Evaluation of antiangiogenic and antioxidant activity of *Harpagophytum procumbens* (devil’s claw)

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Malath Faisal Ghazi¹

**ABSTRACT**

**Introduction:** *Harpagophytum procumbens* an indigenous plant to southern Africa which has a wide pharmacological activity. It is also known as Devil’s Claw and it historically been used to treat a wide range of conditions, including pain and arthritis. The plant is traded internationally and it is difficult to cultivate. **Objective:** The present study is designed to investigate the possible anti-angiogenic activity and to search for the presence of different phytochemicals in *Harpagophytum procumbens* methanol extract. **Materials and Methods:** The method includes qualitative analysis of various secondary metabolites by specific chemical tests which is carried out on the extract and the ex vivo rat aorta ring assay is used to screen the extract for possible anti-angiogenesis activity. This assay is also used to determine the dose-response effect of the active extract. Moreover, five concentrations of crude extract are tested (100μg/ml, 50μg/ml, 25μg/ml, 12.5μg/ml & 6.25μg/ml) on rat aortic rings to assess the dose response relationship. **Results:** preliminary phytochemical investigation of alcohol extract indicates the presence of various chemical constituents and it is showed a significant (P*<0.001) dose-dependent blood vessels inhibition in comparison to the 1% DMSO. **Conclusion:** The extract of *H. procumbens* is shown an antioxidant and antiangiogenic so substance neutralizing the oxidative stress and highly angiogenesis process can be used as a modulatory or adjuvant treatment to maintain the hemostasis of the organisms and diminish the risk of diseases.

**KEY WORDS:** Antiangiogenic, Antioxidant, *Harpagophytum procumbens*, Phytochemical analysis

**INTRODUCTION**

Harpagophytum spp. belongs to some of the most studied medicinal plants with pharmacological activity analysis and clinical tests dating back to the 1960s.¹ *Harpagophytum procumbens*, also known as devil’s claw, is an herbaceous plant species that has extended medicinal uses in Southern Africa.¹² In addition, historically, the plant has been used to treat several disorders such as fever, malaria, indigestion, and pain.¹³ Moreover, it is demonstrated that *Harpagophytum procumbens* extracts have a beneficial effect to treat rheumatic diseases according to animal and clinical studies.¹⁸ Many reports verifying that *H. procumbens* has anti-inflammatory effects on acute or subchronic inflammation in rat model.¹⁵ Furthermore, *H. procumbens* roots are used in traditional medicine in the treatment of fever and other blood diseases, digestive disorders, arthritis, and rheumatic disorders.¹⁷ Tubers extract was used in the treatment of degenerative rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation, and heart disease.¹⁹

Angiogenesis is a process that involves the formation of new blood vessels from preexisting one; this process is typically initiated within hypoxic tissues where additional new blood vessels are required to maintain oxygenation and nutritional supply.¹⁰ The molecular and cellular changes associated with angiogenesis such as recruitment and integration of pericytes in vessel wall for capillaries and smooth muscles for large vessels.¹¹ Furthermore, the angiogenesis process is an important for tumor growth, metastasis, and the mechanism of resistance most therapies still unknown.¹¹ Strict regulation of this system is very important for the human being because both excessive and inadequate development of blood vessels lead to serious diseases.¹² However, scientific studies are lacking regarding the *H. procumbens* antiangiogenic and antioxidant effect. The aim of this research is to study the phytochemical properties, antiangiogenic activity of *H. procumbens* methanol extract.

**MATERIALS AND METHODS**

**Plant Materials Preparation**

Aerial parts of *H. procumbens* were bought from local market in Baghdad and were powdered and stored at

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room temperature 25°C. The plant was authenticated by the National Herbarium at Baghdad.

**Preliminary Qualitative and Phytochemical Analysis**

Chemical tests and screening of various secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, and glycosides were carried out using the methanol extract from plant using standard procedures to identify the active constituents.[13,14]

**Extracts Preparation**

About 100 g of powdered plant materials were extracted by 250 ml of 85% methanol using Soxhlet apparatus until exhaustion for 12 h. Plant extract was filtered through Whatman No.1 filter paper and crude extract obtained by removing solvent in vacuum evaporator at 40°C. Residues were stored at 4°C for further analysis.

