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Research Article

COMPARATIVE ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF ROOTS OF *DACTYLORHIZA HATAGIREA* AND AERIAL PART OF *LAVANDULA STOECHAS*

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ABSTRACT

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. *Dactylorhiza hatagirea* (D.Don) Soo (*D. hatagirea,* Orchidaceae) is native and near endemic to Indian Himalayan region. Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported from Jammu and Kashmir, Sikkim, Arunachal Pradesh, Uttarakhand and Himachal Pradesh. Root paste is externally applied as poultice on cuts and wounds and extract is given in intestinal disorders. *Lavandula stoechas* (*L. stoechas* Lamiaceae), the Spanish lavender or topped lavender or French lavender is a species of flowering plant occurring naturally in several Mediterranean countries, including France, Spain, Portugal, Italy and Greece. It is used commercially in air fresheners and insecticides. Flower spikes have been used internally for headaches, irritability, feverish colds and nausea and externally for wounds, rheumatic pain, antiseptic, digestive, antispasmodic, healing, insect repellent and antibacterial. The aim of the present study was to evaluate *in vitro* antioxidant activities of roots of *D. hatagirea* and aerial part of *L. stoechas* collected from Bhopal region of Madhya Pradesh. The *in vitro* antioxidant activity of hydroalcoholic extract of the roots and aerial part was assessed against DPPH, hydrogen peroxide (H_2O_2) and nitric oxide (NO) radical scavenging assay methods using standard protocols. The activities of hydroalcoholic extracts against DPPH, H_2O_2 and NO assay method were concentration dependent. *D. hatagirea* extract showed higher antioxidant activity than *L. stoechas* extract but less than ascorbic acid in all three models. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Dactylorhiza hatagirea, Lavandula stoechas,* Antioxidant activity, DPPH, H2O2, NO• Assay Method

1. INTRODUCTION

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments [1]. Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defense mechanisms and among them, a key protective role is played by the antioxidant enzymes gluthatione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase [2]. Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage [3]. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation [4, 5]. *D. hatagirea* (D.Don, Orchidaceae) Soo is native and near endemic to Indian Himalayan region. Its distribution extends to Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is reported from Arunachal Pradesh, Uttarakhand, Jammu and Kashmir, Sikkim and Himachal Pradesh [6]. Generally, it is widely and narrowly distributed at an altitudinal ranges between 2500 to 5000 m amsl in open grassy slopes and alpine meadows. It is commonly

known as panja, salam-panja, hath-panja or hatajari in Uttarakhand; salem panja in Kashmir and wanglak or angulagpa in various parts of Ladakh. Generally, the plant is a perennial herb, up to 60 to 70 cm in height, having palmately lobed, divided root tubers with broadly lanceolate leaves arranged more or less along the stem and purple flowers, but some time white [7, 8]. The plant tubers of *D. hatagirea* contain a glucoside, a bitter substance, starch, mucilage, albumen, a trace of volatile oil and ash. Chemically, dactylorhins A to E, dactyloses A and B and lipids, etc is found as major constituents. Young leaves and shoots are eaten as a vegetable. The root is expectorant, astringent, demulcent and highly nutritious. Powdered root is spread over wounds to control bleeding. A decoction of the root is given in cases of stomach trouble. It is also used as aphrodisiac and sexual stimulant [9]. *L. stoechas* (Lamiaceae), the Spanish lavender or topped lavender or French lavender is a species of flowering plant in the family, occurring naturally in several Mediterranean countries, including France, Spain, Portugal, Italy and Greece [10]. An evergreen shrub, it usually grows to 30-100 cm (12-39 in) tall and occasionally up to 2 m (7 ft) high in the subspecies *luisieri*. The flowers, which appear in late spring and early summer, are pink to purple, produced on spikes 2 cm long at the top of slender, leafless stems 10-30 cm (4-12 in) long; each flower is subtended by a bract 4-8 mm long. At the top of the spike are a number of much larger, sterile bracts (no flowers between them), 10-50 mm long and bright lavender purple (rarely white). It blooms in spring and early summer, from the month of March, depending on the climate where it inhabits [11]. *L. stoechas* is used commercially in air fresheners and insecticides. Flower spikes have been used internally for headaches, irritability, feverish colds and nausea, antiseptic, digestive, antispasmodic, healing and externally for wounds, rheumatic pain and as an insect repellent. The flowers are used in aromatherapy, to prepare infusions and essential oils that contain ketones (d-camphor and d-fenchone) and alcohols (borneol and terpineol) [12]. The present study was focused to evaluate the anti oxidant activity of roots of *D. hatagirea* and aerial part of *L. stoechas*.

