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ORIGINAL ARTICLE

In vitro Antioxidant and Anti-inflammatory potential of *Harpagophytum procumbens* leaves for its use in the treatment of Inflammatory Pathologies

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ABSTRACT
The present study aimed to systematically evaluate the antioxidant and anti-inflammatory potential of the ethanol extract of Harpagophytum procumbens leaves. The study evaluated the total phenolic content of the extract that was represented in terms of gallic acid equivalent. Further, the antioxidant potential of the extract was assessed using FRAP assay and DPPH radical scavenging potential whereas the anti-inflammatory property was evaluated using xanthine oxidase inhibition and albumin denaturation inhibition studies. The total phenolic content of the extract was 14.5% that also demonstrated potent antioxidant ability. It was observed that the antioxidant activity using FRAP assay was 1558.4 FRAP units at a concentration of 100 µg/ml extract whereas the DPPH radical scavenging activity revealed 68.5% inhibition of the free radicals as against that of the standard ascorbic acid. Furthermore, the activity of xanthine oxidase was inhibition by 76% that fared much better than that of the standard oxypurinol. Similarly, the albumin denaturation was also inhibited by 65%. Overall, the study provides a strong basis for further in vivo studies in terms of the anti-inflammatory potential of the plant for the treatment of various pathological conditions.
Keywords: *Harpagophytum procumbens; anti-inflammatory; xanthine oxidase; antioxidant; albumin denaturation.*

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INTRODUCTION

Reactive oxygen species (ROS) are produced in the mammalian cells as a result of endogenous and exogenous activities primarily in the mitochondria[1]. They are the chemical molecules containing one unpaired electron produced from molecular oxygen. Under normal circumstances, the cells possess mechanisms to counter these ROS by the production of antioxidant enzymes[2]. However, an imbalance in this regulation develops owing to the increased production or impaired regulation of ROS resulting in oxidative stress condition. While an acute stage is a resultant of certain illness, if this condition is left untreated it results in a chronic state leading to damage to various organs of the body. This can likely prove fatal if left untreated [3]. Furthermore, inflammation is one such pathway that leads to an excessive and uncontrollable elevation in the production of ROS that can cause severe tissue damage. This will augment to the impaired antioxidant pathways of the body resulting in organ failure [4]. In this regard, treatment of the disease condition with an adjunct antioxidant agent has proven beneficiary.

Use of plant materials as a formulation for the treatment of several diseases has been well documented. Traditional formulations from diverse cultural backgrounds have been reported in various medicinal practises. It has been reported that in India around 80% of the population depend on herbal formulations for treating ailments from minor illnesses to major conditions such as diabetes and cardiovascular ailments [5]. Yet, a scientific basis to describe the mechanism of action of these herbal formulations with emphasis on various bioactive components present in the formulation has been lacking. Past few decades

have witnessed a surge in research regarding the identification of bioactive compounds present in the formulation that is responsible for the visible medicinal value. In this regard, *Harpagophytum procumbens* (Devil's claw) is a herbaceous plant with great medicinal value that grows in the Kalahari Desert region of Africa. Ailments including fever, diabetes, diarrhea and blood diseases have been treated using the tubers of this plant since time immemorial[6]. More recently, extracts of the secondary roots of the species were proven to be effective in the treatment of degenerative disorders including rheumatoid arthritis, osteoarthritis, tendonitis, kidney inflammation and heart disease [7]. Therefore, as an alternative to non-steroidal anti-inflammatory drugs formulations from this plant is gaining popularity for the treatment of several types of inflammation. The crude methanolic extract may be attractive for various commercial purposes since it displayed antioxidant activity and it can be conveniently and economically prepared.
With this background, the present study aims at the evaluation of the antioxidant and anti-inflammatory potential of *H.procumbens* using *in vitro* assays.

MATERIAL AND METHODS

Preparation of plant material

H. procumbens leaves were shade-dried and coarsely powdered using a kitchen blender. 500 g of this powder was subjected for extraction sequentially using 100% ethanol in a Soxhlet apparatus. The obtained extract (HPE) was concentrated using a rotary evaporator and stored at 4°C until further use.

