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Evaluation of Phytochemical and Antioxidant Potential of *Lepidagathis Pungens Nees*: A Therapeutic Application

Research Article

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Abstract

This study aimed to investigate the physicochemical properties, heavy metal content, inorganic elements, extract parameters, and antioxidant activities of *Lepidagathis pungens Nees*, a plant with potential health-promoting properties. The physicochemical analysis revealed the presence of ash values, moisture content, total fiber content, swelling index, and foaming index. Heavy metal content analysis showed that cadmium, nickel, mercury, lead, chromium, and arsenic levels were within the safe limits the World Health Organization set, indicating the plant's safety for consumption. Various essential inorganic elements such as calcium, chloride, copper, iron, magnesium, nitrate, phosphate, and potassium were also confirmed. Five different solvent extracts of the plant were obtained, including Petroleum ether, chloroform, ethyl acetate, methanol, and absolute ethanol extracts. from the whole plant, and its extractive values, colours, odours, and consistencies a determination was made. The methanol and aqueous extracts had the highest extractive values. The fluorescent analysis of the whole plant powder revealed various colours under daylight, long UV, and short UV light, depending on the solvent used. The antioxidant properties of the *Lepidagathis pungens Nees* extracts were assessed through their product scavenges superoxide radicals, hydroxyl radicals, and lipid peroxidations. An aqueous and methanol extract demonstrated the highest scavenging activities across all concentrations for both Lipid peroxidation and hydroxyl radicals. The scavenging activity increased in an inverse relationship between dose and response. In conclusion, this study demonstrated the potential of *Lepidagathis pungens Nees* as a source of antioxidant compounds, particularly in methanol and aqueous extracts.

Keywords: *Lepidagathis,* Standardization, Antioxidant activity, Phenols, Extraction.

Introduction

The global population is approximately 80% and continues to rely on herbal remedies for addressing various ailments. The current landscape highlights an escalating demand for herbal products worldwide, with leading pharmaceutical companies diligently exploring plant-based materials for potential therapeutic applications. As such, it is crucial to ensure the efficacy and safety of these herbal products, as underscored by the World Health Organization (WHO) in 2000. The WHO emphasizes the significance of employing qualitative and quantitative techniques to characterize herbal samples, quantify biomarkers or chemical markers, and analyze fingerprint profiles. Herbal medicines are now widely acknowledged as safe alternatives, exhibiting minimal (6) described adverse effects.

As herbal medicine becomes increasingly commercialized, it is increasingly important to ensure its safety, quality, and efficacy a pressing concern. Factors like plant identity and seasonal variation can introduce significant variability in herbal raw materials. The measures implemented by the WHO aim to not only enhance A herbal product's quality, as well as mitigate potential negative impacts (2). The present research encompasses the examination of assessment of Researchers in this study assessed the in vitro antioxidant activity of successive extractives, in addition to quantitatively determining phytochemical constituents, as well as physicochemical constants from the powdered whole plant of *Lepidagathis pungens Nees.*

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Methods and materials

Biological reagents and chemicals

The study was conducted with chemicals supplied by Rankem of Mumbai and Himedia Laboratories Ltd. of Mumbai solvents and reagents.

Plant material

Whole plant of *Lepidagathis pungens Nees* Collection took place in Chennai, Tamil Nadu, in February. In the library, the plant species identified by the voucher specimen were deposited, along with a duly authenticated plant species identification report, for the benefit of future researchers.

Constants of physics and chemistry

A 5-day drying period at room temperature was followed by a proper grounding process and a sieve no. 80 was used to filter the powder and the sample was evaluated for physical constant as well as the number of extractive values, and fluorescent analysis (7). A whole plant's Swelling index, foaming index, fiber content, and foaming index were determined by Evans (15). In (13) heavy metal estimates were determined following a standard procedure.

