PHYTOCHEMICAL STUDIES AND ANTI-BACTERIAL ACTIVITY OF SEEDS OF L. CAMARA LINN

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Abstract
The worrisome rate at which antibiotic resistance is growing necessitates the development of alternative antimicrobial medicines. This in turn has motivated scientists to examine the antibacterial properties of several therapeutic herbs. One of these therapeutic plants is Lantana camara Linn, a large weed with a variety of medicinal benefits. There has been discovered to be effective in vitro antibacterial action against bacteria and fungi. Lantana camara also has larvicidal and anti-tumor properties, according to scientists. The known method was used in this study to examine the antibacterial effect of Lantana camara Linn in methanol and chloroform. Four distinct bacterium isolates, both Gram positive and Gram negative, were used to examine this activity. In order to create the crude extract, methanol and chloroform were used. Zones have detected antibacterial activity.

Keywords: *lantana Camara*, verbenaceae, antibacterial activity, zone of inhibition

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INTRODUCTION

Plants can be used medicinally in herbal medicine. Seeds, flowers, fruit, leaves, roots, stems, rhizomes, and barks are all considered to be herbs. Numerous cultures also make use of other naturally occurring compounds, such as animal and mineral products. The use of plants for therapeutic reasons is the oldest known type of medicine. Hippocrates and Galen's superior medical systems were built on the principles of herbs. All conventional medical systems use herbal drugs as a crucial element. Over all other agents, plants have been utilized as medicine for as long as anybody can remember [1-3]

Herbal medicines are those that use plant-based ingredients to have a pharmacological effect. The herb is often administered whole, without being synthesized or broken up. The word "botanical" is preferred by the food and drug administration. remedies known as nutraceuticals are based on nutrients and go beyond what are considered to be herbal medicinal remedies yet still have a pharmacological impact. These nutrients include synthesized cations (such chromium, which has been used to treat diabetic mellitus), minerals, and vitamins. [4-6]

India is considered to be the world's largest producer of There isn't many medical herbs that are used economically significant that aren't collected or farmed in this country. Since India gained its independence in 1947, In the areas of agro technology, process technology, standardization, quality control, research and development, etc., it has made considerable advancements. In one form or another, medicinal plants have been employed for thousands of years by traditional medical systems like Ayurveda, Siddha, and Unani. India is anticipated to develop into a market for herbal medicines worth Rs. 4,000 crore over the next five years and a significant exporter of herbal products that adhere to international standards. [7-9] Leading Indian research organizations have begun employing cutting-edge genetic editing techniques to explore for patentable genes. About 400 useful plants have been discovered, and more screening is being done to locate more. With over 45,000 unique agroclimatic regions [10-13]

Numerous herbs are utilised as natural dietary supplements, flavourings, colours, and cosmetics

due to their pharmacological effects. They have gained popularity recently not only in India but also in nations like the. In actuality, these countries have built high-end herbal cosmetics businesses. [14-16]

EXPERIMENTAL WORK

Plant authentication

The L. Camara plant's seeds, which belong to the Verbenaceae family, were gathered in the nearby Loni area.. Authentication of plant on basis of Pharmacognostic study and organoleptic characteristics was done by Dept. of Botany and Research Centre PVP College, Loni Ref. No./PVPC/Bot/2022-23/55.

Pharmacognostic Study: The plant materials were examined under a microscope and for several macroscopic characteristics as listed below.

Extraction:

The Lanata Camara medicine was gathered. After that, a grinder is used to pulverise the dried seed material. The extraction process employed the coarse powder.

• Approach: soxhlet

• Solvents: Using chloroform and methanol as solvents

Powdered dry seed was created. A 500 ml round bottom flask was used to extract 50 g of Lanata Camara seed using methanol and chloroform at 400 C for 5 hours. A rotary evaporator was used to evaporate the filtrate after the had been extracted for five hours.

