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Exploring the Antimicrobial Potential of Allium Sativum and Lawsonia Inermis Emulgels: A Comparative Insight

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ABSTRACT:

Plant-based treatments have been used for centuries to address various human ailments. Phytochemicals in herbal extracts help improve skin appearance, nourish the body, and provide healing properties. Topical drug delivery involves applying drug formulations directly to the skin to treat conditions, offering advantages in dermatology, pain management, wound healing, and certain systemic conditions where oral administration may not be effective. Emulgels, a combination of emulsions and gels, are stable systems that incorporate poorly water-soluble drugs. These can deliver both hydrophilic and lipophilic drugs due to their dual aqueous and non-aqueous phases. Allium sativum (garlic) contains sulfur compounds like allicin and ajoene, responsible for its antimicrobial activity. Similarly, Lawsonia inermis (henna) is traditionally used for its antibacterial and anti-inflammatory properties, helping treat skin infections, burns, and wounds. The compound lawsone in henna exhibits antimicrobial effects against bacteria, fungi, and viruses, while other constituents like tannins, flavonoids, and essential oils enhance its antimicrobial activity.The study aimed to formulate an emulgel incorporating extracts from garlic and henna and compare their antimicrobial activity. Ingredients like Tween 80, Span 40, liquid paraffin, polyethylene glycol, herbal extracts, methyl paraben, and vanilla essence were used to prepare the emulgel. After formulation, the emulgel was evaluated for pH, viscosity, grittiness, spreading coefficient, swelling index, centrifuge test, skin irritation test, in vitro diffusion studies, and antimicrobial activity. The results indicated that the herbal emulgel is cost-effective, convenient, and does not cause skin irritation while demonstrating effective antimicrobial properties.

Key Words: Emulgel, Allium sativum, Lawsonia inermis, Anti-microbial, extracts.

I. INTRODUCTION:

SKIN

Skin functions as a soft outer tissue that provides external coverage for vertebrates. Human skin represents the largest human organ because it spans across 20 square feet of the body[1]. Our body defends organs inside through three protective systems which include cushioning layers and cellular elements and protective skin oils. Our skin functions as more than just a boundary because it actively maintains bodily health functions by sweating and flushing for temperature regulation as well as generating goosebumps for heat conservation[2]. Vitamin D formation in sun exposure also occurs through skin reactions that help maintain bone health. Circulation between skin and its environment makes it vulnerable to foreign intruders that produce multiple skin-related medical issues.

Topical Drug Delivery:

The term "topical drug delivery" refers to the widespread patient acceptance of drug delivery through the skin. It is a practical method of administering strong, low molecular weight medications that are prone to first-pass metabolism. Topical medication administration is the application of a medication-containing solution to the skin in order to address a cutaneous issue. When oral, sublingual, rectal, and parental drug administration methods are ineffective or a local skin infection—like a fungal infection—occurs, this method is employed. For both local and systemic disorders, topical medication delivery is a popular therapeutic approach[3]. Topical delivery system increases the contact time and mean resident time of drug at the applied site. Topical drug delivery offers advantages for treating conditions such as dermatological disorders, pain management, wound healing, and even certain systemic conditions where oral administration may not be effective[4]. The primary benefit of a topical

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delivery system is that it avoids the first-pass metabolism. Topical Preparations are of mainly:

1. Conventional Topical preparations[5].

Liquid preparations Liniments	Semi-solid preparations Ointments	Solid preparations Topical powders
Lotions Paints	Creams Pastes	Poultices Plaster
Topical solution Topical tinctures	Gels	

2. Advanced Topical Delivery System[6]:

Micro Emulsions Micro emulsions droplets have a particle size (> 0.5 µm). In addition, they are spontaneously produced in a narrow range of oil-water-surfactant composition. **Nano Emulsions/Sub micro-Emulsions/Mini Emulsions** These are oil-in-water emulsions with an average droplet size ranging from 100 to 500 nm. They have very good stability and they do not undergo phase separation during storage.

Multiple Emulsions Multiple emulsions are novel carrier system which are complex and poly dispersed in nature where both w/o (water/oil) and o/w (oil/water) emulsion exists simultaneously in a single system.

INTRODUCTION TO EMULGEL

Emulgel is known as an emulsion that has been gelled by using a gelling agent. They can be made either o/w or w/o type. Emulgel is a stable and superior system that incorporates poor water-soluble drugs. In brief, emulgel is a combination of emulsion and gel. Emulgel can deliver both hydrophilic and lipophilic drugs due to the presence of both aqueous and non-aqueous phases [7]. In recent years, they have been used as a control release formulation. These are biphasic systems that have better drug loading capacity and better stability. Emulgel has several good properties, such as good spreadability, greaseless, thixotropic, good shelf life, odorless, and a pleasant appearance over the conventional topical formulation. Emulgel has both gel and emulsion properties and functions as a dual control release system. Emulgel dosage form is used for steroids, some antibiotics and it was extended to analgesics and antifungal drugs[8].

