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Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities

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PEER REVIEW

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Comments

The research work is highly valuable for identification of newer antioxidant principles. The plant possesses significant antioxidant, radical scavenging and reducing potency and also the plant has reported vitamin C which usually presents in fruits, but the plant sample reported here is something interesting. The more numbers of photochemical observed in methanol extract as usual that is high polarity solvent. The experimental data were analyzed with appropriate manner and well discussed.
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ABSTRACT

Objective: To analyse qualitative and quantitative phytochemical and evaluate *in vitro* antioxidant properties of various alcoholic and aqueous extracts of leaf and root parts of *Hypochaeris radicata*.

Methods: Preliminary phytochemical analysis for alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids and quantitative phytochemical analysis for alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid were made by following standard procedures. *In vitro* antioxidant properties were evaluated by assessing DPPH$^{\bullet}$, NO$^{\bullet}$ and ABTS$^{\bullet+}$, radical scavenging abilities and assaying the reducing power, β-carotene and antihemolytic activities by adapting standard methods.

Results: The quantitative phytochemical analysis of this species exhibited the presence of alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid in considerable quantity. The *in vitro* antioxidant activity of the species, *Hypochaeris radicata* clearly demonstrated that both the leaf and root parts have prominent antioxidant properties.

Conclusions: From this study, it can be concluded that the species is effective in scavenging free radicals and has the potential to be a powerful antioxidant.

KEYWORDS

Hypochaeris radicata, Phytochemical analysis, *In vitro* antioxidant activities

1. Introduction

A free radical is defined as any atom or molecule possessing unpaired electrons. The reactive oxygen species are oxygen derived free radicals such as superoxide anion (O_2), hydroxyl (OH$^{\bullet}$), hydroperoxyl (OOH$^{\bullet}$), peroxyl (ROO$^{\bullet}$) and alkoxyl (RO$^{\bullet}$) radicals and non free radicals such as hydrogen peroxide (H_2O_2), hypochorous acid (HOCl), ozone (O_3) and singlet oxygen (O_2)[1]. It can be formed in living organisms by both endogenously (respiration, peroxisomes stimulation of polymorphonuclear leucocytes and macrophages) and exogenously (ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents)[2]. These free radicals are produced by our body and to stabilize the body's natural function, but the excess amount could cause the cell and tissue damage[3]. It can also cause oxidative damage to proteins, lipids and DNA and chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans[4].

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule[5]. The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quencher[6,7]. The

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free radicals can be scavenged by the natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol and tetra butyl hydro quinone) antioxidants[8]. But the usages of these synthetic antioxidants are now replaced because the natural antioxidants could be considered as safer without any side effects[9]. In recent decades, many researchers are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of human diseases[10].

Hypochaeris radicata (*H. radicata*), belonging to the family Asteraceae, is an edible perennial herb, distributed in high hills of Nilgiris, the Western Ghats at 2000 m above mean sea level. The whole plant is said to be medicinally important by having antiinflammatory, anticancer, antioxidant[11], antibacterial[12], antifungal[13] and antidiuretic properties. It is being used for the treatment of jaundice, rheumatism, dyspepsia, constipation, hypoglycemia and kidney related problems in traditional medicinal practice of Tamil Nadu, India[14]. However, no much scientific validation has been made for this species for its medicinal uses. To address this lacuna, the present study was carried out for qualitative and quantitative phytochemical analysis and *in vitro* antioxidant activities of leaf and root parts of *H. radicata* using various alcoholic (petroleum ether, chloroform, ethyl acetate and methanol) and aqueous extracts.

2. Materials and methods

2.1. Chemicals

In the present study, all the chemicals were purchased from HI-MEDIA Pvt. Ltd., Bombay. The chemicals used were of analytical grade.

2.2. Collection and identification of plant materials

The plant *H. radicata* was collected from Nilgiris, the Western Ghats, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2010-11/Tech.153. The fresh leaf and root parts of this species were washed under running tap water, shade dried at room temperature and powdered.

2.3. Extract preparation

The powdered plant samples (50 g/250 mL) were extracted successively with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus at 55-85 ℃ for 8-10 h in order to extract the polar and non-polar compounds[15]. For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4 ℃ for further use. The dried plant extracts were then redissolved in dimethyl sulfoxide and to get the solution of 10 mg/10 mL for each extract which was subjected to analysis of *in vitro* antioxidant activities.

2.4. Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various alcoholic and aqueous extracts of leaf and root parts of *H. radicata*[16,17].

2.5. Quantitative estimation of chemical constituency

2.5.1. Determination of alkaloids

A total of 200 mL of 20% acetic acid was added to 5 g of leaf and root powders taken in a separate 250 mL beaker and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed[18]. The percentage of total alkaloid content was calculated as:
Percentage of total alkaloids (%)=Weight of residue×100/Weight of sample taken

2.5.2. Total phenolics content

The total phenolics content of *H. radicata* was estimated using Folin-Ciocalteau reagent by the method of Sidduraju and Becker[19]. About 20 μg of leaf and root extracts were taken separately and it was made up to 1 mL with distilled water. Then 500 μL of diluted Folins-phenol reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na_2CO_3 (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of colour. After incubation, the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 μg/mL. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

2.5.3. Total flavonoids content

The total flavonoids content was estimated using the procedure described by Zhishen *et al*[20]. A total of 1 mL of plant extracts were diluted with 200 μL of distilled water separately followed by the addition of 150 μL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 μL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

2.5.4. Estimation of tannins content

Tannins content of *H. radicata* was estimated by the method of Siddhuraj and Manian[21]. A total of 500 μL of the extracts were taken in test tube separately and treated with 100 mg of polyvinyl polypyrrolidone and 500 μL of distilled water. This solution was incubated at 4 ℃ for 4 h. Then the