PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF *PLUCHEA LANCEOLATA* LEAVES

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ABSTRACT: Historically, medicinal plants have been the mainstay of human disease therapy. In the past, traditional systems of medicine have used the *Asteraceae* family member *pluchea lanceolata (Rasna)* as a bitter, laxative, and nervine tonic. The leaves of the plant, which are a part of the *pluchea lanceolata* species, are the focus of the current study's attention. It examines their pharmacological activity, pharmacognostic profile, and preliminary phytochemical research. Using a variety of criteria, such as ash value, extractive value, moisture content, etc., we investigated the antibacterial and antioxidant activity. *P. lanceolata* leaf extracts were used to investigate the in vitro antibiotic efficacy of four microorganisms. Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and Bacillus subtilis at a temperature of 37 °C, these bacteria were cultivated in nutrient broth, Mueller Hinton agar and the Cup-Plate method were used to conduct an anti-microbial assay. A 400 ug/ml concentration of *P. lanceolata* extract and a 200 ug/ml concentration of the common antibiotic Amoxicillin were dissolved in DMSO solvent. The second activity, antioxidant activity, was evaluated spectrophotometrically by the DDPH (2, 2-Diphenyl-1-picrylhydrazyl) assay using ascorbic acid as a reference. Concentrations ranging from 100 to 500 g/ml of *P. lanceolata* leaf extracts in petroleum ether, ethyl acetate, and alcohol were created. According to the findings of the current investigation, *pluchea lanceolata* is an important source of many therapeutically useful components. Researchers have identified a variety of therapeutic characteristics for the plant after doing significant pharmacological research on it.

KEYWORDS: *Pluchea lanceolata, Extraction, Antioxidant activity, TLC, Screening method, Antibacterial activity.*

INTRODUCTION

Ayurveda is one of the oldest forms of medicine, having its roots in India. Before 3500, for example, Indian medicinal plants have a very long history. The Rig-Veda and Atharvaveda (3500–1500) discuss the therapeutic use of plants. A large range of plants and their therapeutic benefits are discussed in Ayurveda. Ayurveda is well-taught in the Charak Samhita and the Susruta Samhita. [1] There is a plant called *Pluchea lanceolata (Rasna)* that is native to Punjab, Rajasthan, upper west Bengal, and Gujarat, Uttar Pradesh, and Asian nations. Terete, elongated, and lightly pubescent stem and branches. *Pluchea lanceolata* transverse section of the stem has a nearly round, thick circle-covered appearance. A single layer of thick-walled cells with a coating and granular trichomes make up the epidermis. In contrast to granular trichomes, which are sessile and stalked, covering trichomes are uniseriate, multicellular, and have two to many thick-walled cells. Compound corymbs with multiple heads have flowers that are white, purple, yellow, or violet. Ovoid or campanulate capitulum, 6-7mm in corymbs and compound pubescent The outer involucral scale is 2.5-4.0 mm long, 2 mm wide, 5-3 serrate, obtuse, silky pubescent, and tinted purple outside tip. Involucres are ovoid or widely campanulate and have imbricated scales. The leaves are sessile, 2–6 cm long, oblanceolate or oblong, coriaceous, delicately silky, and pubescent on both surfaces. Near the apex, the margins are whole or subtly dentate. The leaf has both covering and granular trichomes; the covering trichomes were uniseriate, multicellular (ranging from 2 to 5 cells), and around 90 um in size. The granular trichomes were sessile and packed. [2, 3] *Pluchea lanceolata, or Rasna*, is a natural remedy for everyone. Issues with the neurological system, particularly the nerves. Rasna is beneficial for illnesses including sciatica, neuritis, and nervous system inflammation that is ongoing. Since the last Vata has good control over a portion of the gut, issues including flatulence and indigestion, which are connected with the intestine's last segment, are healthy *Rasna* treated people *rasna* functions as a drug and a rasayana of choice to slow the aging process. [4, 5] Plant extracts or antioxidant substances generated from plants strengthen the body's defense against free radicals and are preferred because they are secure. Antioxidant main characteristic is their capacity to absorb free radicals. Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids can stop the oxidative processes that result in degenerative diseases by scavenging free radicals, including peroxide, hydroperoxide, and lipid peroxyl. It has anti-inflammatory and analgesic properties and is widely used to treat neurological disorders, rheumatoid arthritis, sciatica, edema, bronchitis, dyspepsia, cough, psoriasis, and piles. Various secondary metabolites are present in the plant. Chemicals with antibacterial activity prevent or slow down bacterial growth locally while posing no risk to nearby tissue. A molecule's antibacterial activity is exclusively linked to substances that selectively eradicate bacteria and viruses or inhibit their growth without significantly damaging adjacent tissues. There are various ways to conduct a microbiological assay. These include the urease assay, the luciferase assay, the tube assay (also called the turbidimetric method), and the disc diffusion method (also known as the cylindrical cup plate method). The agar layer containing the microorganisms is diffused with an antibiotic-laden cylinder in the cup plate method. The cylinder forms the perimeter of the zone. The second technique uses the disc diffusion method, which measures the zone of inhibition surrounding the antibiotic disc.[6] The disc diffusion experiment was used to investigate the antibacterial activity of a petroleum ether, ethyl acetate, and hydro-alcoholic