Studies on phytochemistry

Incorporation

A total of 1.5 kg of plant material was shade-dried and subsequently ground into a coarse powder (using sieve no. 40). The powdered material was (3,9) carried out successive solvent extractions with ether, chloroform, ethyl acetate, and methanol (60-80°C) using a Soxhlet apparatus. The evaporation process was carried out using a rotary vacuum evaporator the extracted samples, and dry weight of the powdered whole plants was used to calculate the final yield. Different chemical constituents were analyzed in these dried extracts, including Alkaloids and carbohydrates are not the only substances found in plants. Other substances include phenols, steroids, flavonoids, gums, mucilages, proteins, volatile oils, fixed oils, fats, and saponins (8).

The extracts of *Lepidagathis pungens Nees* were further analyzed using Infrared (365 nm-Nano Meter), short UV (254 nm), and visible light fluorescence. With petroleum ether, fixed oils were extracted continuously for 3 hours from powder. A reduction in pressure at 40°C was used to concentrate the extracts after they were filtered (10). An apparatus of the Clevenger type was used to hydro-distil 100 g of powdered material for 3 hours in order to separate the essential oils. (12) followed the standard method for extracting essential oils and drying them (anhydrous Na2SO4).

The activity of antioxidants in vitro

The in vitro antioxidant activity of the extracts was analyzed according to previously published procedures with some minor modifications (17).

Scavenging activity against superoxide radicals

There were 5 mL of extract/standard drug (ascorbic acid) in varying concentrations (10-50 g/mL, dissolved in 90% ethanol) added to 0.5 mL of 100 mM phosphate buffer (pH 7.4), 0.5 mL of 0.4 mM NADH(Nicotinamide adenine dinucleotide) and 1.0 mL of 0.156 mM NBT added to the reaction mixture. A suitable blank was used to determine the amount of formazan produced in the mixture after 1 hour of incubation at 25°C (11).

Scavenging of hydroxyl radicals

The mixture of reagents was prepared by combining 0.1 mL of 10 mM 2-deoxy-2-ribose, 0.1 mL of 0.1 mM FeCl3, 0.1 mL of 0.1 mM phosphate buffer (pH 7.3), 0.33 mL of 50 mM phosphate buffer (pH 7.4) ethylenediaminetetraacetic acid Ascorbic acid, H2O2, and EDTA in equal concentrations and volume as necessary of various concentrations (5-50 µg/mL) of extracts. After 45 minutes of incubation, 1.0 mL of trichloroacetic acid (TCA, 2.8% v/v) and 1.0 mL of thiobarbituric acid (TBA, 0.5% v/v in 0.025 mol/L NaOH solution containing 0.2% w/v butylated hydroxyanisole) were added. After incubation at 95°C for 15 minutes, chromogen was developed. A suitable blank solution was used to measure absorbance at 532 nm after cooling (14).

Activity at scavenging lipid peroxidation

An 0.5-milliliter reaction mixture was prepared consisting of 0.1 milliliter of 25% w/v rat liver homogenate, 30 milliliters of KCl, 0.16 milliliters of FeCl3, and 0.06 milliliters of ascorbic acid in a buffer containing 40 milliliters of Tris-HCl pH 7.0, 0.16 mg of FeCl3, and 0.06 milliliters of ascorbic acid. An hour of incubation was performed at 37°C on this mixture with and without the Various concentrations (50-250 grams/ mL) of isolated extracts/standard drugs. The formation of lipid peroxide was assessed by measuring the substances that are reactive to thiobarbituric acid (TBARS). In order to generate the incubation mixture, 0.4 mL of it was mixed with 1.5 mL of 0.8% TBA, 1.5 mL of 20 acetic acid (pH 3.5), and 0.2 mL of 8.1% sodium dodecyl sulfate (4.1% salt). We adjusted the total volume to 4.0 mL by bathing the sample in distilled water at 100°C for 1 hour. As a final step, 1.0 mL of distilled water was added to the reaction mixture and 5.0 mL of a mixture of n-butanol and pyridine (10:1 v/v) was added to the mixture and centrifuged for 10 minutes at 4000 rpm for 10 minutes. A layer of butanol-pyridine was measured at 532 nm to determine its TBARS content.