**Antioxidant Activity (AA) (2,2-diphenylpicrylhydrazyl [DPPH] Radical Scavenging Assay)**

The scavenging activity of free radical of the active extract was assessed using the DPPH protocol. 200 μl of 0.1 mM DPPH dissolved in methanol was poured to 100 μl of the plant’s extract of various concentrations (200, 150, 100, 50, and 25 μg) and incubated for 30 min. Furthermore, this procedure was executed using 96-well plate and each concentration was tested in triplicate and the absorbance was observed at 517 nm using an ELISA reader. Moreover, ascorbic acid (Vitamin C) was used as a positive control and the blank was methanol only. The negative control was prepared from 100 μl of methanol and 200 μl DPPH. The percentage of AA was calculated according to the formula below:[15]

\[
\text{AA}\% = 1 - \left( \frac{AS - AB}{AC - AB} \right) \times 100
\]

Where,

- \( AS \) = Absorbance of sample
- \( AB \) = Absorbance of blank
- \( AC \) = Absorbance of control

**Rat Aorta Ring Antiangiogenic Assay**

The experimental procedures were revised and approved by Ethics Committee of Al-Nahrain University/College of Medicine. The assay was performed according to the standard protocol.[13] Albino male rats were sacrificed through cervical dislocation under anesthesia with diethyl ether. Then, thoracic aorta was excised and rinsed with serum-free media, and cleaned from the fibroadipose tissue and was cross-sectioned into thin rings of 1 mm thickness. After that, 300 μl of M199 growth medium (prepared by addition of fibrinogen 3 mg/mL and aprotinin 5 μg/ml to M199) was used as lower layer and loaded in 48-well plate and one aortic ring was seeded in each well. Hence, 10 μl of thrombin was added to each well and then incubated to solidify at 37°C in 5% CO₂ for 30–60 min. Then, the top layer medium was prepared by addition of 20% heat-inactivated fetal bovine serum, 1% L-glutamine, 0.1% aminoacaproic acid, 1% amphotericin B, and 0.6% gentamicin to M199 medium. A stock solution of *H. procumbens* extract was prepared by dissolving the sample in dimethyl sulfoxide (DMSO) and diluted in M199 growth medium to make the final DMSO concentration 1%. Serial dilutions of the active extract were prepared in the following concentrations: 100, 50, 25, 12.5, and 6.25 μg/ml. Wells without test samples were received medium with 1% DMSO used as the negative control. The tissue rings were incubated at 37°C, 5% CO₂ in a humidified incubator. The results examined on day 5 under inverted microscope and the extent of blood vessel growth was quantified under ×10 magnification with the aid of camera and software package. The magnitude of blood vessel growth inhibition was determined according to the technique developed by Brown *et al.*[16] The experiment was repeated 3 times using six replicate per sample, and the percentage of blood vessels inhibition was calculated according to the following formula:

\[
\text{Blood vessels inhibition} = 1 - \left( \frac{A_0}{A} \right) \times 100
\]

Where,

- \( A_0 \) = distance of blood vessels growth for the test substance in mm.
- \( A \) = distance of blood vessels growth in the control in mm.

The concentration that inhibits 50% of the growing blood vessels “IC₅₀” was calculated using the logarithmic equation for the extract.

**RESULTS AND DISCUSSION**

**Phytochemical Screening of *H. procumbens***

Preliminary screening of *H. procumbens* extracts is indicated the presence of flavonoids, glycosides, sterols, and terpenoids in methanol extracts, as shown in Table 1.

**AA of *H. procumbens***

The scavenging activity of free radicals for *H. procumbens* extract was assessed using the DPPH assay. A serial of five concentrations was used to evaluate the scavenging activity as shown in Table 2. Ascorbic acid has significantly (\( P^* < 0.01 \)) higher scavenger activity compared to *H. procumbens* extract at concentrations of 25, 50, and 100 mg/ml; however, 200 mg/ml of *H. procumbens* was shown no significant difference (\( P > 0.01 \)) (i.e. similar AA to ascorbic acid) [Table 2].