extract of *P.lanceolata* leaves (200ug/ml). Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae were the test organisms used in the study. In Ayurvedic treatment, the entire plant is employed. Pluchea lanceolata is recognized as a traditional arthritic treatment. Its decoction is administered for fever, edema, rheumatoid arthritis, and muscular discomfort. It can also be used topically as massage oil. The laxative, analgesic, and antipyretic properties of the leaves make them aperients. [7]

PHARMACOGNOSTIC STUDY

PLANT PROFILE

TABLE: 1. scientific classification

Botanical Name	*Pluchea lanceolata*	**Kingdom**	Plantae
Common Name	*Rasana*	**Subkingdom**	Tracheobionta
Synonyms	Berthelotia lanceolata	**Division**	Magnoliopsida
Hindi	Phaar	**Order**	*Asterales*
Subclass	*Asteridae*	**Family**	*Asteraceae* (Sunflower family)
Genus	*Pluchea*	**Species**	*Lanceolata*
Part used	Leaves, Whole plant		

Fig. 1: Plant of *Pluchea lanceolata*

PLANT COLLECTION: Gujarat, Uttar Pradesh, Punjab, Rajasthan, and Asian countries are all places where this plant can be found.

STEM AND BRANCHES: Stem and branches are terete, thin, and lightly pubescent. *Pluchea lanceolata* stem's transverse section has an almost round form that is covered in a thick circle. Several layers of thick-walled cells make up the epidermis.

FLOWERS: Flowers are found in many-headed compound corymbs and might be white, purple, yellow, or lilac in color. The involucres of the capitulum are ovoid or campanulate, and the outer involucral scales are 2.5–4.0 mm long, 2 mm wide, 5–3 serrate, obtuse, silky pubescent, and tinged purple outside the apex.

LEAVES: The leaves are sessile, oblanceolate or oblong, coriaceous, finely silky, and pubescent on both surfaces. Near the apex, the margins are whole or obscurely dentate. The trichomes on the leaf are both covering and granular. [3, 8]

MATERIAL AND METHODS

Collection and Extraction of plant material

The leaves of *P.lanceolata* were used raw material for the extraction and other investigation process. *Pluchea lanceolata* plant was collected from Manas Ayurveda in Aura Park, Nagpur and authenticated from Botany Department of RTMNU, Nagpur University, and Nagpur (Specimen Voucher no. 10562). The leaves were air dried in the shade at room temperature, crushed or ground into a coarse powder, and then roughly 250g of the powder was extracted using Petroleum ether, Ethyl acetate solvent was used in the process of soxhlation method temperature was set around 65°C and Hydroalcohol in a maceration technique. The resultant filtrate was heated in a water bath to dryness before being stored in a clean container for later use. [9, 10]