All three methods were the scavenging activity inhibition equation is calculated using the following formula percentages:

$$\textit{Percent inhibition (\%)} = (A_{control}-A_{test})/A_{control}\times 100 \quad (1)$$

Test substances (Extracts and standard drugs) have an absorbance of the latest, while controls have an absorbance of control.

Analyses of statistics

Three replicates of each experiment were performed, with three independent studies each reporting the mean and standard deviation (SEM) as the results.

Results

Physicochemical constants

The physicochemical parameters of *Lepidagathis pungens Nees* leaves, as presented in table 1, provide essential information regarding their composition and properties. The ash values, which include total ash (6.9±0.5%), acid-insoluble ash (3.2±0.2%), water-soluble ash (1.1±0.7%), and sulphated ash (0.8±0.3%), indicate the mineral content of the plant material. These values are crucial in understanding the plant's nutritional composition and potential therapeutic

applications. Moreover, the moisture content (loss on drying) of 10.64±1.62% highlights the plant material's stability and potential vulnerability to microbial contamination, which is vital when considering storage and shelf-life. The total fiber content of 15.7±3.3% suggests the plant's nutritional value, as fiber is an essential component of a balanced diet and plays a significant role in maintaining digestive health. Additionally, the swelling index (17.5±3.6%) and foaming index (86.4±7.5%) offer insights into the potential uses of *Lepidagathis pungens Nees* leaves in various industries. The swelling index is indicative of the plant material's capacity to absorb water and expand, which can be relevant in food, pharmaceutical, and cosmetic applications. The foaming index demonstrates the plant's potential to produce stable foam, an essential characteristic for certain applications in the food and cosmetic industries. Overall, the table provides a comprehensive understanding of the physicochemical properties of *Lepidagathis pungens Nees* leaves, which is crucial for further research and development of potential applications.

Table 1: Physicochemical parameters of the leaves of *Lepidagathis pungens Nees*

S. No.	Parameters	Average % W/W
1	**Ash values** a) *Total ash* *b) Acid-insoluble ash* *c) Water-soluble ash* *d) Sulphated ash*	6.9±0.5 3.2±0.2 1.1±0.7 0.8±0.3
2	**Moisture content** *Loss on drying*	10.64±1.62
3	Total fiber content	15.7±3.3
4	Swelling index	17.5±3.6
5	Foaming index	86.4±7.5

Values were expressed as Mean ±SD

Table 2 presents the heavy metal content found in the crude drug powder of *Lepidagathis pungens Nees* leaves and compares them with the acceptable limits set by the World Health Organization (WHO, 2007). It is crucial to evaluate heavy metal content in plant materials, as excessive amounts can pose health risks when consumed. Cadmium was found in a concentration of 0.23±0.08 ppm, which is within the acceptable limit of 0.3 ppm. Nickel was detected at 2.28±0.67 ppm, with no defined limit by WHO. Mercury levels were below the quantifiable limit (BQL), indicating that the concentration was well within the safe limit of 1.0 ppm. Lead was found at 3.08±0.75 ppm, significantly lower than the WHO limit of 10 ppm. Chromium was detected at a concentration of 0.91±0.38 ppm, well within the acceptable limit of 2.0 ppm. Lastly, arsenic levels were 1.37±0.62 ppm, which is also within the safe range of the 3.0 ppm limit established by WHO. The values are expressed as mean ± standard deviation (SD).