Materials:

Tween 40 was obtained from Vasudha chemicals, Span 40 was procured from Alpha Chemika, liquid paraffin was obtained from Lodha petro, Methyl Paraben was obtained from mathangi industries, Carbopol was procured from Rudra chemicals, and Polyethyleneglycol 400 was obtained from Akhil health care Private limited.

Plant materials:

Allium sativum:

Bulbs of Allium sativum belongs to the family Amaryllidaceae having anti-microbial properties. Garlic has strong antibacterial, antiviral, and antifungal properties, which make it useful for treating infections and boosting the immune system. Besides this, it has anti-inflammatory, anti-oxidant properties. It promotes Gut health as well as inhibiting the growth of cancer cells. Garlic can reduce the risk of cardiovascular diseases as well as acts as a detoxifier[9].

Chemical Constituents: sulfur-containing compounds allicin, ajoene, diallyl polysulfides, vinyldithiins, and S-allylcysteine.

Lawsonia inermis:

Lawsonia inermis, commonly called henna, used cosmetically and medicinally in the Indian traditional folk medicines for thousand years. It belongs to the family Lythraceae. Leaves of henna is having anti-inflammatory, anti-bacterial, anti-septic, anti-fungal activities[10].

Chemical Constituents: Lawsone, flavonoids, coumarins, tannins.

Preparation of Extract:

First of all, Allium sativum bulbs & leaves of henna are dried separately under shade drying until it completely dried. Dried contents are poured in the mixer and grinded separately. Grounded powders were kept in the sieving machine for the purpose of proper uniform dried powder. The powders collected were kept in ethanol for 3 days. This method is known as Maceration[12]. After Maceration process is completed, the extracts were filtered to remove the solid material. The liquid content is then distillate through rotatory evaporator to eliminate all ethanol content. The extracts are then kept in heating mantle. In this process alcohol is evaporated and remains the extract in a container. Then extracts were collected in a suitable air tight containers and stored in a desiccator[11].

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Formulation of emulgels:

S. No	Ingredients	Allium sativum	Lawsonia inermis
1.	Tween 80	2ml	2ml
2.	Span 40	1.8ml	1.8ml
3.	Liquid Paraffin	10ml	10ml
4.	Carbapol	3g	3g
5.	Extract of Allium sativum	1g	-
6.	Extract of Lawsonia inermis	-	1g
7.	Polyethylene glycol	10ml	10ml
8.	Methyl Paraben	0.12g	0.12g
9.	Vanilla essence	0.1 ml	0.1 ml
10	Distilled water	Q. S	Q. S

Procedure[13,14]:
Preparation of oil phase: 1.8 ml of Span 40 is mixed with 10ml of liquid paraffin. **Preparation of aqueous Phase:** 2ml of Tween 80 is mixed with 10 ml of distilled water.
Preparation of Emulsion: The two phases then slowly mixed in mortar and pestle at a continuous interval. Finally, an emulsion was obtained.

Formulation of gel:
3 gm of Carbopol was mixed with 100 ml of distilled water and left overnight.
Finally, a thick gel was obtained

Procedure for extract phase: First of all 1 gm of extract were taken in a mortar and pestle. Then mixed with 10ml of polyethylene glycol and 0.12gm of Methyl paraben. After mixing 0.1ml of vanilla essence were added for fragrance. Finally, an extract phase was obtained.
Formulation of Emulgel: First of all, emulsion was mixed in a mortar and pestle and slowly the extract phase was added to form a thick paste. After that equal quantities of emulsion and gel were mixed together to form an emulgel. Finally, emulgel was obtained and kept in a well closed container.

CALIBRATION CURVE OF ALLIVIUM SATIVUM: Prepared Allium sativum extracts with concentrations 2, 4, 6, 8, 10, 12µg/mL in methanol. pure methanol was used as a blank and calibrated the instrument to zero absorbance. Then measured the absorbance of each standard solution at 420 nm. A graph was plotted by taking concentration on x-axis and absorbance y-axis[15].

CALIBRATION CURVE OF LAWSONIA INTERMIS: Prepared lawsone extracts with concentrations 2, 4, 6, 8, 10, 12µg/mL in methanol. pure methanol was used as a blank and calibrated the instrument to zero absorbance.Then measured the absorbance of each standard solution at 480 nm. A graph was plotted by taking concentration on x-axis and absorbance y-axis. A graph was plotted by taking concentration on x-axis and absorbance y-axis[16,17].

FTIR Studies:
FTIR analysis was performed for extracts and formulated gels separately[18].

