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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*. Methanol and aqueous extract of aerial part of *Lepidagathis cuspidata*was prepared and tested for qualitative phytochemical screening basis of their entahno-medicinal potential. The *in-vitro* antioxidant potential has been shown to be important in recovery from many diseases. The antioxidant was evaluated by DPPH [2, 2,-diphenyl -1-picrylhydrazyl] and ferric reducing antioxidant power (FRAP). Thin Layer Chromatography (TLC) of methanolic and aqueous extracts was performed by using various solvent systems. The present study revealed that the methanol extract of *Lepidagathis cuspidata* (MELC) and aqueous extract of *Lepidagathis cuspidate* (AELC) contained flavonoids, glycosides, phenols, tannin, saponins, steroids and terpenoids. However, alkaloids were detected only in MELC. Qualitative analysis of MELC showed presence of flavonoids, glycosides, phenols, saponins, steroids and terpenoids spots at R_F0.63, 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 R_F0.085, 0.74, 0.72, 0.86, 0.23 etc. respectively. The *in-vitro* antioxidant potential MELC and AELC was evaluated. The 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 e.g.- anti-inflammatory and antipyretic activity, mouth ulcer, 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, TLC and *In-vitro* antioxidant.

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

the botanist 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 (aerial part) was washed with water & then samples were air-dried at room temperature with dehumidifier about 12 days. Dried samples were ground to powder using a mechanical grinder, and stored in a sealed plastic container.

2.2 Extraction method [9]

In brief, 2 Kg coarse powders of the sample were extracted in soxhlet extractor with methanol. Then the extract was evaporated by using rotatory vacuum evaporator to get semisolid mass. The concentrated extract of aerial part of *Lepidagathis cuspidata* was stored in airtight close container in dark place.

Aqueous extraction was done by Hot water extract: 200 g of the weighed plant leaves powder was soaked in 2 L of boiled hot water. That mixture was boiled for thirty minutes into a conical flask and put for 24 h. The extract was filtered using filter paper and evaporated. The extractives were further concentrated below 40^0C and lyophilization were done for both the extract. The extracts were stored in airtight closed 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 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, their distances were measured, and R_F values were calculated.

$$\text{Retention Factor } (R_F) = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front}}$$

2.4.1 Detection of Spots

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

2.5 Evaluation of *in-vitro* antioxidant activity

Antioxidant *(in-vitro)* activity of methanol extract of *Lepidagathis cuspidate* (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.

2.5.1 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 and AELC was determined using method of Chen et al and compared with standard 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 37^0C for 30min and absorbance measured at 517nm.

% Inhibition of DPPH radicals$= \{(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\} \times 100$

Where, $A_{control}$= Absorbance without extract A_{sample}= Absorbance with extract.

2.5.2 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 $FeCl_3$ (20 mM) in a ratio of 10:1:1 (v/v). FRAP reagent was warmed to 37^0C 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 methanol and aqueous extracts of aerial part of *Lepidagathis cuspidata* contained flavonoids, glycosides, phenols, tannins, saponins, steroids and terpenoids **(Table 1).** However, alkaloids were detected only in methanolic extracts of aerial parts of *Lepidagathis cuspidata*.

Table 1: Phytochemical screening of methanol and aqueous extract of *Lepidagathis cuspidata*

Constituents	Extracts	
	MELC	AELC
Alkaloids	+	-- -
Flavonoids	+++	----
Glycoside	+++	+
Phenols	++	+
Saponins	++	+
Steroids	+++	+
Tannins	++	+
Terpenoids	++	+

Where, +++ Highly present, ++ Moderately present, + Less Present

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)**.