Phytochemical screening for total phenolic content

Total phenolic content (TPC) was evaluated as per Jayanthi *et al*.[8]. Briefly, the plant HPE along with the positive control (gallic acid) were prepared at a concentration of 1 mg/ml from which working standard solutions were prepared at a concentration of 25 mg/ml. To this solution, 750 µL Folin-Ciocalteu reagent was added, mixed and incubated room temperature for 5 min. To this mixture, 750 µL of 6.0% (w/v) sodium carbonate was added, mixed well and incubated at 25°C for 60 min. Finally, the absorbance of the tube was recorded at 765 nm using a spectrophotometer. The reaction was carried out in triplicates and the TPC of the sample was represented as gallic acid equivalent/g dry weight.
TPC = (C) V/M where,
TPC = Total phenolic content (mg GAE/g DW)
C = Concentration of GA from calibration curve linear equation (mg/ml)
V = Volume of the extract solution (ml)
M = Weight of extract used (g).

Evaluation of antioxidant activity

DPPH radical scavenging activity

DPPH radical scavenging ability of the HPE was evaluated as per Mallikarjunaswamy *et al.* [9]. Briefly, varying concentrations of the extract (0.5 ml) was taken with 3.5 ml DPPH solution and incubated in dark conditions at room temperature. The absorbance was then measured at 540 nm was measured with distilled water blank.
% scavenging activity = $(A^0 - A^1)/A^0 \times 100$ where,
$A^0$is the absorbance of the blank and A^1 is the absorbance of the standard or test.

Ferric reducing power assay

The antioxidant potential of HPE was assessed using FRAP assay as per the method given by Jayanthi *et al*. [10]. The freshly prepared FRAP was warmed at 37 °C and mixed with varying concentrations of HPE. The aliquots were mixed thoroughly and incubated at 37°C for 10 min followed by the measurement of absorbance at 593 nm using a spectrophotometer. The results obtained were represented as FRAP equivalent by taking $FeSO_4$ as a standard.

Anti-inflammatory activity

Xanthine oxidase inhibitory potential

Xanthine oxidase inhibition was evaluated as per the method given by Chouikh *et al*. [11]. Varying concentrations of the HPE (0.1% each) at 100 µl volume was taken along with 300µl of 50 mM potassium phosphate buffer (pH 7.5) to which 100 µl freshly prepared xanthine oxdiase was added. The volume was made using 100 µl deionized water and the entire mixture was incubated at 37°C for 15 min. To this mixture 15 mM (200 µl) was added and re-incubated at 37 °C for 30 min. The enzymatic reaction was halted by the addition of 0.5 M hydrochloric acid to all the tubes. The activity of xanthine oxidase was measured at 295 nm using a spectrophotometer. In this assay, oxypurinol was used as a positive control while the assay mixture without HPE was taken as negative control. This assay was carried out in triplicates and the percent inhibition was taken as a mean of the three observations. The activity was represented using the formula,
% XO inhibition = (1−B/A)×100%Where,

B is the absorbance reading of the test sample, and A is the absorbance reading without test sample (negative control).

Albumin denaturation inhibition assay

The albumin denaturation inhibition assay was performed as per Babu & Noor [12]. Briefly, 0.1% (1000 µl each) of varying concentration of HPE was taken along with 200 µl egg albumin and 1400 µl phosphate buffered saline. In addition, egg albumin mixture with diclofenac served as a positive control whereas distilled water with egg albumin mixture served as a negative control. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. After the incubation period, the reaction mixture was cooled and the absorbance was measured at 660 nm using a spectrophotometer. The percent inhibition of albumin denaturation was evaluated using the formula,

% Denaturation inhibition = (1−D/C)×100%Where,

D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics version 17.0. The results were expressed as mean ± standard deviation obtained from the triplicate readings of the assays. The data were evaluated using one-way ANOVA. Further, Tukey's post hoc test was performed for a pair-wise comparison in the means of the control to that of the test.

RESULTS AND DISCUSSION

Total phenol content

The total phenolic content of HPE was expresses as gallic acid equivalent as given in Figure 1. The R2 value of 0.993 was obtained with the gallic acid calibration curve plotted for F-C total phenolic content assay.