Evaluation of Emulgel:
pH: The pH was determined by using digital pH meter[19].
Grittiness: emulgel was observed visually for the particles and then gently rubbing the emulgel between fingers to feel for any gritty sensation. After that it was microscopically evaluated for the particles if any particulate matter was seen under light microscope[20].
Viscosity: A Brookfield digital viscometer with a suitable sample adaptor was used to measure the viscosities of the Carbopol gel in cps. An appropriate amount of emulgel formulation was kept in a suitable beaker, and the spindle groove was dipped, and the rpm was set. Viscosity measurements were started, and the readings were measured after 1 minute, and the viscosity of each formulation was calculated[21].
Spreadability: Spreadability was determined by excess of sample was applied within the two glass slides then compressed to uniform thickness by placing 1kg weight for 5 min. Weight (50 gm) was added to the pan. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of Spreadability (S)[22].
Spreadability (g.cm/s) (S) =M×L/T Where M = weight tide to upper slide, L = length moved on the glass slide, T= time taken.
Centrifuge Test: 6 g of Emulgel was taken in 10 ml graduated centrifuge tubes and were subjected to spin at 3000 RPM for 10 min. The sample was observed for any phase separation occurrence[23].

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Swelling Index: To determine the swelling index,1g of emulgel is placed on a porous material (like aluminum foil) and immersed in a liquid (often a buffer solution) for a specific time period. Then, the swollen gel is removed, dried, and weighed to calculate the swelling percentage. Swelling index is then calculated by using below formula. **Swelling Index (SW)%**=[(Wt−Wo)/Wo] ×100 Where (SW) % = Equilibrium percent swelling, Wt = Weight of swollen emulgel after time t, Wo = Original weight of emulgel at zero time[23].

Skin Irritation Test: Skin Sensitivity The hairs of rats of approximately skin size 4 X 4 cm2 from the dorsal region was removed employing electric clipper without damaging the skin. The control group was treated with normal saline and the Emulgel was applied to the test group thrice a day for 3 consecutive days. The animals were observed for any signs of itching or change in skin such as erythema, papule, flakiness, and dryness[24].

In-Vitro Diffusion Study: The experiments were conducted in Franz diffusion cell with a receiver and donor compartment. The egg's membrane was prepared by dipped the egg into 1M concentrated HCl for 30 minutes. After half an hour, the egg was removed from the HCl solution and the membrane was extracted. The egg membrane is used as dialysis membrane in drug diffusion study and was positioned between the receptor and donor compartments of the Franz-diffusion cell, ensuring direct contact with the formulation's release surface in the donor cell. The receptor compartment contained 10.0 ml of isotonic phosphate buffer pH 6.8 and was maintained at 37 ± 1°C using a diffusion cell. The assembly was mounted to a magnetic stirrer. A 1.0 g gel sample was placed over the egg membrane, and a pH 6.8 phosphate buffer solution was swirled in the receptor compartment at 50.0 rpm with a magnetic bead. Aliquots of 2.0 ml were withdrawn over 4 hours and immediately replaced with an equal volume of fresh PBS. Samples were assayed for drug content spectrophotometrically. Sink condition was maintained throughout the experiments[25].

ANTI-MICROBIAL STUDY: Preparation of nutrient agar medium. Sterilization of medium by autoclaving to ensure free of contaminants. A measured volume of the diluted sample (bacterial culture) is pipetted into the center of a sterile Petri dish. The molten agar (usually kept at 45–50°C to avoid killing the microorganisms) is poured over the sample in the Petri dish. Gently swirl the dish to ensure that the sample is evenly mixed with the agar. Allow the agar to cool and solidify at room temperature. During this process, the microorganisms from the sample are immobilized within the agar. Then wells are made in the plate to add control, sample of Allium sativum and Lawsonia inermis in to the wells. The Petri dish is then placed in an incubator at the appropriate temperature 370C is maintained to allow microbial growth. Typically, incubation is done for 24–48 hours. The zone of inhibition was measured around each well. The results were compared to control samples and standard antimicrobial agents[26].

