al., 2011; Nono et al., 2014; Zhen et al., 2016; Mohanasundaram et al., 2017). In recent years, there has been a resurgence of interest in medicinal plants as sources of compounds with antioxidant and anti-inflammatory properties (Jiang et al., 2014; Lingbeck et al., 2015). In the present study different root extracts of Coleus forskohlii were studied for their antioxidant and anti-inflammatory effects.

**Materials and Methods**

**Plant materials:**

The completely ripe Coleus forskohlii root was harvested from a single plant in Thanjavur, Tamil Nadu, India in January 2018. Dr. S. John Britto, The Director of the Rabinat Herbarium and Centre for Molecular Systematics at St. Joseph’s College in Trichy, Tamil Nadu, India validated the plant root. A voucher specimen was placed in the Rabinat Herbarium at St. Joseph’s College in Thiruchirappalli, Tamil Nadu, India.

**Preparation of extracts:**

The harvested Coleus forskohlii root was washed with distilled water many times to eliminate all signs of contaminants. The root was air-dried and then ground into a coarse powder. For 48 h, the powder was extracted with methanol, ethanol, aqueous and chloroform. After removing the solvent completely at decreased pressure, a semisolid extract was produced. The Coleus forskohlii root extract (CFRE) was refrigerated for further use.

**Antioxidant and anti-inflammatory properties in vitro:**

DPPH radical-scavenging activity was assessed by using Shimada et al. (1992) technique. Using the method of Chandra et al. (2012) anti-inflammatory activity was measured in vitro. Anti-inflammatory activity was assessed using Membrane stabilising activity as suggested by Singh et al. (2013).

**Results and Discussion**

**Radical scavenging action of DPPH:**

Figure 1 depicts the DPPH radical scavenging activity of Coleus forskohlii root extract and the standard ascorbic acid. The half inhibition concentration (IC50) of methanol extract of Coleus forskohlii root was 42.30 g/ml, ethanol extract was 44.37 g/ml, aqueous extract was 45.67 g/ml, chloroform extract was 48.52 g/ml, and ascorbic acid was 38.40 g/ml. The root extract of Coleus forskohlii showed a strong dose-dependent suppression of DPPH activity (Table 1). L-ascorbic acid’s capacity to scavenge DPPH radical is proportional to its concentration. The methanolic extract of Coleus forskohlii root exhibited the highest DPPH activity, followed by the ethanolic, aqueous, and chloroform extracts.

The 1,1-Diphenyl-2-picrylhydrazyl radical is a standard technique for evaluating the capacity of different materials to scavenge free radicals. DPPH
Fig. 1: DPPH radical scavenging activity of Coleus forskohlii root

Table.1: DPPH radical scavenging activity of Coleus forskohlii root

<table>
<thead>
<tr>
<th>Samples</th>
<th>% inhibition (µg/ml)</th>
<th></th>
<th></th>
<th></th>
<th>IC50 value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract</td>
<td>20 (µg/ml)</td>
<td>40 (µg/ml)</td>
<td>60 (µg/ml)</td>
<td>80 (µg/ml)</td>
<td>42.30</td>
</tr>
<tr>
<td></td>
<td>25.55±1.78</td>
<td>48.01±3.36</td>
<td>71.81±5.02</td>
<td>86.34±6.04</td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>23.34±1.63</td>
<td>45.37±3.17</td>
<td>70.48±4.93</td>
<td>84.14±5.88</td>
<td>44.37</td>
</tr>
<tr>
<td></td>
<td>23.34±1.63</td>
<td>45.37±3.17</td>
<td>70.48±4.93</td>
<td>84.14±5.88</td>
<td>44.37</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>22.46±1.57</td>
<td>43.17±3.02</td>
<td>69.16±4.84</td>
<td>83.25±5.82</td>
<td>45.67</td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>19.82±1.38</td>
<td>40.08±2.80</td>
<td>65.63±4.59</td>
<td>80.61±5.64</td>
<td>48.52</td>
</tr>
<tr>
<td>Std. (Ascorbic acid)</td>
<td>27.31±1.91</td>
<td>54.62±3.82</td>
<td>76.21±5.33</td>
<td>91.62±6.41</td>
<td>38.40</td>
</tr>
</tbody>
</table>

Values expressed as Mean ± SD for triplicate

is a nitrogen-centered radical whose colour changes from violet to yellow following reduction by hydrogen or electron donation. Substances with the potential to catalyse this reaction may be quantified as antioxidants and, therefore, radical scavengers. It was noticed that the activities of Coleus forskohlii root extract to scavenge free radicals increased with increasing concentration. The antioxidant ingredient in the extract neutralised the DPPH free radical solution and transformed it to a reduced state by either giving an electron or donating a hydrogen atom followed by a proton (Nuutila et al., 2003; Sounder et al., 2018). The DPPH radical scavenging activity of Coleus forskohlii root extract is compared to the standard ascorbic acid. The plant extract inhibited DPPH activity significantly and dose-dependently. L-ascorbic acid’s ability to scavenge the DPPH radical is proportionate to its concentration. The methanolic extract of Coleus forskohlii root exhibited the highest DPPH activity, followed by the ethanolic, aqueous, and chloroform extracts. The DPPH test activity was comparable to the ascorbic acid standard.