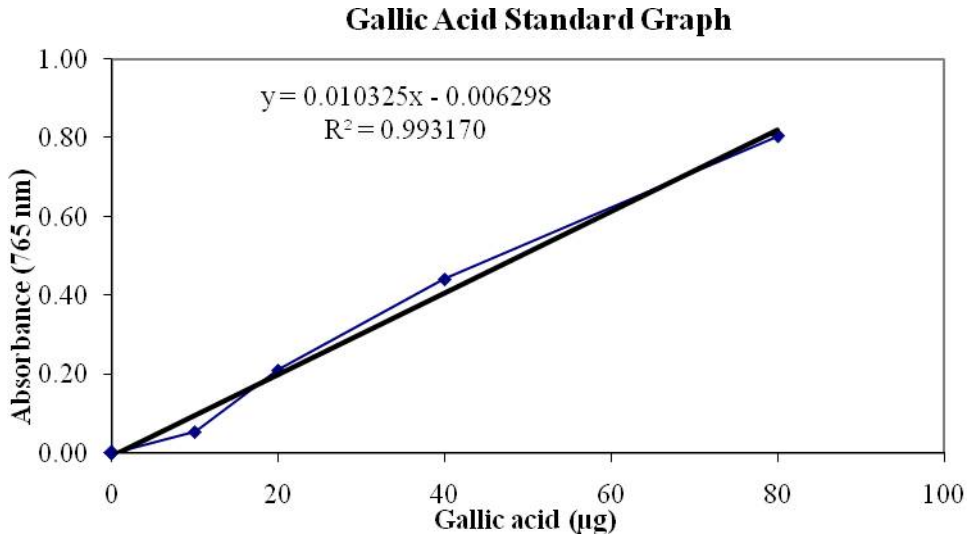

Figure 1: Gallic acid calibration curve plotted for total phenolic content of *H. procumbens*.

Phenolic acids are predominantly present as plant secondary metabolite. The extraction using ethanol could be a likely reason for the high phenolic content from HPE. Reports of greater antioxidant potential from plant extracts which are rich in phenolic content have been recorded [13] [14] [15]. In this regard, in the present study HPE showed relatively high percentage phenolic acid content (14.5%) in agreement with several studies.

Antioxidant activity

The antioxidant activity of HPE was evaluated using DPPH free radical scavenging ability and FRAP assay. In our study, remarkable antioxidant activity was observed in HPE in a dose-dependent manner. It was seen that at a concentration of 50 µg/ml highest antioxidant activity was witnessed in terms of FRAP units (Figure 2).

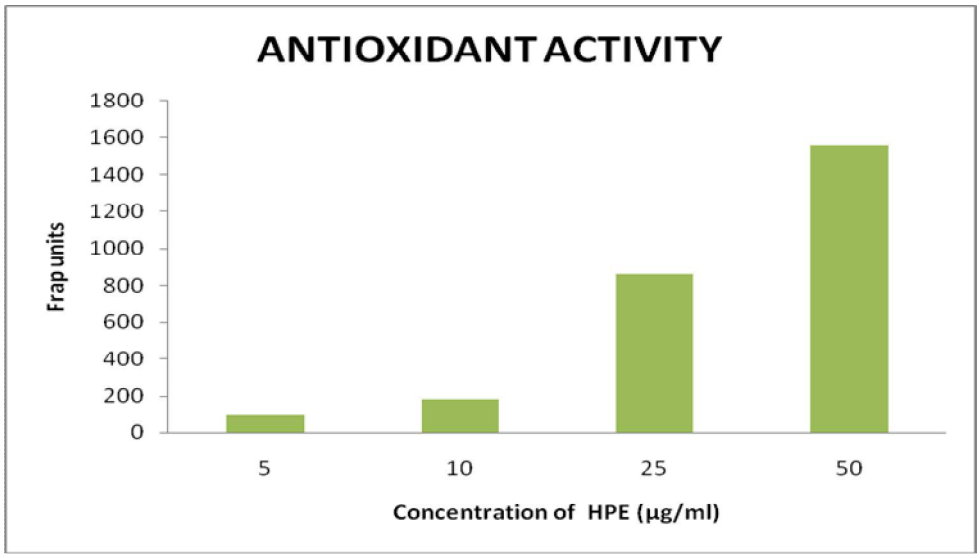

Figure 2: Antioxidant activity of *H. procumbens* in terms of FRAP activity

Furthermore, the antioxidant potential was also evaluated using the radical scavenging ability of DPPH free radical. In our study, it was observed that free radical scavenging potential of the extract was remarkable in comparison with the standard ascorbic acid/vitamin C. The activity was increasing in a dose-dependent manner with the highest activity seen at a concentration of 100 µg/ml(**Figure 3**).