Table 2: Powdered crude drugs containing Heavy metals

Heavy metal	Quantity ppm	Limits ppm (WHO, 2007)
Cadmium	0.23±0.08	0.3
Nickel	2.28±0.67	ND
Mercury	BQL	1.0
Lead	3.08±0.75	10
Chromium	0.91±0.38	2.0
Arsenic	1.37±0.62	3.0

Values were expressed as Mean ±SD; BQL-Below quantifiable limit

Table 3 presents the extract parameters of the whole plant of *Lepidagathis pungens Nees*, obtained using various solvents. These parameters include extractive value (percentage weight/weight), colour, odour, and consistency. Extractive value is an essential aspect to consider, as it indicates the relative amount of phytochemicals that can be obtained from the plant material using different solvents. The petroleum ether soluble extractive exhibited a black-green colour, characteristic odour, and greasy consistency, with an extractive value of 11.36±3.82%. The chloroform soluble extractive showed a dark green colour, characteristic odour, and greasy consistency, with an extractive value of 14.55±4.06%. The ethyl acetate soluble extractive displayed a black-green colour, characteristic odour, and greasy consistency, with an extractive value of 3.19±0.57%. These three extracts, being greasy, might be rich in lipophilic components, which can potentially influence the plant's therapeutic properties. The methanol soluble extractive had a black-brown colour, characteristic odour, and non-greasy consistency, with an extractive value of 26.38±5.71%. The water-soluble extractive demonstrated a brown colour, characteristic odour, and sticky, non-greasy consistency, with an extractive value of 26.04±5.13%. The non-greasy nature of these extracts suggests that they might be rich in hydrophilic components. The relatively high extractive values of methanol and water-soluble extractives indicate that a significant portion of the plant's phytochemicals can be extracted using these solvents.

Table 3: Extract parameters of the whole plant of *Lepidagathis pungens Nees*

Solvent	Extractive value %w/w	Colour	Odour	Consistency
Pet. Ether soluble Extractive	11.36±3.82	Black green	Characteristic	Greasy
Chloroform soluble extractive	14.55±4.06	Dark green	Characteristic	Greasy
Ethyl acetate soluble Extractive	3.19±0.57	Black green	Characteristic	Greasy
Methanol soluble Extractive	26.38±5.71	Black brown	Characteristic	Nongreasy
Water soluble Extractive	26.04±5.13	Brown	Characteristic	Sticky nongreasy

Table 4: Extractive values of the Whole plant of *Lepidagathis pungens Nees* with different solvents

Extraction solvent	Extractive value %
Alcohol soluble extractive value	10.06±2.57
Ether soluble nonvolatile extractive value	0.63±0.18
Volatile extractive value	27.37±4.65

A preliminary phytochemical screening and fluorescent analysis are presented in Tables 5 and 6. In addition to polyphenols, alkaloids, steroids, carbohydrates, proteins, gums, mucilages, and saponins found in the raw material, it also contained alkaloids, steroids, carbohydrates, proteins, and sterols.

The table presents the results of the fluorescent analysis of the whole plant powder of *Lepidagathis pungens Nees* and its extracts, which were examined under daylight, long UV, and short UV light. Fluorescent analysis is a valuable technique for evaluating the presence of various chemical constituents in plant materials and can provide insights into their composition and potential applications. Under daylight conditions, the powder exhibited a green colour, while the various extracts showed different shades of green, ranging from greenish (petroleum ether) to dark green (aqueous). When exposed to long UV light, the powder appeared dark green, and the extracts displayed colours from dark orange (petroleum ether) to dark green (aqueous and chloroform). In short UV light, the powder and most of the extracts (petroleum ether, chloroform, and methanol) showed a dark green to fluorescent green colouration, while the ethyl acetate extract appeared pale green. These variations in fluorescence under different light conditions and solvents indicate the presence of diverse chemical constituents in the plant material. Some of these compounds may have potential therapeutic benefits and could be further explored in the development of new medicines, supplements, or other health-promoting products.