ANTIMICROBIAL ACTIVITY

According to Russell and Furr's 1977 description, Mueller Hington sensitivity test agar (MHA) was the employed as the medium. A loopful of the broth culture was injected into 20ml of sterile, molten MHA that had been made in a Mac Carthney bottle and chilled to 45^{o}C. Shaking the Mac Carthney allowed for full mixing. A Petri plate with clear labels was filled with the culture and agar combination, which was then let to set. Then, using a sterile cork borer, were made into the set infected. The distance between the wells and the plate's edge was around 5 mm. Using sterile syringes, extracts at a concentration of 25 mg/ml were aseptically injected into the wells that had been drilled. Care was taken to keep the extract from spilling onto the agar's surface. To ensure optimal extract diffusion into the MHA, the plates were kept on the bench for roughly an hour. Each bacteria isolate underwent the same process.

RESULTS AND DISCUSSION

Determination of Inorganic Compounds:

Table no. 01: Determination of Inorganic Compounds

1. calcium	+ve
2. Potassium	+ve
3. Iron	+ve
4. Sulphate	+ve
5. Chloride:	+ve
6. Carbonate	+ve
7. Nitrates	+ve

Table no. 02: Phytochemical test

Test	**Procedure**	**Observation**	**Result**
Alkaloid	Mayers Reagent	Yellow Cream ppt	+ve
Carbohydrate	Molish ‘s Reagent	Form violet ring	+ve
Glycoside Test	Modified Brontrager’s reagent	Form red colour	+ve
Flavonoids	Dil NaOH + Dil Hcl add yellow colour appears	become colourless	+ve
Tannin	Gelatin	Milky ppt.	+ve
Saponins	Foam Test	Produce foam lasts than 10 min	+ve
Terpenoids	5ml Extract+2ml ChCl3+3ml Conc. H2SO4	Rdish brown colouration of the interface	+ve

Table no. 03: Thin layer Chromatography

Sr. no	**Name of plant**	**Mobile Phase**	**Ratio**
1.	**• Chloroform Extract • Methanol Extract**	**Chloroform : ethyl acetate**	**9:1**

Rf value:

- Rf Value = distance traveled by solute / distance traveled by the solvent
- Rf Value = 3.4/6.2 = 0.5

