Phytochemical and in-vitro antioxidant evaluation of methanolic and aqueous extract of Lepidagathis cuspidata

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Abstract

The objective of present study was to evaluate preliminary phytoconstituents and in-vitro antioxidant potential of Lepidagathis cuspidata. Methanolic and aqueous extract of aerial part of plants was prepared and tested for the presence of different phytoconstituents. The in-vitro antioxidant potential of extracts was evaluate by different in-vitro methods. TLC of extracts was performed by using various solvent systems. The present study revealed that the MELC and AELC contained flavonoids, glycosides, phenols, saponins, steroids and terpenoids. However, alkaloids were detected only in MELC. Tannins were absent in methanolic as well as in aqueous extracts. The in-vitro antioxidant potential MELC and AELC was evaluated by DPPH and FRAP. In both methods MELC possesses good antioxidant activity as compared to AELC. Ascorbic acid is used as a standard.

Keywords: Lepidagathis Cuspidata, Thin Layer Chromatography, Antioxidant activity, DPPH, FRAP.

1. Introduction

Medicinal plants have been historically used for the treatment of various diseases, as it is well aware that plants are the potential source of variety of phytoconstituents with nutritive and therapeutic value[1].

Lepidagathis genus belonging to the family acanthaceae, represent more than 100 species, widely distributed in tropical and subtropical regions of Asia and Africa.[2] This family is known to possess broad spectrum bio-active components eg- anti-inflammatory and antipyretic activity, mouth ulcer, antidiabetic, antioxidant, antiviral, hepatoprotective and anti-platelet aggregation activity[3,4]. Lepidagathis cuspidata is a spiny shrub and found in the tropical Himalayas and Western Ghats at altitudes of 300-400 m. Traditionally use of Lepidagathis cuspidata whole plant is used for the cure of inflammation[5, 8], itchy infections, mouth ulcer and aqueous extract is used for fever. The human body has complex system of enzymatic and non-enzymatic antioxidant defenses [6], which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases [7]. The present study was aim to preliminary phytochemical screening, in-vitro antioxidant and antimicrobial activity.

2. Material and Methods

2.1 Plant Material

Aerial part of Lepidagathis cuspidata was collected from Keshavshrushsti botanical garden, Bhayandar, Mumbai. The collected plant material was authenticated by Dr. Rajendra D. Shinde, Head, Department of Botany & Director, Blatter Herbarium, St. Xavier’s college, Mumbai. (Herbarium Specimen number NI-4205) The collected plant material was washed with water & then samples were air-dried at room temperature with dehumidifier. Dried samples
were ground to powder using a mechanical grinder, and stored in a sealed plastic container.

2.2 Extraction method [9]

100g coarse powders of the sample were extracted in soxhlet extractor with methanol and water for 6 hrs. Then the extract was evaporated by using rotatory vacuum evaporator to get semisolid mass. The concentrated extract of aerial part of Pentatropis nivalis was stored in air tight close container in dark place.

2.3 Phytochemical screening of extracts [9-11]

The qualitative phytochemical screening of above extracts was performed to evaluate the types of phytoconstituents present in the extracts.

2.4 Analytical Instrument

UV spectrophotometer (Make and model: JASCO V 550 UV spectrophotometer). The software used data acquisition and evaluation was Spectra Manager.

2.5 Thin Layer Chromatography (TLC) [9, 12]

TLC was performed with various solvent systems by using Pre-coated TLC GF254 plate was obtained from Merck Pvt. Ltd. TLC plate Size of 1.5 cm X 10 cm was taken for analysis. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-μl of sample by using capillary at distance of 1 cm plates were observed under UV chamber and specific spray reagents were sprayed and allowed to dry. The colored spots developed on the stationary phase were marked and identified by specific reagent and their distances were measured and Rf values were calculated.