Figure 1: TLC of MELC

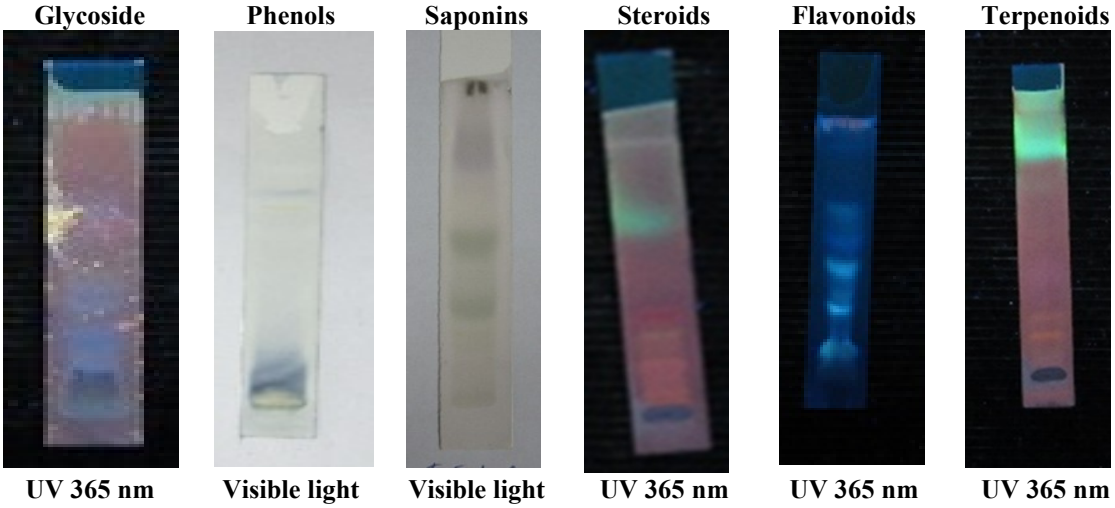

Figure 2: TLC of AELC

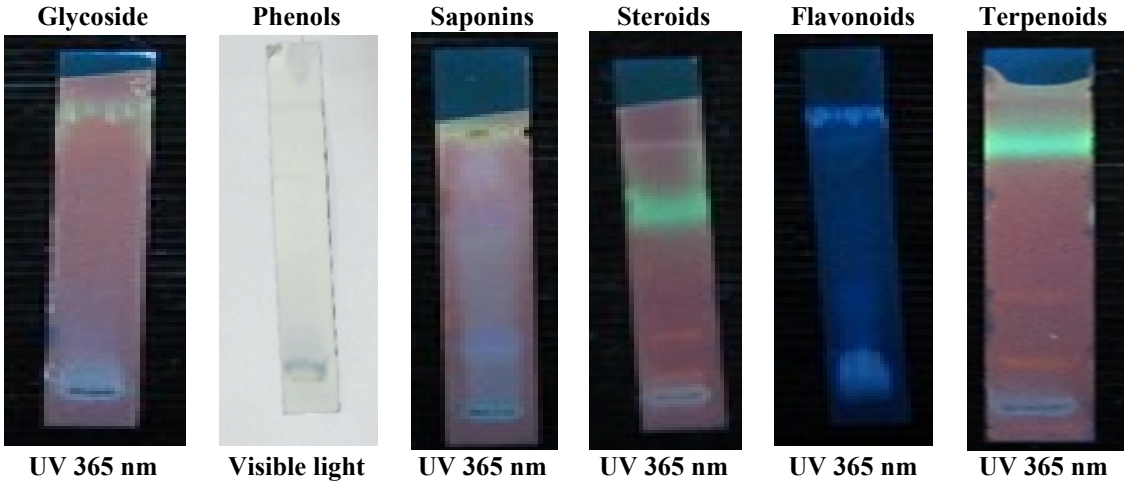

Table 2: Rf values for various phytochemicals in MELC and AELC

S. No	Phyto-constituents	Mobile phases	Spraying reagent		R_F value	
				Spot colour	MELC	AELC
1	Alkaloids	Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v)	Dragendroff's reagent followed by 10% ethanolicsulphuric acid reagent.	No Spot	--	---
2	Flavonoids	Ethyl acetate: Formic Acid: Glacial Acetic Acid: Water (10: 1.1: 1.1: 2.6 v/v/v/v)	1% Ethanolicaluminium chloride reagent	Yellow	0.63, 0.53, 0.40, 0.27.	---
3	Glycoside	Ethyl acetate: Methanol: Water (10: 1.35: 1 v/v/v)	Anisaldehdye sulphuric acid reagent	Blue	0.39, 0.25.	0.085.
4	Phenols	Toluene: Acetone: Formic Acid (4.5: 4.5: 1 v/v/v)	20% Sodium carbonate solution followed by Folin-Ciocalteau reagent	Blue	0.78	0.74.
5	Saponins	Chloroform: Glacial Acetic acid: Methanol: Water (6.4: 3.2: 1.2: 0.8 v/v/v/v)	Anisaldehdye sulphuric acid reagent	Blue	0.92, 0.54, 0.34	0.72, 0.48, 0.20.
6	Steroids	Toluene: Methanol (9:1 v/v)	Anisaldehdye sulphuric acid reagent	Purple	0.39, 0.24, 0.20, 0.14.	0.86, 0.23, 0.10.
7	Terpenoids	n-hexane: Ethyl acetate (7.2: 2.9 v/v)	Anisaldehdye sulphuric acid reagent	Pink	0.26, 0.19.	0.37, 0.17.