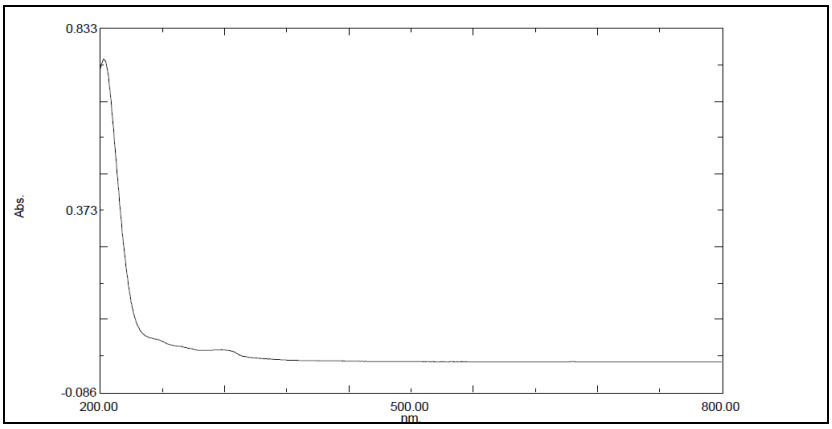


Fig. no.01: UV spectrum of Extract Fraction-I

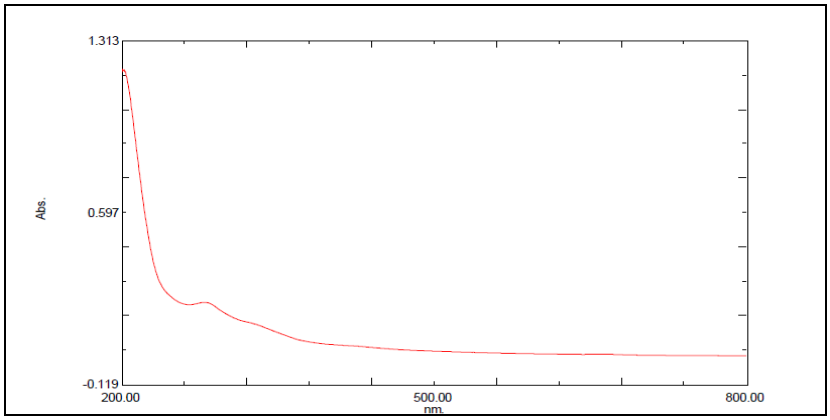


Fig. no.02: UV spectrum of Extract Fraction-2

The extract shows absorption maxima at 270nm indicates the presence to unsaturated compound in the extract.

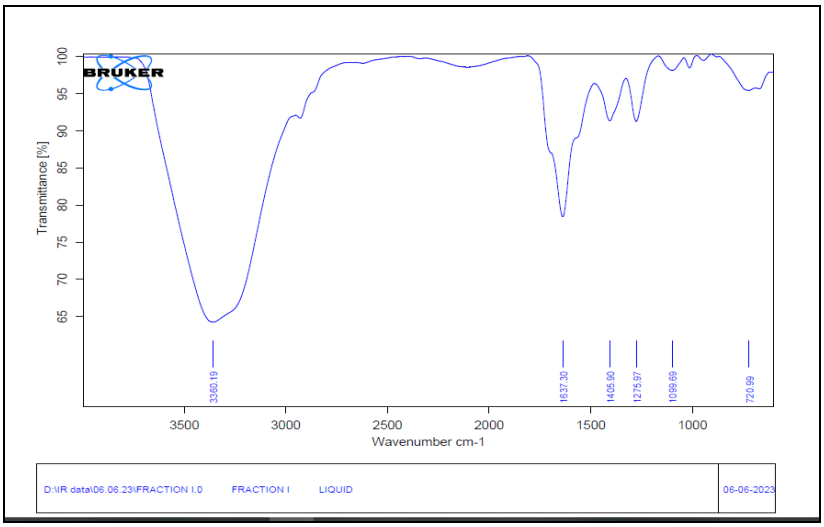


Fig. no.03: FTIR of Extract Fraction I

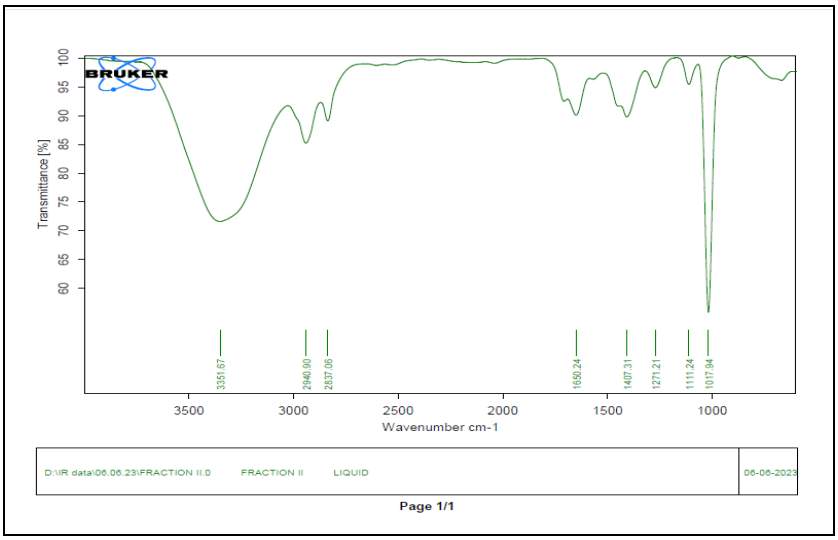


Fig. no.04: FTIR of Extract Fraction II

The charteristics presence of vibrational frequencies as per the FTIR shown indicates the presence of flavones and flavonoids in the plant extract.