Distance traveled by the compound
\[ R_f = \frac{\text{Distance traveled by the solvent front}}{\text{Distance traveled by the compound}} \]

Distance traveled by the solvent front

2.5.1 Detection of Spots

Spots were detected by UV 254 nm and UV 366 nm, also dervatized by various spraying reagent. [15, 16]

2.6 Evaluation of in-vitro antioxidant activity

Antioxidant (in-vitro) activity of methanolic extract of Lepidagathis cuspidata (MELC) and aqueous extract of Lepidagathis cuspidata (AELC) was determined by compared with ascorbic acid, which is used as a standard. All the experiments were performed in triplicate.

2.6.1 DPPH radical scavenging activity [13]

The DPPH assay measures the free radical scavenging capacity of the extract and has been used to evaluate the free radical scavenging ability in-vitro. Free radical scavenging capacity of MELC&AELC was determined using method of Chen et al and compared with ascorbic acid. Briefly MELC, AELC and ascorbic acid concentration were prepared in methanol and mixed with 1ml of 0.1mM DPPH methanolic solution. The reaction mixture was incubated at 37°C for 30min and absorbance measured at 517nm.

\[ \% \text{ of inhibition} = \frac{\text{A1-A2/A1}}{100} \]

Where,
\( A1=\text{absorbance without extract} \);
\( A2=\text{absorbance with extract} \).

2.6.2 Ferric reducing (FRAP) assay [14]

The ability to reduce ferric ions was measured using the method described by Benzie and Strain the antioxidant capacity based on the ability to reduce ferric ions of extract was calculated from the linear calibration curve. The FRAP reagent was prepared fresh daily by mixing 100 ml of sodium acetate buffer (300mM, pH 3.6), 10 ml TPTZ solution (10 mM TPTZ in 40mM HCl), 10ml FeCl3 (20 mM) in a ratio of 10:1:1 (v/v). FRAP reagent was warmed to 37°C on a water bath prior to use. Sample at different concentration (200, 400, 600,800 and 1000 µg) was added to 3 ml of the FRAP reagent and the mixture sample incubated for 30 min. The increase in the absorbance at 593 nm was measured. Freshly prepared solution of FeSO4 was used for calibration.

3. Result

3.1 Preliminary qualitative phytochemical analysis

The present study revealed that the methanolic and aqueous extracts of aerial part of Lepidagathis cuspidata contained flavonoids, glycosides, phenols, saponins, steroids and terpenoids (Table 1). However, alkaloids were detected only in methanolic extracts of aerial parts of Lepidagathis cuspidata. Tannins were absent in methanolic and aqueous extracts.

Table 1: Preliminary qualitative phytochemical analysis of methanolic and aqueous extracts of aerial part of Lepidagathis cuspidata

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Plant Constituents</th>
<th>Extracts</th>
<th>MELC</th>
<th>AELC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Glycoside</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>


3.2 Thin Layer Chromatography (TLC)

TLC was performed on methanolic and aqueous extracts of aerial part of Lepidagathis cuspidata. The results are shown in Table 2.
Table 2: Rf values of MELC and AELC

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Phyto-constituents</th>
<th>Mobile phases</th>
<th>Spraying reagent</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spot colour</td>
<td>MELC</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v)</td>
<td>Dragendorff’s reagent followed by 10% ethanolicsulphuric acid reagent.</td>
<td>No Spot</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>Ethyl acetate: Formic Acid: Glacial Acetic Acid: Water (10: 1:1: 2.6 v/v/v/v)</td>
<td>1% ethanolic aluminium chloride reagent</td>
<td>Yellow</td>
</tr>
<tr>
<td>3</td>
<td>Glycoside</td>
<td>Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v)</td>
<td>Anisaldehyde sulphuric acid reagent</td>
<td>Blue</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>Toluene: Acetone: Formic Acid (4.5: 4.5: 1 v/v/v)</td>
<td>20% sodium carbonate solution followed by Folin-Ciocalteau reagent</td>
<td>Blue</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Chloroform: Glacial Acetic acid: Methanol: Water (6.4: 3.2: 1.2: 0.8 v/v/v/v)</td>
<td>Anisaldehyde sulphuric acid reagent</td>
<td>Blue</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>Toluene: Methanol (9:1 v/v)</td>
<td>Anisaldehyde sulphuric acid reagent</td>
<td>Purple</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>n-hexane: Ethyl acetate (7.2: 2.9 v/v)</td>
<td>Anisaldehyde sulphuric acid reagent</td>
<td>Pink</td>
</tr>
</tbody>
</table>
3.3 DPPH Assay