*Harpagophytum procumbens* is an indigenous plant which is grown in southern Africa and it has a wide pharmacological activity. That *H. procumbens* is a potent source of natural antioxidant and has a
comparable antioxidant effect to ascorbic acid at high concentration of 200 mg/ml [Table 2]. This AA may be attributed to the polyphenolic compounds like flavonoids which are discovered by phytochemical analysis [Table 1].[17,18] Interestingly, free radicals are generated as a by-product of biological reaction or exogenous factors which are involved in many disorders such as cancer, neurodegenerative disease, and AIDS.[19,20] Altogether, *H. procumbens* can express its antioxidant activity due to the presence of flavonoid compound. The mechanism of the action of this polyphenolic flavonoid may be related to its scavenging or chelating process against the free radicals.[21] Antiangiogenic Activity of *H. procumbens*

Five dilutions of *H. procumbens* extract (100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, and 6.25 μg/ml) were prepared and added to the embedded rat aortic rings to detect the dose-response curve. After 5 days of the experiment, the results are indicated a significant ($P < 0.01$) dose-dependent inhibition of blood vessels growth when compared to DMSO 1% as found in Table 3. Furthermore, the dose-response effect of samples on blood vessels growth is shown in Figure 2. The IC$_{50}$ for each concentration of methanol extract was determined from the logarithmic equation that is shown in Figure 3, and it was found to be 2.71 μg/ml.

### Table 1: Qualitative phytochemical analysis of *H. procumbens* methanol extract

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Chemical test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Borntrager’s test</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>Liebermann–Burchard test</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Sulfuric acid test</td>
<td>+ve</td>
</tr>
</tbody>
</table>

### Table 2: The percentage of DPPH free radical scavenging activity for methanol extract of *H. procumbens* which is compared to ascorbic acid activity

<table>
<thead>
<tr>
<th>Concentration <em>H. procumbens</em> (mg/ml)</th>
<th>Ascorbic acid (mg/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>54.27±1.74</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>50</td>
<td>63.70±0.2</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>100</td>
<td>71.25±0.2</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>150</td>
<td>82.5±2.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>200</td>
<td>85±0.00</td>
<td>&gt;0.01</td>
</tr>
</tbody>
</table>

Scavenger activity presented as mean±SEM. *Significant difference.

DPPH: 2,2-diphenylpicrylhydrazyl.

### Table 3: Serial concentrations and their inhibition percentage %

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>77</td>
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<tr>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>12.5</td>
<td>14</td>
</tr>
<tr>
<td>6.25</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1: The antioxidant activity of *Harpagophyllum procumbens* extract in compare with ascorbic acid which shows that *H. procumbens* has comparable antioxidant activity to ascorbic acid as its concentration increased.

Figure 2: Dose-response inhibition effect of *Harpagophyllum procumbens* methanol extract in rat aortic ring assay, (a) 1% dimethyl sulfoxide, (b) 6.25 μg/ml, (c) 12.5 μg/ml, (d) 25 μg/ml, (e) 50 μg/ml, (f) 100 μg/ml.

Figure 3: (a and b) Dose-response curve of methanol extract of *Harpagophyllum procumbens* in rat aortic rings model versus concentrations mg/ml which is shown that antiangiogenic activity increased with concentration.

Where $Y$ = the inhibition percentage and $X$ = the concentration.

Furthermore, angiogenesis, the formation of new blood vessels, is a part of physiological and pathological process. Angiogenesis can contribute in menstrual cycle and wound healing while disturbance of this process can lead to several disease conditions such as cancer, stroke, and atherosclerosis.[22] Many studies have reported that free radicals may regulate the physiology and pathology of angiogenesis process. More in the point, free radicals can influence the production of vascular endothelial growth factor and its receptor also modulates many transcription factors (CREB, hypoxia-inducible factor-1, and activator protein-1).[23,24] *H. procumbens* contains different compounds such as...
flavonoid, glycosides, and terpenoids [Table 1] which express antioxidant, anti-inflammatory, and importantly antiangiogenic.[25-26] Antioxidants of these constituents can affect the physiology of angiogenesis in vivo through modulation of nitric oxides synthase expression and activation.[27] Therefore, this antiangiogenic effect can reduce the oxygen and nutrition as a result tumor growth slowing and reduce the likelihood of metastasis.

CONCLUSION

The phytochemical investigation of H. procumbens is appeared a presence of several constitutions of the secondary metabolites including glycosides, flavonoids, terpenoids, and steroids. These secondary metabolites can be assessed more to investigate further activity and pharmacological effect. Importantly, the extract of H. procumbens is demonstrated an antioxidant and antiangiogenic activities. Disturbance of angiogenesis and oxidative stress can lead to several serious disorders such as cancer, diabetes, and ischemic heart diseases. Therefore, substance neutralizing the oxidative stress and highly angiogenesis process can be used as a modulatory or adjuvant therapy to maintain the hemostasis of the organisms and diminish the risk of diseases.

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REFERENCES