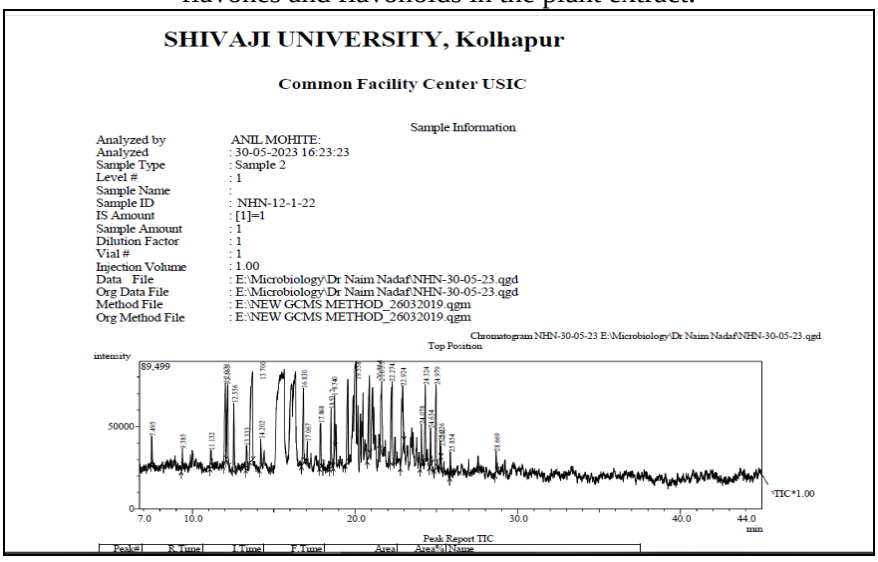


Fig. no.05: GC-MS of Extract

min

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	7.495	7.450	7.565	40093	1.09	.alpha.-Pinene
2	9.385	9.315	9.425	24022	0.65	.alpha.-Phellandrene
3	11.132	11.070	11.210	39736	1.08	trans-Linalool oxide (furanoid)
4	12.031	11.900	12.075	235310	6.39	Linalool
5	12.163	12.070	12.240	204383	5.55	(2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl)t
6	12.556	12.480	12.630	128566	3.49	(2S,4R)-4-Methyl-2-(2-methylprop-1-en-1-yl)t
7	13.333	13.270	13.400	41302	1.12	l-Menthone
8	13.700	13.500	13.725	479820	13.02	Cyclohexanone, 5-methyl-2-(1-methylethyl)-,
9	14.202	14.115	14.265	47143	1.28	Cyclohexanol, 1-methyl-4-(1-methylethyl)-
10	16.830	16.705	16.890	207180	5.62	2,6-Octadien-1-ol, 3,7-dimethyl-, formate, (Z)
11	17.067	17.000	17.105	30572	0.83	(-)-trans-Myrtanyl acatate
12	17.868	17.790	17.940	85650	2.32	2,6-Octadiene, 2,6-dimethyl-
13	18.532	18.425	18.645	125655	3.41	Copaene
14	18.740	18.660	18.775	108312	2.94	(-)-.beta.-Bourbonene
15	19.558	19.390	19.640	237560	6.45	Caryophyllene
16	20.884	20.745	20.920	220234	5.98	Germacrene D
17	21.635	21.490	21.715	244565	6.64	Citronellyl butyrate
18	22.274	22.140	22.355	264477	7.18	Butanoic acid, 3,7-dimethyl-2,6-octadienyl est
19	22.924	22.775	23.015	266722	7.24	2-Phenylethyl tiglate
20	24.078	23.995	24.155	80999	2.20	(2E,6E)-Farnesyl pentanoate
21	24.324	24.215	24.385	168754	4.58	Citronellyl tiglate
22	24.634	24.560	24.715	61125	1.66	Pentanoic acid, 4-methyl-, 3,7-dimethyl-6-octe
23	24.979	24.845	25.055	237579	6.45	Geranyl tiglate
24	25.226	25.175	25.260	30773	0.84	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl es
25	25.281	25.260	25.310	13175	0.36	Pentanoic acid, 4-methyl-, 3,7-dimethyl-6-octe
26	25.854	25.815	25.900	30688	0.83	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl es
27	28.669	28.595	28.740	30333	0.82	6-Octen-1-ol, 3,7-dimethyl-, propanoate