Microscopical Analysis of P. Lanceolata Leaf

Transverse Section through the Midrib of a Leaf: [5, 8, 13]

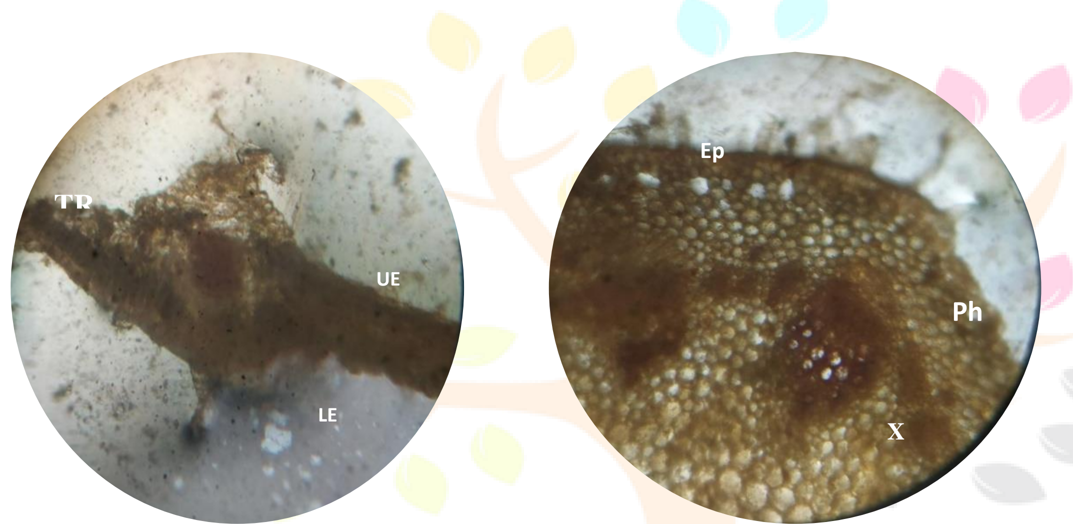


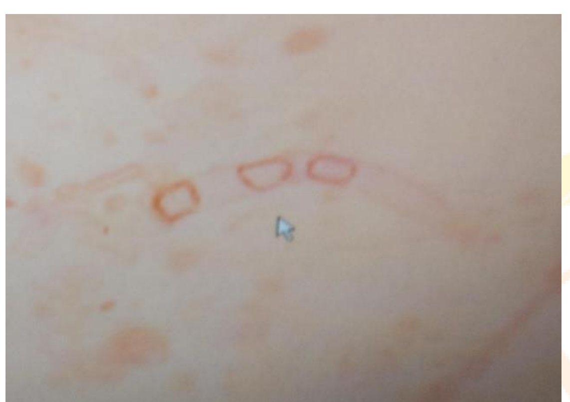
Fig 2: T.S. of Leaf **Fig. 3: T.S of Stem**

Fig 2: Microscopy of the leaf of *P.lanceolata* (DC.) View Transverse section of leaf passing through the midrib and shows that the upper and lower epidermis is made up of single layered cells covered with cuticle. Both the epidermis is multicellular covering trichomes. Single layer palisade cells are present bellow epidermis. Mesophyll showed the presence of parenchymatous cells.

Fig 3: Microscopy of the stem of P.lanceolata (DC.) view transverse section of stem. Shows that upper layer epidermis is multicellular covering trichomes. Midrib shows presences of vascular bundles. Shows presences of covering (non-glandular) trichomes which are 2-3 celled, uniseriate with pointed apex and are plenty on lower epidermis and the multicellular glandular trichomes in which the stalk is 2-4 celled and head is unicellular. Ep-epidermis, Tr-trichomes, Vb-vascular bundle, X-xylem, Ct-cortex, Ph-phloem.