Table 5. Fluorescent analysis of Whole plant powder of *Lepidagathis pungens Nees*

Treatments	Observations		
	Day light	Long UV	Short UV
Powder as such	Green	Dark green	Dark green
Pet. Ether	Greenish	Dark orange	Fluorescent green
Chloroform	Yellow-green	Dark green	Fluorescent green
Ethyl acetate	Pale green	Yellowish brown	Pale green
Methanol	Dark green	Brownish Green	Fluorescent green
Aqueous	Brownish green	Dark green	Dark green

Table 6. Different extracts were screened for phytochemical properties of *Lepidagathis*

Sl. No.	Test	P. ether	Choloform	Ethyl acetate	Methanol	Aqueous
1	Carbohydrates	-	-	-	+	+
2	Alkaloids	+	+	-	+	+
3	Glycosides	-	+	-	+	+
4	Tannins	-	-	-	+	+
5	Steroids	+	+	-	+	-
6	Triterpenoids	+	+	+	+	-
7	Volatile oils	-	-	-	-	-
8	Fats and fixed oils	-	-	-	-	-
9	Flavanoids	+	+	-	+	+
10	Polyphenols	-	-	-	+	+
11	Saponins	-	-	-	+	+
12	Aminoacids	-	-	-	+	+
13	Gums and mucilages	-	-	-	-	+

"+" represents **Presence** **"-"** represents **Absence**

In vitro antioxidant activity

Table 7 presents the superoxide radical scavenging activity of *Lepidagathis pungens Nees* extracts at varying concentrations (10, 20, 30, 40, and 50 µg/mL). The extracts tested include Petrol ether, chloroform, ethyl acetate, methanol, and ethyl alcohol are used in the preparation of extracts of aqueous extracts. The positive control for comparison was ascorbic acid, a known antioxidant. At 10 µg/mL, the petroleum ether extract exhibited a scavenging activity of 35.62±4.46%, while the chloroform extract showed 42.61±4.92%. The ethyl acetate extract had lower activity of 23.33±3.68%, whereas the methanol and aqueous extracts displayed higher activities of

46.52±4.14% and 45.17±3.66%, respectively. Ascorbic acid exhibited the highest activity with 62.54±5.84%. With increasing concentrations, the scavenging activity for all extracts improved. At 50 µg/mL, the petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts demonstrated scavenging activities of 65.55±9.27%, 84.16±8.54%, 42.26±8.32%, 97.76±7.91%, and 92.43±10.57%, respectively. Ascorbic acid maintained the highest activity with 99.25±9.07%.

Table 8 illustrates the scavenging activity of different concentrations of Lepidagathis extracts (10, 20, 30, 40, and 50 g/mL). Petroleum ethers and chloroforms were tested along with petroleum ethers, chloroforms, ethyl acetates, methanol, and aqueous extracts. A positive control was used for comparison, ascorbic acid, which is known to be an antioxidant. At the lowest concentration of 10 µg/mL, the petroleum ether extract showed a scavenging activity of 22.93±4.16%, while the chloroform extract exhibited a higher activity of 50.47±3.62%. The ethyl acetate extract had a moderate activity of 31.15±4.78%, and the methanol and aqueous extracts displayed similar activities of 50.16±5.98% and 49.85±5.55%, respectively. Ascorbic acid had an activity of 51.02±6.16%. As the concentration increased to 50 µg/mL, the scavenging activity for all extracts improved. The petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts demonstrated scavenging activities of 64.07±10.51%, 85.76±11.26%, 49.32±11.06%, 98.77±10.57%, and 96.75±13.52%, respectively. Ascorbic acid maintained a high activity with 93.25±10.05%.

Table 9 presents the lipid peroxidation scavenging activity of *Lepidagathis* extracts at the same concentrations as in Table 8. At 10 µg/mL, the scavenging activities for Extracts were made from methanol, chloroform, petroleum ether, and aqueous solutions 40.36±4.16%, 46.91±3.66%, 17.49±3.17%, 50.16±3.95%, and 53.16±3.08%, respectively. Ascorbic acid had an activity of 58.14±4.81%. At the highest concentration of 50 µg/mL, the scavenging activities for the petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts were 70.46±9.57%, 81.29±10.58%, 52.07±8.76%, 96.35±10.58%, and 95.82±11.68%, respectively. Ascorbic acid maintained the highest activity with 97.26±5.93%. These tables indicate that *Lepidagathis pungens Nees* extracts possess antioxidant properties, with the methanol and aqueous extracts demonstrating the highest scavenging activities across all concentrations for both hydroxyl radicals and lipid peroxidation. The scavenging activity increased in a dose-dependent manner, suggesting the potential for these extracts to effectively neutralize free radicals and prevent lipid peroxidation. Further investigation into the specific compounds responsible for the antioxidant activity could lead to the development of health-promoting products or therapeutic interventions utilizing the extracts' antioxidant properties.

