

Preliminary Phytochemical Screening, Antioxidant and Antifungal Activity of *Lepidagathis cuspidata*

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Abstract

The aim of present study was to evaluate antioxidant and antifungal potentials of *Lepidagathis cuspidata*. Ethanol extract (80%) of *Lepidagathis cuspidata* roots and its fractions (CCL₄ and ethyl acetate) were prepared and preliminary screened for the presence of different chemical constituents. Total polyphenolic and flavonoids contents of the plant were also measured. DPPH assay was used for the evaluation of antioxidant potential whereas the antifungal activity was performed with disc diffusion method on the plant pathogen. The IC₅₀ value was measured in µg/ml, and zone of inhibition was expressed in millimetres. *Lepidagathis cuspidata* extract showed the presence of steroids, tannins, phenolics, saponins, alkaloids, flavonoids, carbohydrates and amino acids. Total phenolic and total flavonoid contents in the extract were found to be 8.14 ± 0.005 and 11.3 ± 0.002 mg/g, respectively. In DPPH assay, the IC₅₀ value of ethanol extract and fractions (CCL₄ and ethyl acetate) prepared from this extract was found to be 91.01 µg/ml 4788 µg/ml and 928 µg/ml, respectively. While standard drug (Trolox) showed 11.89 µg/ml IC₅₀ value. Moreover, ethanol extract showed significant antifungal activity against all tested fungal strains in comparison to Mancozeb (standard drug). Ethyl acetate fraction also showed moderate antifungal activity against all tested strains while CCL₄ showed least potential only against *Aspergillus flavus* and *Aspergillus fumigates*. This study showed that *Lepidagathis cuspidata* may be used for controlling plant pathogen due to its antifungal potential.

Keywords: *Lepidagathis cuspidata*; Acanthaceae; Antioxidant; Antifungal

Introduction

Plants are the potential source of food and therapeutic aids. India has one of the richest biodiversity across the globe. In addition, herbs have provided a valuable lead in establishing the lifesaving drug formulations as modern medicines [1]. However, among the estimated 250,000-400,000 plant species, only 6% have been screened for biological potential, and only 15% have the phytochemical exploration [2]. The plant kingdom has provided a variety of chemical compounds for controlling the plant and human pathogens and related diseases. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world [3-5]. Fungi destroy the grains and foodstuffs during storage and also decreasing the nutritive value by producing mycotoxins [6,7]. In the world, a significant portion of foodstuffs become unfit for human consumption every year, because of contamination with mycotoxins [8-10]. Approximately 25% of cereals are contaminated with known mycotoxins and around 300 fungal toxic metabolites are reported for man and animals [11]. The major toxic effects are teratogenicity, genotoxicity, hepatotoxicity, nephrotoxicity, carcinogenicity, and reproductive disorders etc. [12,13].

Lepidagathis genus belongs to the acanthaceae and represented by 100 species, widely distributed in tropical and subtropical regions of Asia and Africa. This family is known to possess broad spectrum bio-active component like cytotoxic, antifungal, anti-inflammatory, antioxidant and insecticidal etc. The major isolated chemical constituents of this genus include essential oils, sesquiterpenes, alkaloids, inorganic minerals and saponins [14]. In India, the species

Lepidagathis cuspidata (Adhusa) is a spiny shrub and found in the tropical Himalayas and Western Ghats at altitudes of 300-700 m. Ban-gujjar tribes used the *Lepidagathis cuspidata* whole plant for the treatment of inflammation in fingers and itchy infections of the skin. The paste of the roots is used in the treatment of boils and blisters of the mouth while water extract is used as a tonic in fever. The present study was aimed to preliminary check the antioxidant activity, and antifungal activity of *Lepidagathis cuspidata* roots against plant pathogen fungal species *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium nodatum* and *Aspergillus fumigates*.

Materials and Methods

Plant material

The plant specimen was collected from Dist. Kangra, Himachal Pradesh, India. The plant specimen *Lepidagathis cuspidata* was identified and authenticated by Department of Biodiversity, IHBT, Palampur with accession number PLP-17043. A voucher specimen was deposited in the herbarium of IHBT, Palampur, Himachal Pradesh, India.

Extraction and fractionation

The plant material (roots) of *Lepidagathis cuspidata* was collected and dried under shade for about two weeks. The dried plant material was powdered to get 1 kg dry material and stored in airtight container for further analysis. The powdered material was extracted with 80% ethanol in water at room temperature for six days with occasional shaking. The extract was dried under vacuum at 40°C to get crude extract (40 gm). The 10 gm of this extract was retained and rest 30 gm was fractionated with CCL₄ (1 L) and ethyl acetate (1 L), and each solvent was removed under similar conditions to get the CCL₄ (5 gm) and ethyl acetate (8 gm) fractions of ethanol (80%) extract.

Phytochemical screening

The phytochemical screening of above extracts was performed to establish the type of constituents present in ethanol extract and its fractions as per standard methods [15,16].

Determination of total polyphenolic content

Total polyphenolic content of ethanol extract was estimated using Folin Ciocalteu reagent. Polyphenols were expressed as mg/g equivalent to gallic acid. The experiment is performed in triplicate as described by Mahdavi et al. [17].

Determination of total flavonoids content

The total flavonoid content was estimated in ethanol extract using aluminium chloride colorimetric assay and expressed as mg/g equivalent to quercetin. Experiment is performed in triplicate as described by Zhishen et al. [18].