The DPPH method was performed and revealed that scavenging of free radical of MELC was found to be 27.00, 41.67, 55.00, 68.67, and 75.33 at concentration of 200, 400, 600, 800 and 1000 µg/ml, respectively. The inhibition of the DPPH radical by the AELC was 18.67, 23.67, 33.00, 40.00 and 55.67 at concentration of 200, 400, 600, 800 and 1000 µg/ml respectively. In the DPPH assay, the IC$_{50}$ of ascorbic acid was found to be 346.30 µg/ml where MELC and AELC was 543.15 and 949.11 µg/ml respectively.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (µg/ml)</th>
<th>Percentage of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MELC</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>27.00±1.00</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>41.67±0.88</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>55.00±1.53</td>
</tr>
<tr>
<td>4</td>
<td>800</td>
<td>68.67±1.20</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>75.33±1.76</td>
</tr>
</tbody>
</table>

The values presented are mean ± standard deviation, n = 3. Results were analyzed using descriptive statistics.

![Figure 3: DPPH radical Scavenging activity](image)

3.4 FRAP Assay

Antioxidant activity methanolic and aqueous extracts of plants determined by the FRAP assay. The results are shown in Fig 5. In the FRAP assay the absorbance of MELC was found to be 0.12, 0.17, 0.24, 0.50, 1.99 and the absorbance of AELC was found to be 0.09, 0.13, 0.17, 0.37, 1.65 at 200, 400, 600, 800 and 1000 µg of sample. Ascorbic acid is used as standard which had an absorbance 3.967 at the maximum concentration at 1000 µg.

![Figure 4: IC$_{50}$ values (µg/ml) - DPPH radical Scavenging activity](image)
4. Discussion and Conclusion

Aerial part of *Lepidagathis Cuspidata* was collected, authenticated, dried and ground to powder, further extracted by methanol and water separately by soxhlet method. Phytochemicals screening of MELC and AELC was performed which showed the presence of glycosides, steroids, saponins, flavonoids, phenols and terpenoids. Methanolic extract showed better qualitative tests for presence of secondary metabolites than aqueous extract.

TLC plate of methanolic and aqueous extracts of *Lepidagathis Cuspidata* were subjected to derivatisation, various spots were observed in different solvent system. Quantitative analysis of MELC showed presence of flavonoids, glycosides, phenols, saponins, steroids and terpenoids spots at Rf 0.63, 0.39, 0.78, 0.92, 0.59, 0.26 etc. respectively and AELC by TLC showed presence of glycosides, phenols, saponins, and terpenoids spots at Rf 0.085, 0.74, 0.72, 0.86, 0.23 etc. respectively. Also, The TLC studies showed that among the two solvents, methanol extracted higher quantity of secondary metabolites of medicinal importance viz., flavonoids, glycosides, phenols, saponins, steroids and terpenoids from the aerial parts of *Lepidagathis Cuspidata*.

Antimicrobial study of MELC and AELC were performed by bore plate method but there was no significant antimicrobial activity was observed on *E. coli*, *S. aureus* *S. pyrogen* and *S. typhi*.

The *In-vitro* antioxidant activity of both extracts of was evaluated by DPPH and FRAP, and compared with ascorbic acid as standard. From the result of both the methods it was concluded that the plant possesses good antioxidant activity, specifically methanolic extract shore more activity in comparison to aqueous extract.

In the recent year, the use of herbal medicine and phytochemical processing of antioxidant properties have been rise due to potential effects on in the therapy of various chronic and infectious diseases.[17] The result obtained from the extract of *Lepidagathis Cuspidata* have good antioxidant activity. However, Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy [18, 19]. The plants may be considered as good sources of natural antioxidants for medicinal uses such anti-inflammatory, wound healing, mouth ulcer and other diseases related to radical mechanisms[20,21]. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

References


