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Free radical scavenging activity of aqueous extract of *Rhus succedanea* galls

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Abstract

Objective: To evaluate the free radical scavenging activity of aqueous extract of *Rhus succedanea* galls. Material and methods: Aqueous extract was prepared and free radical scavenging activity was studied using DPPH assay and nitric oxide scavenging assay. Result: Aqueous extract of *R. succedanea* galls showed remarkable and concentration dependent free radical scavenging activity comparable to that of standard ascorbic acid in the studied models. IC_{50} was found to be 27.33µg and 32.63µg in DPPH assay and NO scavenging assay respectively. Conclusion: The present results indicate that aqueous extract of *R. succedanea* galls exhibit free radical scavenging activity.

Keywords: *Rhus succedanea*, free radical scavenging, DPPH assay.

1. Introduction

Galls of *R. succedanea* L (Anacardiaceae) has been reported to possess astringent [1], antiviral [2], tonic, expectorant and stimulant properties [3]. In Indian ethnomedicine, this plant is locally known as Kakrasingi and its galls have been used as Ayurvedic remedy for diarrhea and dysentery [4].

Free radicals are believed to be involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, ageing and neoplastic diseases [5]. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals [6]. Hence, the present study was aimed at evaluating the free radical scavenging activity of aqueous extract of *R. succedanea* galls.

2. Materials and methods

2.1 Plant material

R. succedanea galls were collected from Jammu and Kashmir in March 1999. The sample was authenticated at our Pharmacognosy department where the voucher specimen (hb/99/06) has been preserved.

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2.2 Preparation of extract

Air-dried, powdered galls of *R. succedanea* weighing 100g was extracted with 500 ml of distilled water for 3 h. below 50°C. The extract was filtered with Whatman No. 2 filter paper. The filtrate was collected and water was evaporated under reduced pressure using vaccum evaporator. Phytochemical screening [7, 8] of aqueous extract gave the positive tests for flavonoids, catechins, saponins and tannins.

2.3 Free radical scavenging activity

2.3.1 Diphenyl-picryl-hydrazyl (DPPH) assay

The free radical scavenging capacity of aqueous extract of *R. succedanea* galls was tested by its ability to bleach the stable 2,2 diphenyl 2-picryl hydrazyl radical (DPPH) [9]. A stock solution of DPPH (1.5 mg /ml of methanol) was prepared such that 75 µl of it in 3 ml methanol gave initial absorbance of 0.9. This stock solution was used to measure the antiradical activity. Decrease in absorbance in the presence of aqueous extract of *R. succedanea* galls at different concentrations was noted after 15 min. IC_{50} was calculated from percentage inhibition. Ascorbic acid was used as reference standard.

2.3.2 Nitric oxide scavenging activity

The interaction of aqueous extract of *R. succedanea* galls with nitric oxide was assessed by the nitrite detection method. The chemical source of NO was sodium nitroprusside (10mM) in 0.5 M phosphate buffer, pH 7.4, which spontaneously produces nitric oxide in an aqueous solution. Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites.

After the incubation for 60 min at 37°C, Greiss reagent (α-naphthyl-ethylenediamine 0.1% in water and sulfanilic acid 1% in H_3PO_4 5%) was added. The same reaction mixture without the aqueous extract of sample but with equivalent amount of distilled water served as control [10]. Ascorbic acid was used as positive control.

3. Results and discussion

Recently much attention has been focused on reactive oxygen species and free radicals, which play an important role in the genesis of various diseases such as inflammation, cataract, atherosclerosis, rheumatism, arthritis, liver cirrhosis, ischemia reperfusion injury [11]. Herbal drugs containing radical scavengers are gaining importance in prevention and treatment of such diseases. Phenolic compounds and flavonoids are the major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity [12].

The free radical scavenging capacity of aqueous extract of *R. succedanea* galls was tested by its ability to bleach the stable DPPH radical. This assay provided information on the reactivity of the test compound with a stable free radical since its odd electron DPPH gives strong absorption band at 517 nm in visible spectroscopy (deep violet color).

Table 1
Antiradical activity of aqueous extract of *R. succedanea* galls observed with DPPH.

Sample	Concentration (µg/ml)	% Inhibiton ± S.D.	IC_{50} (µg/ml) r*
Aqueous	3.33	16.33 ± 2.3	
Extract	6.66	27.69 ± 3.2	
	13.33	36.73 ± 0.8	27.33(0.9816)*
	26.66	48.54 ± 2.0	
	39.33	69.90 ± 1.7	
	53.33	75.80 ± 1.2	
Ascorbic acid			1.99(0.963)*

n=3 Values are mean±S.D. r*- regression coefficient

As this electron become paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. It showed excellent antiradical activity by inhibiting DPPH radical with an IC_{50} value of 27.33 µg/ml (Table1). The scavenging activity was comparable to that observed with ascorbic acid.

Table 2
In vitro NO scavenging activity of aqueous extract of *R. succedanea* galls.

Sample	Concentration (µg/ml)	% inhibiton ± S.D.	IC_{50} (µg/ml) r*
Aqueous	8.33	22.18±2.1	
Extract	16.66	36.25±3.4	
	33.33	52.30±1.5	32.63(0.9932)*
	50.00	69.62±4.6	
	66.66	81.52±2.7	
Ascorbic acid			5.2(0.9450)*

n=3 Values are mean±S.D. r*- regression co-efficient

Nitric oxide (NO) exhibits numerous physiological properties and it is also implicated in several pathological states [13]. It is an important second messenger, acts as a neurotransmitter, and plays an important role in the defence against pathogens as well as in the control of blood pressure. NO is produced in various cells including neurons, endothelial cells and neutrophils by three isoforms of nitric oxide synthase enzyme (encoded by a unique gene), from nitrogen of the guanidine group of L-arginine and from molecular oxygen [14].

The interaction of NO with other radicals leads to the formation of more hazardous radicals such as peroxynitrite anion and hydroxyl radical. In fact, NO reacts more rapidly with superoxide than the latter does with superoxide dismutase.

Aqueous extract significantly decreased with IC_{50} value 32.63 µg/ml, in a dose-dependent fashion, the concentration of nitrite after spontaneous decomposition of sodium nitroprusside, indicating that aqueous extract may contain compounds able to scavenge nitric oxide (Table 2).

However, the specificity of this assay has been questioned since nitrite is one final product of the reaction of nitric oxide with oxygen, through intermediates such as NO_3, N_2O_4 and N_2O_3 [15]; therefore the decrease in the nitrite production could also be due to an interaction of the extract with other nitrogen oxides.

Our results indicate that aqueous extract of *R. succedanea* galls possess free radical scavenging activity. Further studies are needed to better characterize the important active constituents responsible for the free radical scavenging activity.

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