Table 7. Superoxide radicals scavenging activity of *Lepidagathis*

Group	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
Pet Ether	35.62±4.46	42.68±6.44	51.82±7.39	59.82±7.69	65.55±9.27
Chloroform	42.61±4.92	60.86±5.87	73.03±6.11	80.17±7.41	84.16±8.54
Ethyl acetate	23.33±3.68	29.75±6.04	34.61±7.09	38.27±6.89	42.26±8.32
Methanol	46.52±4.14	64.82±5.13	78.45±6.38	90.08±7.06	97.76±7.91
Aqueous	45.17±3.66	63.29±5.22	73.54±6.2	86.99±7.54	92.43±10.57
Ascorbic acid	62.54±5.84	71.75±6.24	81.09±6.87	89.76±8.41	99.25±9.07

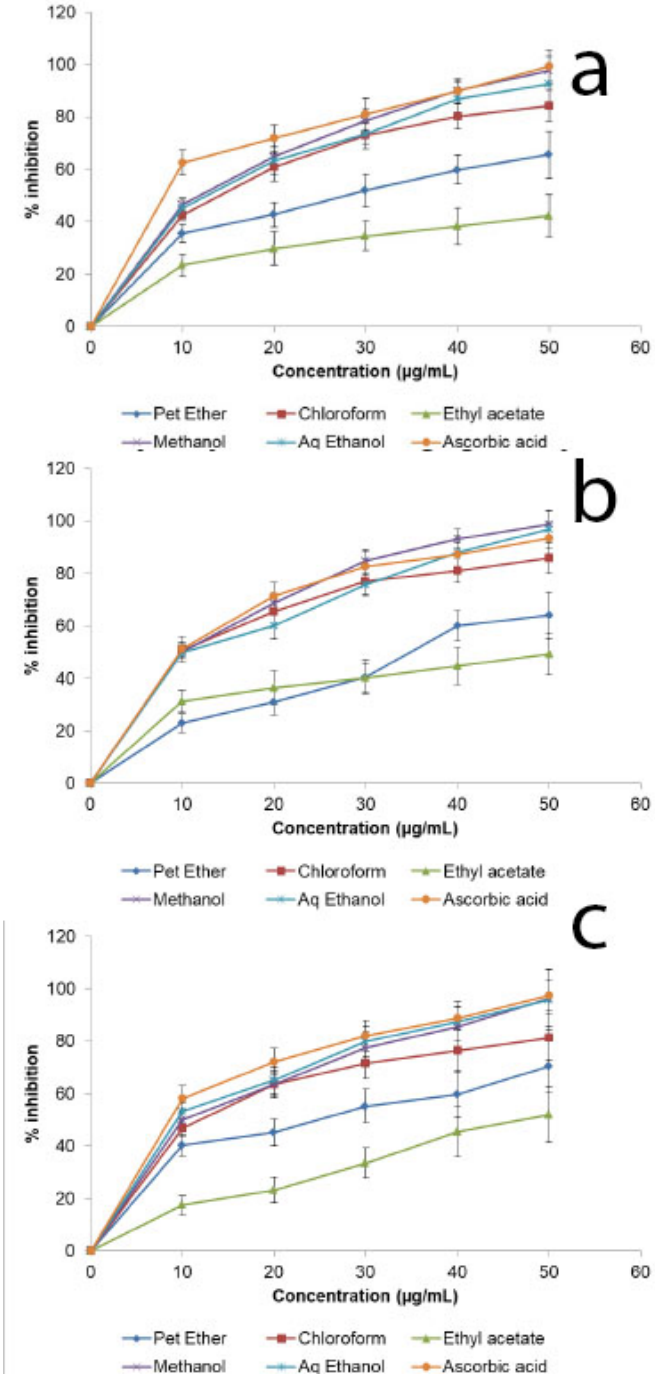
Table 8. Hydroxyl radical scavenging activity of *Lepidagathis* extracts