POWDER MICROSCOPY

According to WHO guidelines, glycerol reagent was made. The combination was put to a little amount of powder on a slide, covered with a cover slip, and inspected under a microscope.[10] Lignified tissue could be clearly seen under powder microscopy, along with multicellular trichomes. Starch grain and root hair are both very distict.



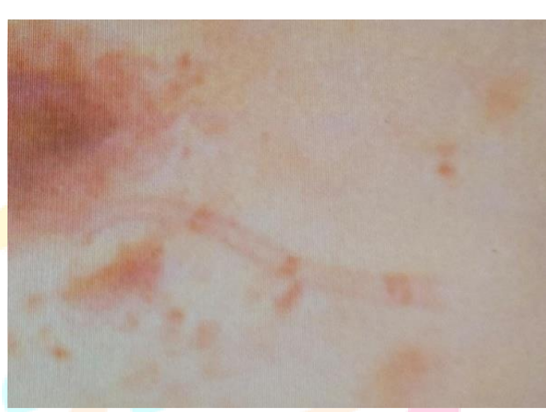


Fig 4: Trichomes

Fig 5: Starch Grain

PHYTOCHEMICAL STUDIES

Initial phytochemical analysis of *P. lanceolata* leaves revealed the following: *P. lanceolata* leaf extract was put through phytochemical screening to look for the presence of primary and secondary metabolites such as proteins, amino acids, lipids and oils, sugars, alkaloids, glycosides, flavonoids, tannins, terpenoids, and steroids, among other substances.[13,14,15]

Table 2: Phytochemical Screening of Various Extracts of *P.lanceolata* Leaves [13]

Sr. no	Phytochemical	Test Performed	Extracts		
			PE	EA	HA
1	Carbohydrates Test	Molish Test	+ve	+ve	+ve
		Felling Test	+ve	+ve	+ve
		Barfoed Test	+ve	-ve	+ve
		Selwinoff's Test	-ve	-ve	-ve
2	Protein & Amino acid Test	Biuret Test	-ve	+ve	+ve
		Millon's Test	+ve	-ve	+ve
		Ninhydrine Test	+ve	+ve	-ve
3	Fixed Oils & Fats	Spot Test	+ve	-ve	-ve
4	Flavonoids Test	Shinoda Test	-ve	+ve	+ve
		Lead acetate Test	+ve	-ve	+ve
		Alkali Test	+ve	-ve	-ve
5	Alkaloids Test	Mayer's Test	+ve	-ve	+ve
		Wagner's Test	-ve	+ve	+ve
		Hanger's Test	+ve	+ve	-ve
		Dradrendroff's Test	+ve	+ve	-ve
6	Sponine Test	Foam Test	-ve	-ve	-ve

7	Cardiac Glycoside	Keller Killiani Test	-ve	-ve	+ve
		Legal's Test	-ve	-ve	-ve
		Baljet's Test	-ve	-ve	-ve
8	Anthraquinone Glycoside	Borntrager's Test	-ve	-ve	-ve
9	Steroids Test	Salkowski's Test	+ve	+ve	+ve
10	Tannin & Phenol Test	Ferric chloride Test	-ve	+ve	+ve
		Lead acetate	-ve	-ve	-ve
		Gelatine solution	-ve	+ve	+ve

Note: - +ve indicates presence and –ve indicate absence of phytoconstituents P.E- Petroleum Ether, E.A-Ethyl Acetate, H.A- Hydroalcohol

Table: Indicates that petroleum ether extract contains carbohydrates, fixed oil & fats, protein and amino acid, steroids, flavonoids, alkaloids. Ethyl Acetate extract contains carbohydrate, protein and amino acid, steroids, tannin and phenol, alkaloid. Hydro-alcoholic extract contains carbohydrate, steroids, tannin, alkaloids, and flavonoids. [5, 8]

PHARMACOLOGICAL EVALUATION

Physico-Chemical Characterization of *P.lanceolata* Leaves: [16, 17, 18, 19]

The Leaves powder was subjected for physical standard such as pH, Ash content, Extractive value, Moisture Content.