2. MATERIALS AND METHODS

2.1. Plant material

The roots of plant *D. hatagirea* and aerial part of *L. stoechas* were collected from local area of Bhopal (M.P), India in the month of Jan 2017. The taxonomical identification and authentication of the plant material was done by Dr. Zia Ul Hasan, Department of Botany, SAFIA College Bhopal (M.P). The specimens of voucher have been submitted and preserved in the herbarium of SAFIA College Bhopal (M.P).

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

2.3. Preparation of extract

2.3.1. Defatting of plant material

Powdered material of *L. stoechas* and *D. hatagirea* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

2.3.2. Extraction by hot continuous percolation process

100 gm of *L. stoechas* and *D. hatagirea* dried material were exhaustively extracted with 80% ethanol (Hydroalcoholic) using hot continuous percolation for 24 hrs. Appearance of colorless solvent in the siphon tube was taken as the end point of extraction. The extracts were concentrated to ¾ of its original volume by distillation. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [13].

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Olufunmiso *et al.,* 2011 [14]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken

immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

2.4.2. Hydrogen peroxide (H_2O_2) scavenging assay

Hydrogen peroxide scavenging activity of the plant extract was determined using the procedure explained by *Jayaprakasha et al.,* 2004 [15]. A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS; pH7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230nm by using the molar absorptivity of $81 M^{-1}cm^{-1}$. Different concentrations of extract (20 to 100µg/ml) in ethanol were prepared.1 ml of ethanolic standard and test were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract.

The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

$$\% \text{ scavenged [H2O2]} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

2.4.3. Nitric oxide (NO•) radical scavenging assay

The determination of NO• radical scavenging ability of the extracts is based on the inhibition of NO• radical generated from sodium nitroprusside in phosphate buffer saline solution by Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Scavengers of nitric oxide act against oxygen, prompting to lessened production of nitrite ions which can be monitored at 546 nm [16]. Briefly, sodium nitroprusside (0.6 ml, 5 mM) solution was mixed with and without varying the concentration of the extracts or Ascorbic acid (2 ml, 10-200µg/ml) and incubated at 25 ± 2°C for 5 h. Incubated solution (2 ml) was mixed with equal volume of Griess reagent and absorbance of the purple colored azo dye chromophore was measured at λmax 546 nm using UV-Vis spectrophotometer. The NO• radical scavenging ability was calculated using following formula:

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x100$$

3. RESULTS AND DISCUSSION

The roots of *D. hatagirea* and arial parts of *L. stoechas* were collected from the local area of Bhopal, MP, India. Air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of hydroalcoholic extract of *D. hatagirea* and *L. stoechas* was found to be 6.2 and 8.5%w/w respectively. DPPH radical scavenging assay measured hydrogen donating nature of extracts [17]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *D. hatagirea and L. stoechas* hydroalcoholic extract was found to be 65.31 and 67.38µg/ml as compared to that of ascorbic acid (15.22µg/ml). A dose dependent activity with respect to concentration was observed Table 1 & Figure 1.

Table 1: % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* hydroalcoholic extract using DPPH method

Conc. (µg/ml)	Ascorbic acid % Inhibition	*D. hatagirea* % Inhibition	*L. stoechas* % Inhibition
20	47.88	16.88	9.74
40	63.47	25.97	25.97
60	81.33	40.74	42.53
80	84.57	63.79	63.79
100	89.44	80.84	76.46
IC 50	15.22	65.31	67.38

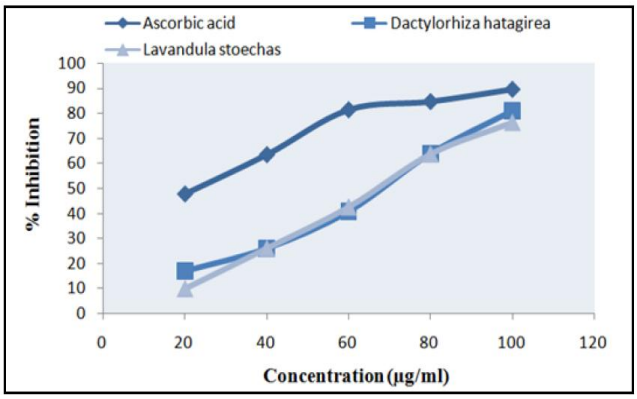

Fig. 1: Graph of % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* using DPPH method