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 346.30µg/ml where MELC and AELC was 543.15 and 949.11 µg/ml respectively.

Table 2: **DPPH scavenging potential of the MELC and AELC**

S. No	Concentration (µg/ml)	Percentage of Inhibition		
		MELC	AELC	Ascorbic acid
1	200	27.00±1.00	18.67±1.20	41.33±1.20
2	400	41.67±0.88	23.67±1.45	54.67±0.88
3	600	55.00±1.53	33.00±1.53	62.67±1.20
4	800	68.67±1.20	40.00±1.53	70.00±1.15
5	1000	75.33±1.76	55.67±0.88	79.67±0.88

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

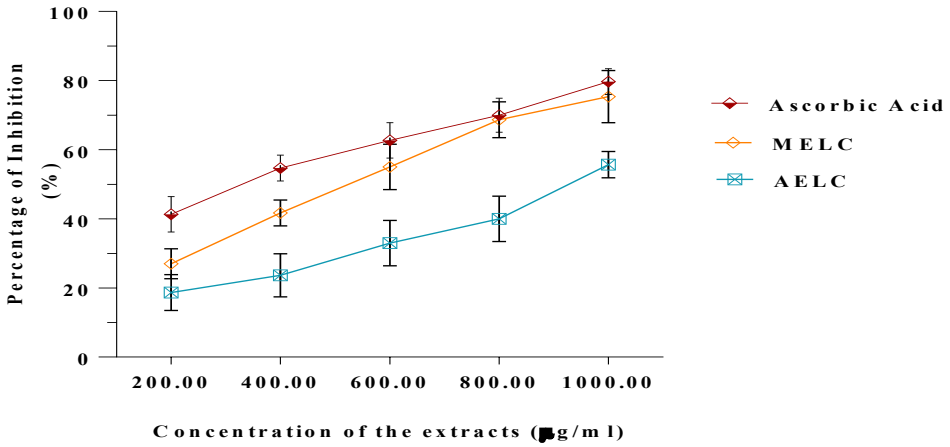

Figure 4: DPPH radical Scavenging activity

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 at1000 µg.

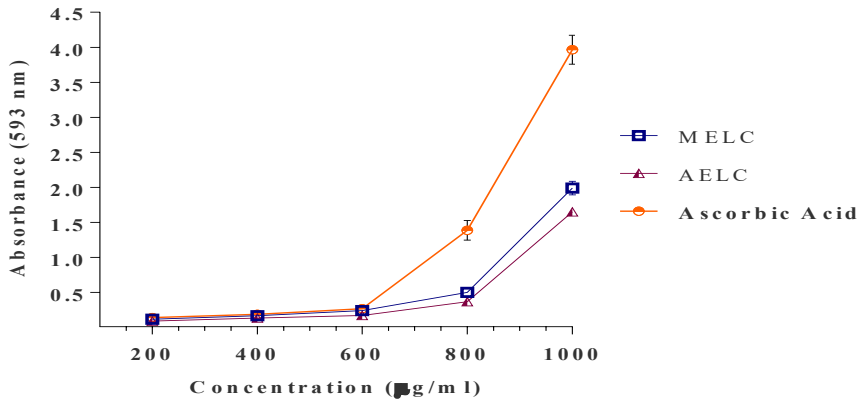

Figure 5: Ferric reducing antioxidant power (FRAP) assay

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

4. Discussion and Conclusion

Aerial part of *Lepidagathis Cuspidata* was collected, authenticated, dried and ground to powder, further extracted by methanol and water. Phytochemicals screening of MELC and AELC was performed which showed the presence of Glycoside, steroids, tannins, 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. Qualitative analysis of MELC showed presence of flavonoids, glycosides, phenols, saponins, steroids and terpenoids spots at R_f0.63, 0.39, 0.78, 0.92, 0.59, 0.26 etc. respectively and TLC for AELC showed presence of glycosides, phenols, saponins, and terpenoids spots at R_f0.085, 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.*

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 shows better 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]. Therefore further investigation along these lines would be worthwhile for isolation and characterization of extracts of the *Lepidagathis Cuspidata*and screening for the pharmacological action for the isolated compounds to identify an efficient antioxidant and various -inflammatory disorders.

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