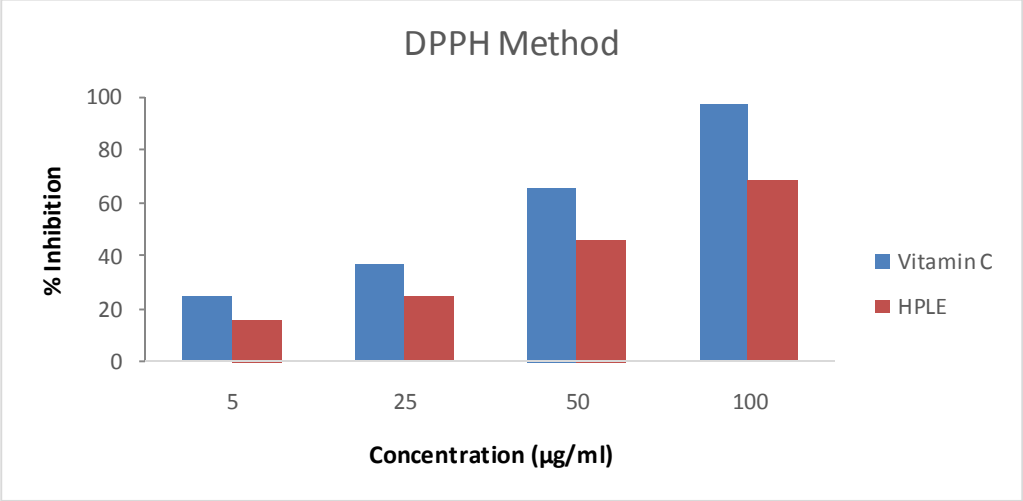

Figure 3: Estimation of radical scavenging activity of *H. procumbens* by DPPH method

This property as stated earlier is rendered because of the rich phenolic compounds present in the extract as confirmed by several studies. The structural feature of these phenolic compounds is expected to be the deciding factor of the antioxidant properties of the extract. The extent of hydroxylation on the aromatic phenols gradually enhances its antioxidant potential. A detailed phytochemical screening will reveal the various phenolic compounds that are responsible for the expected results. The results obtained in our study are on comparable to that of the activity exerted by the standard ascorbic acid, suggesting that the extract contains primary antioxidant compounds that are able to counter the released free radicals thereby blocking the chain reaction associated with the generation of free radicals [16] [17] [18]. Overall, the antioxidant property exerted by HPE was on par with that of the standard ascorbic acid in DPPH assay. HPE also showed remarkable antioxidant potential as observed in the FRAP assay.

Anti-inflammatory property

Xanthine oxidase inhibitory potential

HPE showed a remarkable anti-inflammatory activity as evaluated by xanthine oxidase inhibition. From the study, it was evident that at a concentration of 50 µg/ml 76% inhibition of xanthine oxidase activity was seen. Yet, there was a dose-dependent increase in the activity and the results were much better than

that of the standard used i.e., oxypurinol (65.66%). Figure 4.Xanthine oxidase is an enzyme that catalyzes the oxidative hydroxylation of purine residues and corresponding production of reactive oxygen species by the generation of O_2. In inflammatory diseases such as rhumetoid arthritis and others xanthine oxidase catalyzes the conversion of hypxanthine to xanthine which in turn results in the formation of uric acid that accumulates in the joints. Levels of this enzyme provide significant information on the pathological condition in patients suffering from ischemia-reperfusion, inflammation, hepatitis cancer and aging [19] [20]. Therefore, inhibition of this enzyme can be a useful in the treatment of inflammation. The most popularly available drug for the treatment of inflammation caused by gout is allopurinol that competitively inhibits the action of xanthine oxidase. The adverse effects observed during this treatment such as skin rashes and some time death due to drug reactions have led to the research on safer alternatives. In this regard, HPE has provided promising evidence for its use as a potent anti-inflammatory agent.

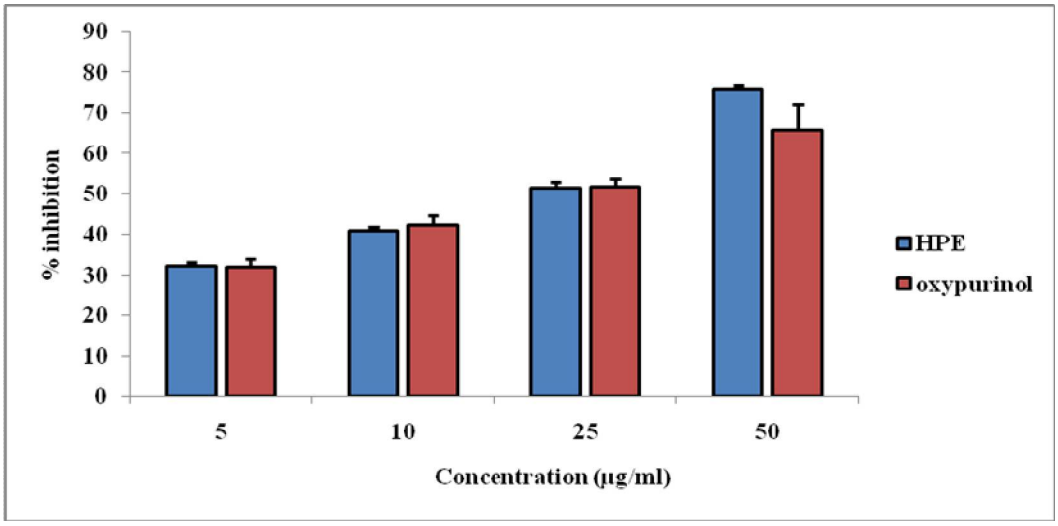

Figure 4: Xanthine oxidase inhibitory activity exerted by *H. procumbens*.