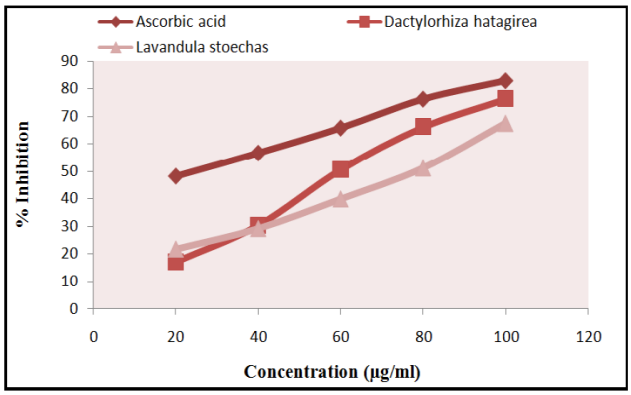

Fig. 2: Graph of % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* using NO• method

Table 2: % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* hydroalcoholic extract using NO• method

Conc. (µg/ml)	Ascorbic acid % Inhibition	*D. hatagirea* % Inhibition	*L. stoechas* % Inhibition
20	48.176	17.173	21.884
40	56.687	30.395	29.331
60	65.805	50.608	39.970
80	76.292	66.109	51.216
100	82.979	76.292	67.477
IC 50	24.17	62.50	74.24

Table 3: % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* hydroalcoholic extract using H_2O_2 method

Conc. (µg/ml)	Ascorbic acid % Inhibition	*D. hatagirea* % Inhibition	*L. stoechas* % Inhibition
20	49.063	29.977	27.986
40	61.124	43.208	34.895
60	72.482	53.747	43.208
80	81.733	68.970	53.747
100	86.885	74.122	73.302
IC 50	17.92	53.01	63.10

In comparison to both the plant *D. hatagirea* have higher antioxidant activity as comparison to *L. stoechas*. Extracts showed $NO^{\bullet}$ scavenging effects by competing with oxygen to react with NO directly hence inhibited the nitrite ion formation [18]. *D. hatagirea and L. stoechas* extracts showed nitric oxide (NO•) radical scavenging activity with IC_{50} value of 62.50 and 74.24 µg/ml, respectively, as compared to that of ascorbic acid (IC_{50} 24.17µg/ml). *D. hatagirea* extract showed significant activity as compared to *L. stoechas* extract (Table 2 and Figure 2).

Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains [19]. There is increasing evidence that, hydrogen peroxide, either directly or indirectly via its reduction product, $OH^{\bullet}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators [20]. When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured.

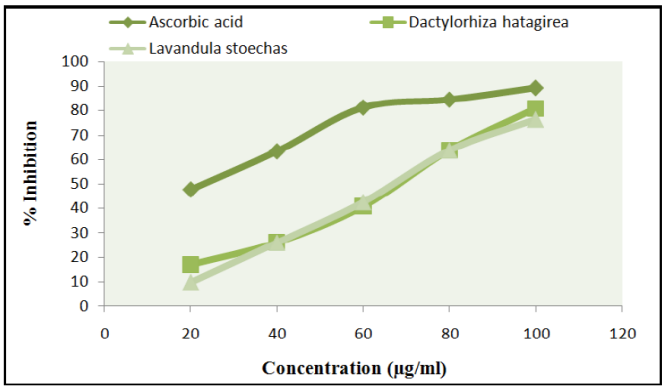

Fig. 3: Graph of % Inhibition of ascorbic acid, *D. hatagirea and L. stoechas* using H_2O_2 method

Table 3 and Figure 3 show the scavenging ability of *D. hatagirea, L. stoechas* hydroalcoholic extract and ascorbic acid on hydrogen peroxide at different concentrations. Extracts was capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur containing molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper (I), cobalt (II) and nickel(II) also take part in the process [19]. Thus, the removing is very important for antioxidant defense in cell or food systems.

4. CONCLUSION

The results obtained in the present study clearly demonstrate that the extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the both plants. However, the in vivo safety of both plants needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. The above results showed that *D. hatagirea extract* could exhibit antioxidant properties more comparable to *L. stoechas* extract. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

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