Table 3: Physicochemical evaluation parameter

Powder of P.lanceolata Leaves	**Observation**
pH 1% solution	7.23 ± 0.4
Moisture content (loss of drying)	7.2 (%w/w)
Extractive Value	**Value (%w/w)**
Water Soluble Extractive	20%
Petroleum ether Soluble	23%
Ethyl acetate Soluble	19%
Hydroalcohol Soluble	32%
Type of Ash	**Value (%w/w)**
Total Ash	7.5 ± 0.65
Acid in Soluble Ash	2.75 ± 0.36
Water Soluble Ash	1.25 ± 0.22

THIN LAYER CHROMATOGRAPHY [20, 21, 22, 23]

To determine the quantity of components present in the petroleum ether and ethyl acetate extracts of the root of H. schulli, thin-layer chromatography was used. The steps that were taken in detail were as follows:

Getting the platter ready:

Silica gel G served as the adsorbent in thin-layer chromatography. A glass pestle was used to combine 25 g of silica gel G with 35 ml of distilled water. A glass rod was used to swirl the fluid until it was homogeneous. After that, 15 ml more of distilled water was added while stirring. Then, this suspension was poured into a 150-ml flask with a plastic stopper and violently shaken for roughly two minutes. The Continental instruments' thin layer chromatography (TLC) applicator was then instantly spread with this suspension.

Plate drying and storage:

The newly coated plates were then air-dried until the layer's transparency vanished. After that, the plates were placed in an oven and baked to 1100 °C for 30 minutes.

Utilizing the sample:

Glass capillaries were employed to apply the test sample to the TLC plates. A transparent template was used to aid apply the spots, and a minimum of 1 cm was left between each pair of neighboring spots. On the top of the plate, the sample areas were designated to help identify them.

Chromatographic chamber, saturation conditions, and TLC plate creation

The studies were conducted in a chromatographic rectangular glass chamber (20-15). A smooth piece of filter paper measuring between 15 and 40 cm in size was inserted in the developing solution in order to prevent inadequate chamber saturation and the unfavorable edge effect. The paper was then applied to the chamber's walls and pressed against them after being wet in this manner. Allowing the chamber to saturate. The tests were conducted in diffused sunshine at room temperature.

Detection of spots: UV light (UV chamber) and iodine vapors (rectangular glass chamber 15 cm by 25 cm with 1 gram of iodine crystals) were used to find spots.

Table 4: TLC of *P.lanceolata* Leaves

Sr. No	Solvent system	Ratio	Spraying Reagent	No. of constituent	R value
			Petroleum ether		
1	Toluene: Chloroform: Methanol	4: 4: 1	Iodine chamber	5	0.30
					0.33
					0.46
					0.53
					0.96
			Ethyl acetate		
1	Petroleum ether: Ethyl acetate: Formic acid	4: 2: 1	Iodine chamber	7	0.26
					0.35
					0.36
					0.43
					0.61
					0.76
					0.86
			Hydroalcohol		
				3	0.41

1	Ethyl acetate: Methanol: Water	10: 2.2: 2	Iodine chamber		
					0.83
					0.88

Antibacterial Activity: [24, 25]

The disc diffusion experiment was used to investigate the antibacterial activity of petroleum ether, ethyl acetate, and hydro-alcoholic extract of P. lanceolata leaves (200ug/ml). Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae were the test organisms used in the study. [24]

Creating the inoculums: The bacterial cultures were cultivated for 24 hours at 37 °C in an incubator on nutrient agar slants and in nutrient broth. After one week of storage at 4 °C, subculturing was completed. A 24-hour fresh culture of bacteria floating in nutritional broth was tested for antibacterial activity .