Albumin denaturation inhibitory property

HPE exerted a remarkable inhibition of albumin denaturation in comparison with that of diclofenac standard (**Figure 5**).

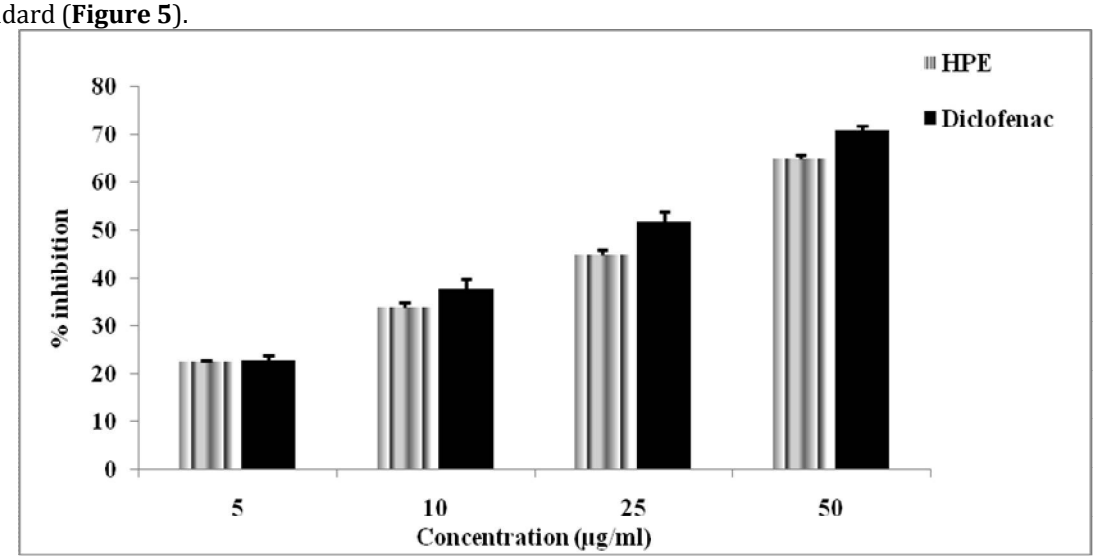

Figure 5: Percentage of inhibition of albumin denaturation exerted by *H. procumbens*.

There is a commendable dose-depended inhibition exerted by HPE whereby the more concentrated extract (50µg/ml) shows the highest percentage of inhibition of 65%. Denaturation of protein is a well-documented cause of inflammation, which has been implicated in the pathogenesis of several diseases including cardiac disorders. Drugs such as phenylbutazone, salicylic acid, flufenamic acid used to treat inflammation have shown a dose-dependent ability to thermally induced protein denaturation. As per

Opie [21] denaturation of proteins within the intracellular components is representative of tissue injury that is an indication of inflammation. As a part of the investigation on alternative anti-inflammatory agents for synthetic drugs, the ability of the extract to inhibit protein denaturation was studied. It was effective in inhibiting heat-induced albumin denaturation at different concentrations with optimal activity seen at 50µg/ml. In the present study, diclofenac was used as a standard that showed a dose-dependent inhibition with optimum of 71% at a concentration of 50µg/ml which was on par with that of HPE.

CONCLUSION

The present was carried out to evaluate the antioxidant and anti-inflammatory potential of *Harpagophytum procumbens* leaves ethanol extract. The study demonstrated a remarkable antioxidant potential as observed by FRAP and DPPH radical scavenging ability of the HPE which could be associated with the high phenolic content present in the extract. Furthermore, anti-inflammatory activity evaluated using xanthine oxidase inhibition and albumin denaturation inhibition showed that the inhibitory potential of the extract was comparable with that of the standard oxypurinol and diclofenac, respectively. Overall, this preliminary study can be taken as a basis for further evaluation of the anti-inflammatory studies of the extract *in vivo*.

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