Group	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
Pet Ether	22.93±4.16	31.07±5.92	40.49±7.79	59.98±8.76	64.07±10.51
Chloroform	50.47±3.62	65.28±6.26	76.91±8.49	81.07±9.46	85.76±11.26
Ethyl acetate	31.15±4.78	36.48±5.88	39.99±7.64	44.76±8.55	49.32±11.06
Methanol	50.16±5.98	68.58±6.33	84.67±7.44	93.16±9.86	98.77±10.57
Aqueous	49.85±5.55	60.11±7.07	75.46±7.04	87.91±7.36	96.75±13.52
Ascorbic acid	51.02±6.16	71.24±5.87	82.45±8.74	87.24±9.14	93.25±10.05

Table 9. Lipid peroxidation scavenging activity of *Lepidagathis* extracts

Group	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL
Pet Ether	40.36±4.16	45.18±5.07	55.27±6.27	59.82±8.74	70.46±9.57
Chloroform	46.91±3.66	63.55±4.91	71.62±5.84	76.36±9.67	81.29±10.58
Ethyl acetate	17.49±3.17	23.27±4.13	33.49±5.99	45.46±8.47	52.07±8.76
Methanol	50.16±3.95	63.57±5.14	77.49±6.04	85.49±7.65	96.35±10.58
Aqueous	53.16±3.08	65.22±5.11	79.81±5.87	87.46±7.44	95.82±11.68
Ascorbic acid	58.14±4.81	72.16±5.12	82.04±5.97	88.75±4.57	97.26±5.93

Figure 1: Invitro Extracts with antioxidant properties of *Lepidagathis pungens Nees.*



a. Scavenging activity of superoxide radicals b. Scavenging activity of hydrogen peroxide radicals c. Scavenging activity of lipid peroxidation radicals

Conclusion

In conclusion, the study explored the physicochemical parameters, heavy metal content, inorganic elements, extract parameters, and antioxidant properties of *Lepidagathis pungens Nees*. The analysis of physicochemical parameters revealed the presence of This laboratory also measures fiber content in addition to ash content, moisture content, swelling index, and foaming index. The heavy metal content was within the safe limits the World Health Organization set, indicating the plant's safety for consumption. Various inorganic elements such as calcium, chloride, copper, iron, magnesium, nitrate, phosphate, and potassium were also established. Different solvent extracts, including Chemicals analyzed, including petroleum ethers, chloroforms, ethanols, methanols, and aqueous extracts, were obtained from the whole plant, and their extractive values, colours, odours, and consistencies were determined. The methanol and aqueous extracts had the highest extractive values. The fluorescent analysis of the whole plant powder revealed various colours under daylight, long UV, and short UV light, depending on the solvent used.

The antioxidant properties of the *Lepidagathis pungens Nees* extracts were assessed through their product scavenges superoxide radicals, hydroxyl radicals, and lipid peroxidations. Aqueous extracts and methanol extracts demonstrated the highest scavenging activities across all concentrations for both Peroxidation of lipids by hydroxyl radicals. The scavenging activity increased in an inverse relationship between dose and response. Overall, this study demonstrated the potential of *Lepidagathis pungens Nees* as a source of antioxidant compounds. The methanol and aqueous extracts, in particular, showed promising antioxidant activities. Further research into the Antioxidant compounds specific to different compounds' activity could lead to the development of health-promoting products or therapeutic interventions utilizing the extracts' antioxidant properties. Additionally, the safety profile and presence of essential inorganic elements in the plant suggest its potential for use in traditional medicine or as a dietary supplement.

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