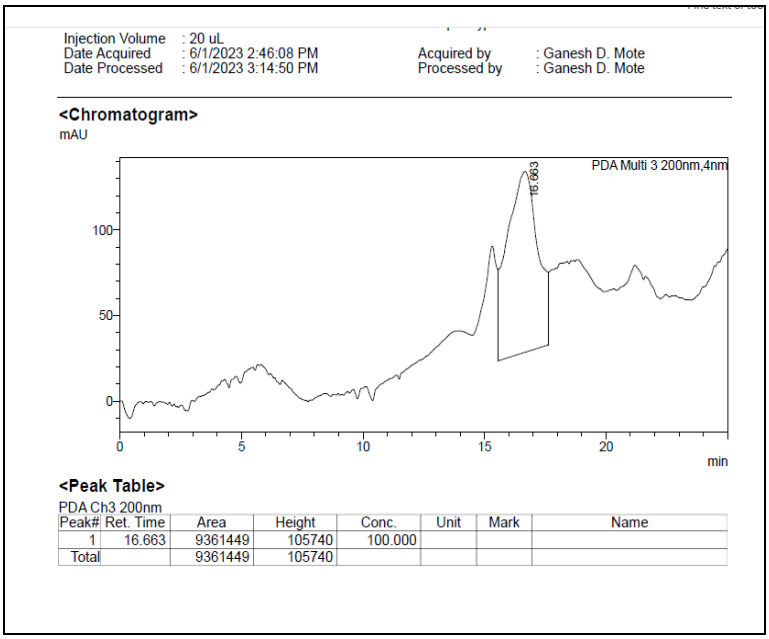
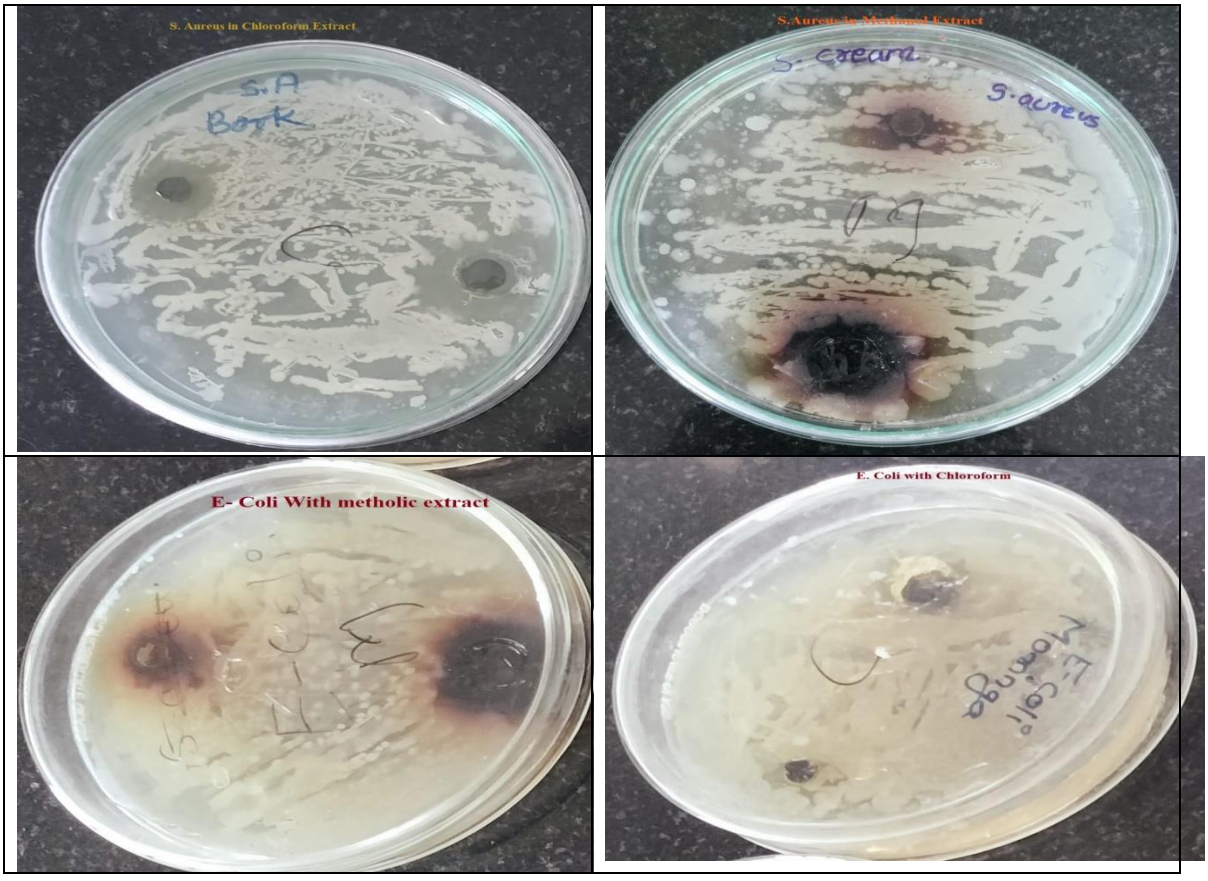