Anti-inflammatory properties:
Using protein denaturation (bovine serum albumin) and membrane stabilisation, the anti-inflammatory efficacy of the root extract of Coleus forskohlii was examined. Coleus forskohlii root extract inhibited the denaturation of Bovine serum albumin in a concentration-dependent manner, with Diclofenac sodium serving as the standard medication. It was discovered that the greatest dosage of Coleus forskohlii root methanol extract (500 g/ml) was comparable to diclofenac sodium. The half inhibition concentration (IC50) of Coleus forskohlii root methanol extract (244.91 g/ml),
ethanol extract (253.08 g/ml), aqueous extract (266.84 g/ml), chloroform extract (281.02 g/ml), and standard was equivalent to 226.01 g/ml. This study indicated that *Coleus forskohlii* root extract had a significant anti-inflammatory effect *in vitro* against protein denaturation (Table 2; Fig. 2). Using a protein denaturation model, the anti-inflammatory efficacy of *Coleus forskohlii* root was best in methanolic extract, followed by ethanol, aqueous, and chloroform extracts.

Using membrane stabilisation, the anti-inflammatory effect of *Coleus forskohlii* root extract was studied. The membranes of human erythrocytes were shown to be protected against hypotonic solution-induced lysis via a concentration-dependent mechanism. It was discovered that the highest dosage of *Coleus forskohlii* root methanol extract (500 g/ml) was comparable to diclofenac sodium. The half inhibitory concentration (IC$_{50}$) of *Coleus forskohlii* root methanol extract was (231.90 g/ml), ethanol extract was (240.45 g/ml), aqueous extract was (257.93 g/ml), chloroform extract was (285.62 g/ml), and the standard was 225.12 g/ml. According to this study, *Coleus forskohlii* root extract showed a significant *in vitro* anti-inflammatory activity against membrane lysis (Table 3; Fig. 3). Through an HRBC membrane lyses model, the anti-inflammatory efficacy of *Coleus forskohlii* root is best in methanolic extract,
followed by ethanol, aqueous, and chloroform extracts.

Protein denaturation includes the breakdown of the secondary, tertiary, and quaternary structures of the molecules and ultimately results in cell death; it is caused by stresses such as high levels of salt, high temperature, and high acidity. Probably, the process of denaturation includes changes in electrostatic, hydrogen, hydrophobic, and disulphide bonds. It is widely recognised that protein denaturation contributes to inflammatory disorders such as rheumatoid arthritis, diabetes, and cancer. The majority of researchers have stated that protein denaturation is one of the causes of RA owing to the development of autoantigens in certain rheumatic illnesses (Aswini et al., 2017). In the current investigation, there was a considerable suppression of protein denaturation in both the standard and extract-treated groups, and the percentage inhibition of protein denaturation generated by the extract at a concentration of 500 µg/ml was comparable to that of the standard (diclofenac sodium). By reducing the in vivo denaturation of proteins in rheumatic illnesses, Coleus forskohlii root extract may inhibit the generation of autoantibodies, according to the findings of this study (Deshpande et al., 2009).

Membrane stability prevents serum protein
and fluid leakage into tissues during a time of elevated permeability produced by inflammatory mediators (Chaitanya et al., 2011). Stabilizing impact on erythrocyte lysis generated by heat and saline is an excellent indicator of anti-inflammatory and, by extension, anti-arthritic action. RBC membranes are comparable to lysosomal membranes. In inflammatory conditions, stabilising the lysosomal membrane prevents the release of lysosomal contents, including as proteases and bactericidal enzymes, that induce additional tissue inflammation and injury upon extracellular release (Deshpande et al., 2009; Leelaprakash and Dass, 2011; Reshma and Arun, 2014). According to the findings, Coleus forskohlii root extract significantly inhibited proteinase activity. Present work concurs with observations of Shilpa et al. (2018) who have revealed anti-arthritic activity (in vitro models) of Hibiscus hispidus Griffith by protein denaturation and membrane stabilisation technique.

Conclusion

In this study, we explored the potential therapeutic benefit of several Coleus forskohlii root extracts in illnesses and diseases associated with inflammatory processes and oxidative stress, either directly or indirectly. For this objective, a rather novel strategy based on the measurement of anti-inflammatory and antioxidant characteristics in combination was used. Using in vitro models, the methanolic extract of Coleus forskohlii root has the highest antioxidant and anti-inflammatory activity, followed by ethanol, aqueous, and chloroform extracts. The root of Coleus forskohlii may serve as a promising material for the future development of innovative and safer plant-based anti-inflammatory and/or antioxidant medicines.

References