Making the culture medium: Nutrient agar, which comprises the following ingredients, served as the experiment's medium: In order to prepare the media for antibacterial activities, 28.0 g of components were dissolved in 1L of distilled water and sterilized in an autoclave for 20 minutes at 121 °o and 15 lbs/inch pressure.

Cup plate method: [24, 25, 26]

Gram-positive S. aureus and gram-negative E. coli were the two bacterial strains against which the produced compounds were tested. Nutrient agar media (0.75 ml) was added, thoroughly mixed, and then put into a sterile Petri dish. A sterile metallic borer was used to create 6 mm wells after the media had a chance to harden. The test material was then dissolved in DMSO (100 L) at a concentration of 1 mg/ml and applied to the appropriate wells. The usual antibiotic medication (1 mg/mL) was utilized as a positive control, while DMSO was used as a negative control. Each bacterial strain had duplicate plates made, which were then cultured for 24 hours in an aerobic environment at 37°C. The diameter of the zone exhibiting full inhibition (measured in mm) was used to calculate the activity. Calculating the growth inhibition in relation to the positive control was done.

Determine the MIC and MIB: The goal of the broth and agar dilution procedures is to identify the minimal inhibitory concentration, or MIC, of the tested antimicrobial agent that, under certain test conditions, prevents the observable growth of the bacterium under investigation. The most popular methods for determining the least inhibitory concentration (MIC) of antimicrobial agents, such as antibiotics and other compounds with bactericidal activity or bacteriostatic activity are agar dilution and broth dilution. The MIC dilution, along with at least two of the more concentrated test product dilutions, are plated and counted to determine viable CFU/ml in order to determine the MBC. When opposed to the MIC dilution (Microchem Lab), the MBC is the lowest concentration that exhibits a predetermined reduction in CFU/ml, such as 99.9%.

Broth dilution technique for MIC: Prepare and label test tubes with nutritional broth (double strength). In the first test tube (UT), which serves as a negative control and a sterility check for the media, no inoculum is added. Inoculums (three to four drops) are applied to each test tube until the ultimate microbial concentration of 106 cells/mL is reached. All test tubes, excluding the uninoculated (negative control) and control (positive) tubes, have a test antimicrobial substance added to them, ranging in volume from 0.5 to 5 mL. The positive evaluates the inoculum's viability and the appropriateness of the medium for the growth of the test microorganism. Using sterile water, adjust

each test tube's final volume to 10 mL. All test tubes are thoroughly shaken before being incubated for two days at 37°C

Formula:

$$\text{Working conc}^{n}\ (\text{ug/mL}) = \text{Vol}^{m}\ \text{of stock taken (mL)} \times \frac{\text{Stock conc}^{n}\ (\text{ug/ml})}{\text{Working volume (ml)}}$$

Table 5: Antibacterial activity of *Pluchea lanceolata* leaves extract [15, 16, and 17]

Sr no.	Extract	Concentration	Cup-Plate Method			
			Test organism (Zone Inhibition in mm)			
			E.coli	S.aureus	K.pneumoniae	B.subtilis
1.	Standard	200ug/ml	34	30	28	29
2.	Petroleum ether	400ug/ml	16	14	15	17
3.	Ethyl acetate	400ug/ml	22	19	14	18
4.	Hydroalcohol	400ug/ml	24	24	16	16