Fig. no.06: HPLC chromatogram of the extract

1. **The Lantana camara seed extract:** After freeze-drying, the extract took on a greenish-black hue.
2. **Antimicrobial Sensitivity** Testing of Lantana Camara Seed Methanolic and Chloroform Extract: The methanolic crude seed extract of Lantana Camara was shown to be efficacious against four bacteria and the zone of inhibition were as given below.



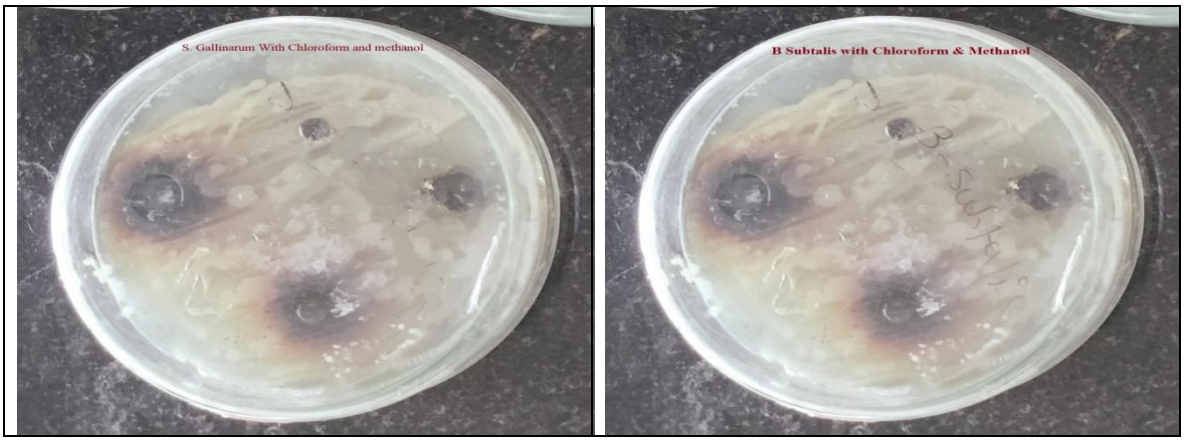


Fig. no. 07: Antimicrobial sensitivity test

Table no. 03: Zone of Inhibition

	Chloroform Extract		**Methanol Extract**	
Microbial strain	Pour plate	Disc plate	Pour plate	Disc plate
E-coli	8mm	5mm	5mm	4mm
S- Aureus	5mm	5mm	14mm	10mm
B Subtalis	5mm	3mm	10mm	5mm
S. Gallinarum	5mm	5mm	10mm	8mm

PHYTOCHEMICAL ANALYSIS OF THE METHANOLIC SEED EXTRACT OF Lantana camara

Table no. 04: The Methanolic seed extract of Lantana camara Linn.

Sr. No	**Phytochemicals**	**Result**
1	**Saponnin**	**+**
2	**Terpenoids**	**+**
3	**Tannins**	**-**
4	**Alkoloids**	**+**

Table no. 05: The Chloroform seed extract of Lantana camara Linn.

Sr. No	**Phytochemicals**	**Result**
1	**Saponnin**	**+**
2	**Terpenoids**	**+**
3	**Tannins**	**+**

Cream preparation:

Take the necessary amount of methyl paraben and propyl paraben and dissolve them in 5 ml of distilled water using a water bath. Propylene glycol was added once the solution was cooled. Additional 1 gramme of lanata Camara seed extract was added to the aforementioned mixture, and distilled water was used to create the final volume.

Table no. 06: Formula for Herbal Cream

Sr. No	**Ingredients**	**Quantity**
1	Lanata Camara Seed Extract	2 gm
2	Hydroxy propyl methyl cellulose	2 gm
3	Methyl paraben	0.4 gm
4	Propyl paraben	0.2 gm
5	Propyleneglycol	10 ml
6	Triethanolamine	2.4 ml
7	Distilled water	q.s 3 ml