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: The free radical scavenging activity of the ethanol extract and its fractions were measured *in vitro* by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [19,20]. Trolox was used as a standard drug.

Radical scavenging activity (%) = $\left[\frac{\text{abs}_{\text{control}} - \text{abs}_{\text{sample}}}{\text{abs}_{\text{control}}} \right] \times 100$

The absorbance was taken at 517 nm against blank as a methanol and IC₅₀ value were also calculated for all samples [21].

Antifungal activity

Test microorganisms and growth media: *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium nodatum*, *Aspergillus fumigates* were selected based on their importance. The fungal strains obtained from Institute of Microbial Technology, Chandigarh, for evaluating antifungal activity.

Disc diffusion method

Antifungal activity was investigated by the disc diffusion method [22]. Fungal suspension (2×10^5) was streaked on the SDA medium containing Petri plates. Then sterile discs (made from Whatman filter paper) each about 5 mm diameter impregnated with extract and its fractions were placed on inoculated plates. All plates were incubated at 28°C for 24-28 hours. The zones of growth inhibition around the disc were measured after 48 hours. The sensitivity of fungal species was determined by measuring sizes of zone inhibition (diameter of the zones in mm) on agar surface around the disc. The experiment is performed in triplicate for all samples [23].

Results and Discussion

Phytochemical screening is necessary to establish the information about the chemical diversity of the plants. Most of these chemical entities are important for the therapeutic usage. The phytochemical screening of *Lepidagathis cuspidata* (Figure 1) has been performed and depicted in Table 1.

Polyphenols and flavonoids are the chemical constituents which play important roles in plant and human life. In current study, the total polyphenolic and flavonoid content were determined in the roots of *Lepidagathis cuspidata*. The polyphenolic and flavonoid content in the roots of *Lepidagathis cuspidata* were found to be 8.14 ± 0.005 and

11.3 ± 0.002 mg/g. DPPH is a protonated radical having absorption maxima at 517 nm which decreases with the scavenging of free radical by ethanol extract and its fraction. Hence, DPPH finds applications in the determination of the antioxidant activity. Ethanol extract showed antioxidant potential as compared to standard which was approximately 8 fold less than the standard and might be due to the antagonistic or synergistic action of chemical constituents. Moreover, fractions of ethanol extract were not significant and the study to isolate the antioxidant potential from this plant is under investigation in our laboratory (Table 2).

Antifungal activity is important in medical mycology and standard methods have shown remarkable progress in the field of antifungal drug discovery and leads. The agar diffusion method enables to determine the antifungal potential of test samples in comparison to standard against various fungal strains. Numerous studies suggest that disk diffusion is a reproducible method. The diameter of the zone of inhibition was used as indicator for antifungal activity. Upon comparison of inhibition diameter recorded, it was observed that ethanol (80%) extract and its ethyl acetate fraction showed maximum inhibition in the range of 7-10 mm against all selected fungal species i.e., *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium nodatum*, *Aspergillus fumigates*. Moreover, ethanol (80%) extract and its ethyl acetate fraction showed highest potential against *Aspergillus flavus* while CCl₄ fraction of ethanol extract showed minimum range of inhibition (4 mm) and only against *Aspergillus flavus* and *Aspergillus fumigates*. All the screening was performed in comparison to Mancozib (standard drug) and control (DMSO). The detailed results were depicted in Table 3.

Lepidagathis cuspidata have antioxidant and antifungal potential and may be a source for controlling plant pathogens and also in biocontrol. Further studies are being carried out in our lab for the characterization of pure molecules which may be responsible for the activity.

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Figure 1: Roots of *Lepidagathis cuspidata*.

Samples	Alkaloids	Carbohydrates	Glycosides	Saponins	Flavonoids	Tannins
Ethanol extract	+++	++	++	+++	++	+++
CCl ₄ Fraction	++	-	+	-	-	+
Ethyl acetate Fraction	+	+	++	+	++	+

+: Present; ++: Strongly present; +++: Very strongly present; -: Absent; CCl₄: Carbon tetrachloride

Table 1: Phytochemical screening of extract and its fractions of *Lepidagathis cuspidata*.

Samples	DPPH IC ₅₀ µg/ml
Trolox	11.896 ± 0.596
Ethanol	91.01 ± 2.569
CCl ₄	4788 ± 57.16
Ethyl acetate	928 ± 12.07

Table 2: IC₅₀ value of crude plant extract and fractions (µg/ml) against DPPH radicals.

Fungal species	Inhibition zone of different plant extracts (in mm)				
	Control (DMSO) 1 ml	Ethanol extract (80%) 10 mg/ml	Ethyl acetate fraction 1 mg/ml	CCl ₄ fraction 1 mg/ml	Mancozib 1 mg/ml
<i>Aspergillus flavus</i>	-	7 ± 1.16	7 ± 0.58	4 ± 1.16	14 ± 2.31
<i>Rhizopus stolonifer</i>	-	11 ± 1.73	10 ± 1.73	-	20 ± 2.31
<i>Penicillium nodatum</i>	-	8 ± 1.73	9 ± 1.16	-	18 ± 1.16
<i>Aspergillus fumigates</i>	-	10 ± 0.58	9 ± 1.16	4 ± 0.58	16 ± 1.16

Mean ± SEM

Table 3: Inhibition zone of different plant extracts (in mm).

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