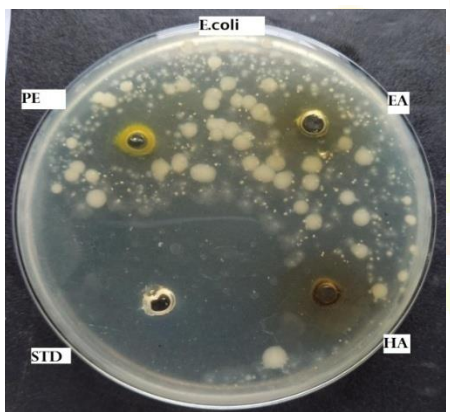


Fig 6: E-Coli

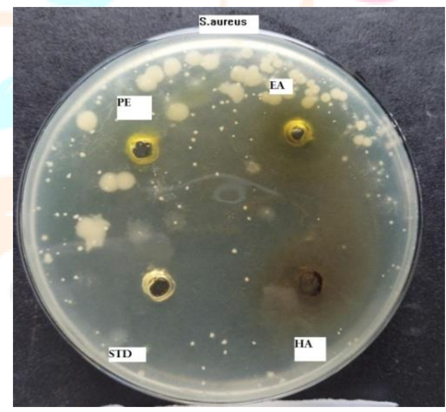


Fig 7: S.aureus

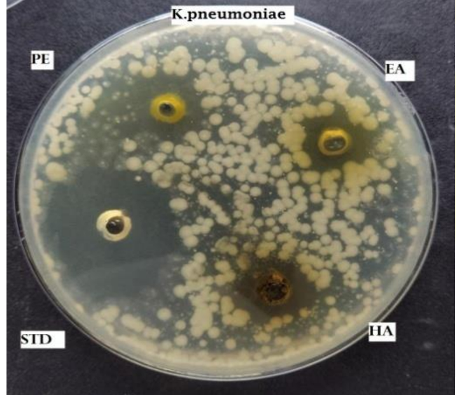


Fig 8: K. pneumoniae

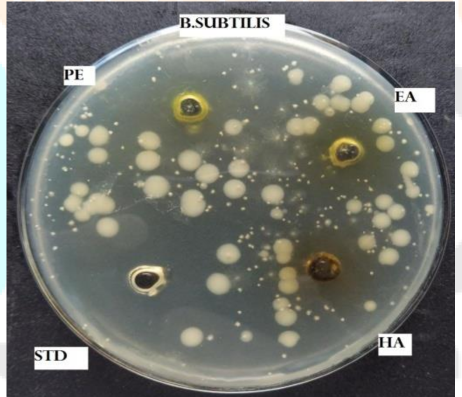


Fig 9: B. subtilis

Antioxidant Activity [27, 28, 29, 30]

By DPPH Method: Using BHT (tert-butyl-1-hydroxytoluene) as a reference, the test extract's antioxidant activity was spectrophotometrically assessed using the DDPH (2, 2-Diphenyl-1-picrylhydrazyl) assay. For concentrations between 0.977 and 500 g/ml, P. lanceolata leaf extract was diluted in petroleum ether, ethyl acetate, and alcohol. Freshly made DPPH solution (2.9 ml; 100 g/ml in ethanol) and 0.1 ml of various extract strengths were added to a 10-ml volumetric flask. A Shimadzu 1601 UV-Vis spectrophotometer was used to measure the mixture's absorbance at 517 nm after 30

minutes of standing in the dark with the mixture thoroughly mixed. 0.1 millilitres of ethanol and 2.9 millilitres of DPPH radical solution make up the control solution. The test solution's concentration was the same as the standard, which was butyrate hydroxyl toluene. By comparing the absorbance between the test combination and the control, percentages of DPPH scavenging in the test extract were calculated. Using the formula the fraction of DPPH that was scavenged was determined.