Cream formulation for evaluation

Research on accelerated stability: An accelerated stability research was conducted on an ointment formulation at 80°C and 45°C over the course of one month. For all formulations, the various criteria including colour, odour, texture traces of gritty, particles, and skin irritation test were examined at the first month [17].

Extrudability:
An ointment-filled closed collapsible tube was strongly pushed at the constricted end. [18]

Spreadability Test [19]
Then positioned between slides under a specific lo ad, to flip out or from cream. Spreadability is improved when two slides are sep arated more quickly.The formula is used to calcula te it.

Table no. 07: Evaluation result of Cream

PhysicochemicalParameters	Result
Colour	Dark Brown
Odour	Characteristics
Texture	Smooth
pH	5.67
Washability	Good
Non Irritancy test	Non Irritant
Consistency	Smooth
Spreadability	Easily Spread
Stability Study	Stable

Table no. 08: Evaluation of Extract For Its Anti-Microbial Potential

	Zone of Inhibition (mm)				
	B. subtilis	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***
Plant Extract	16.3 ± 0.57	18.5 ± 0.13	14.2 ± 0.61	9.7 ± 0.22	16.1 ± 0.46
Formulation	16.7 ± 0.37	19.4 ± 0.23	13.9 ± 0.81	9.4 ± 0.43	15.87 ± 0.52
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Std. (Amoxicillin)	16.8 ± 0.37	20.5 ± 0.24	15.6 ± 0.53	NA	NA
Amphotericin-B	NA	NA	NA	18.7 ± 0.61	13.1 ± 0.35

Table no. 09: MIC's of the most effective plant extract against S. aureus and E. Coli

	Conc. (mg/ml)	S. aureus	E.coli
Plant Extract	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	9.6 ± 0.65	8.3 ± 0.95
	5.00	14.8 ± 0.83	13.2 ± 1.1
Formulation	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	9.47 ± 0.72	7.95 ± 0.55
	5.00	13.55 ± 0.63	13.8 ± 0.98

Table no. 10: MBC of the most effective plant extract against S. aureus and P. aeruginosa

Table	Conc. (ppm)	S. aureus	E.coli
Plant Extract	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	8.33 ± 0.49	6.55 ± 0.35
	5.00	12.45 ± 0.63	11.25± 1.23
Formulation	1.25	0.0 ± 0.0	0.0 ± 0.0
	2.50	9.37 ± 0.42	7.45 ± 0.76
	5.00	12.93 ± 0.53	12.55 ± 0.58

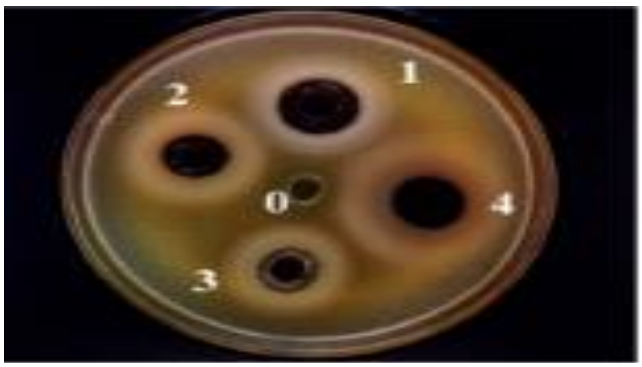


Fig. no. 08: Antimicrobial activity of Plant Extract

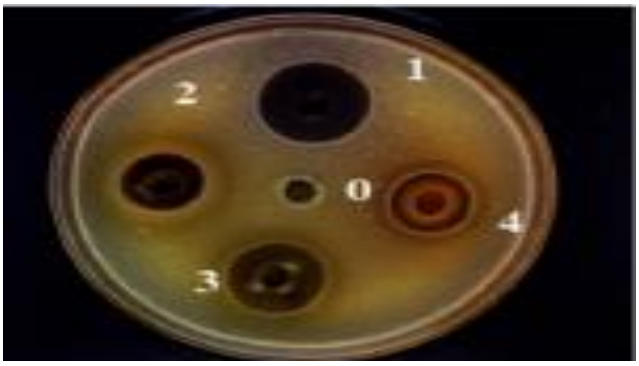


Fig. no.09: Antimicrobial activity of Cream

DISCUSSION

It has been discovered that L. camara seed extracts contain antimicrobial properties. At a dosage of 25 mg/ml, the extract was proven to inhibit four bacterial isolates. The extract also performed well in comparison to streptomycin, which is used in this study as a positive control. External application of L. camara cream has been used to treat eczema swellings, chicken pox, measles, and other skin conditions and itches. Other use include the treatment of scabies, leprosy, and as an antiseptic for wounds. It has additionally been proposed as a biocide. One of the most recent and important public health concerns is the emergence of multidrug resistance bacteria. Therefore, more thorough research on this plant may offer solutions to this issue. The new field of Lantana spp. molecular taxonomy should be the focus of future research.

CONCLUSION

The results obtained indicate that the Methanolic extract of Lantana Camara species exhibited potent antimicrobial activity. Lantana Camara species have been found to possess various pharmacological properties, including analgesic, anti-diuretic, anti-tussive, gastro protective, anti-inflammatory, anti-ulcer, antifertility, and more. A preliminary analysis of the seed extract revealed the presence of terpenoids, tannins, and alkaloids. To further explore the potential therapeutic benefits of the components in Lantana Camara species, comprehensive pharmacognostical, phytochemical, and pharmacological analyses were conducted using the seeds of Lantana Camara. The findings from this study provide valuable insights into the diverse therapeutic effects of the constituents, thus supporting the traditional use of this plant in various medicinal applications. However, additional research is warranted to identify and isolate the specific active compounds present in these Lantana species. Further investigations can help in understanding the mechanisms of action and optimizing the utilization of these plant extracts for therapeutic purposes.

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