(A control – A test) / (A control x 100) = % scavenging of DPPH

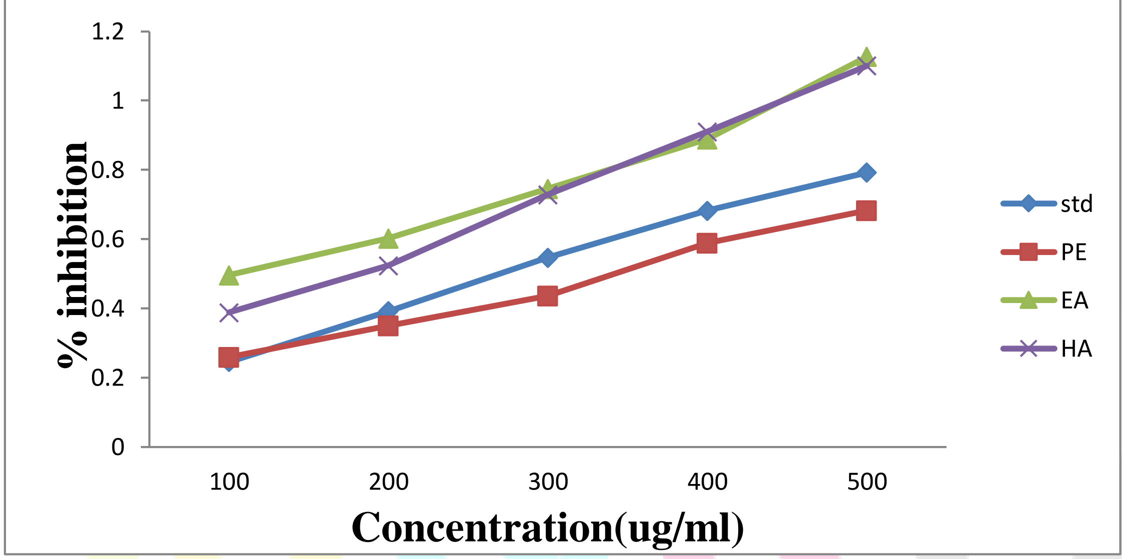
Plots showing the percent inhibition in DPPH radical scavenging against concentrations (0.977-500 g/ml) were made.

Table 6: Antioxidant activity of *P.lanceolata* leaves extract by DPPH assay.

The antioxidant activity found in concentration range 100-500 ug/ml and the highest activity shown in ethyl acetate and hydroalcohol extract.

Sr no.	Concentration	Antioxidant activity Absorbance at 517nm			
		Std	PE	EA	HA
1	100ug/ml	0.2456	0.2589	0.4956	0.3876
2	200ug/ml	0.3917	0.3491	0.6023	0.5236
3	300ug/ml	0.5468	0.4356	0.7452	0.7282
4	400ug/ml	0.6823	0.5883	0.8883	0.9103
5	500ug/ml	0.7924	0.6823	1.1267	1.1002

The graph shows the highest ratio of concentration



Uses:

- The whole plant is utilized in Ayurvedic medicine.
- The leaves are aperients, used as a laxative, analgesic, and antipyretic.
- Pluchea lanceolata is recognized as a traditional arthritic treatment.
- Its decoction is used both internally as a massage oil and externally to treat rheumatic disorders, Muscle aches, edema, and fever. [31 to 38]

Summary and conclusion

Both macroscopic and microscopic characteristics of Lanceolata leaves were characterized, and several aspects were looked into. The petroleum ether, ethyl acetate, and hydroalcohol extract showed the presence of steroid, flavonoids, and terrene components as well as carbohydrates in the preliminary phytochemical examination. Along with the pH and total ash content, the extractive value of the powder material was evaluated and a percentage was computed. Thin-layer chromatography was used to separate the components of petroleum ether, ethyl acetate, and hydroalcohol extracts, and the Rf value for each extract's separated components was calculated. Each extract's anti-microbial

activity was evaluated using the cup-plate method against a range of gram-positive and gram-negative bacteria, including S. aureus, E. coli, B. subtilis, and K. pneumoniae. The findings of this investigation showed significant antibacterial activity against K. pneumoniae and E. coli. Ethyl acetate and hydroalcohol extract demonstrated positive antioxidant activity results. More research is required to isolate and identify the antioxidant component that is found in P. lanceolata because it is a rich source of natural antioxidants for medical applications.

Future scope

- Comparative analysis of the various P. lanceolata plant extracts is required.
- Spectrophotometric methods for isolating and characterizing bioactive compounds
- It is possible to evaluate their pharmacological potential and develop an appropriate dose form.

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