II. RESULTS AND DISCUSSION:

Organoleptic evaluation of powders:

S. No	Properties	Interference	
		Allium sativum	Lawsonia inermis
1.	Nature	Powder	Powder
2.	Colour	Slightly Yellowish	Green
3.	odour	Aromatic	Characteristic earthy smell

PHYSICO-CHEMICAL EVALUATION OF POWDERS:

S.No	Tests	Interference	
		Allium sativum	Lawsonia inermis
1.	Ash Value		
	Total Ash	38.3%	29.3%
	Acid Insoluble Ash	13.6%	10.6%
	Water soluble Ash	24.7%	8.7%
2.	Moisture content	4%	6%
3.	pH	4.8	5.6

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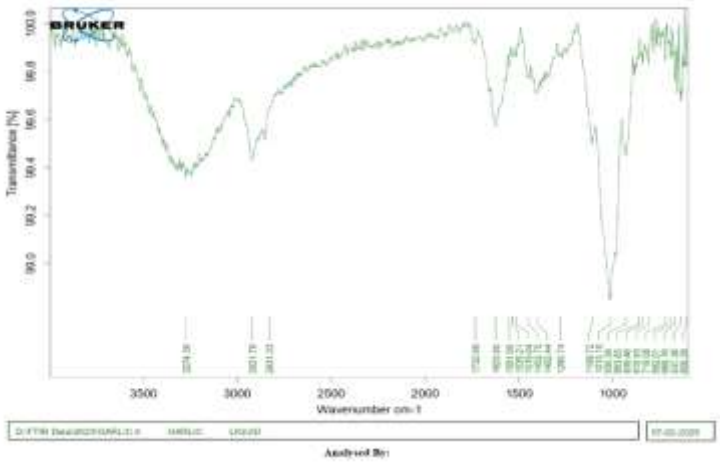
GENERAL POWDER CHARTERISTICS:

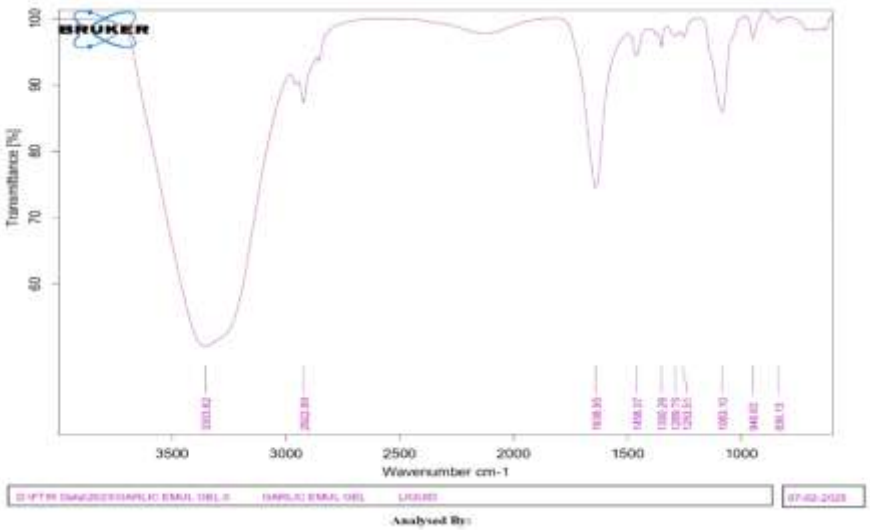
S. No	Parameters	Results		Interference
		Allium sativum	**Lawsonia inermis**	
1.	Bulk density	0.45±0.01	0.50±0.02	-
2.	Tapped density	0.56±0.02	0.60±0.03	-
3.	Carr's index	23.05±0.14	22.12±0.12	Passable
4.	Hausner's ratio	1.32±0.01	1.29±0.2	Passable
5.	Angle of repose	37.46±0.2	39.43±0.3	Fair

FTIR Spectra Analysis:

FTIR Spectra of Garlic



FTIR Spectra of Garlic emulgel



Interpretation of Garlic emulgel:

This FTIR revealed the presence of specific functional groups in garlic emulgel and showed Hydroxyl, and carbonyl compounds. The O-H stretching appeared at 3353 cm^{-1} while the C-H stretching vibrations at 2922 cm^{-1}.The characteristic C=C stretching wad observed at 1638 cm^{-1}. while C-H bending vibrations at and C-O stretching are observed at1458 cm^{-1} and 1083 cm^{-1} respectively suggesting that the functional groups of garlic extract were retained after formulation, indicating that the bioactive

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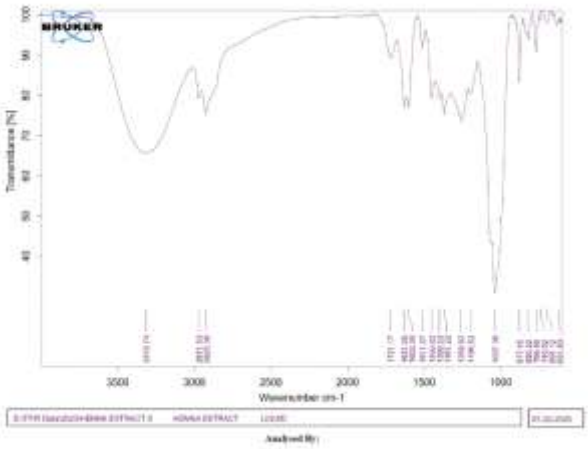
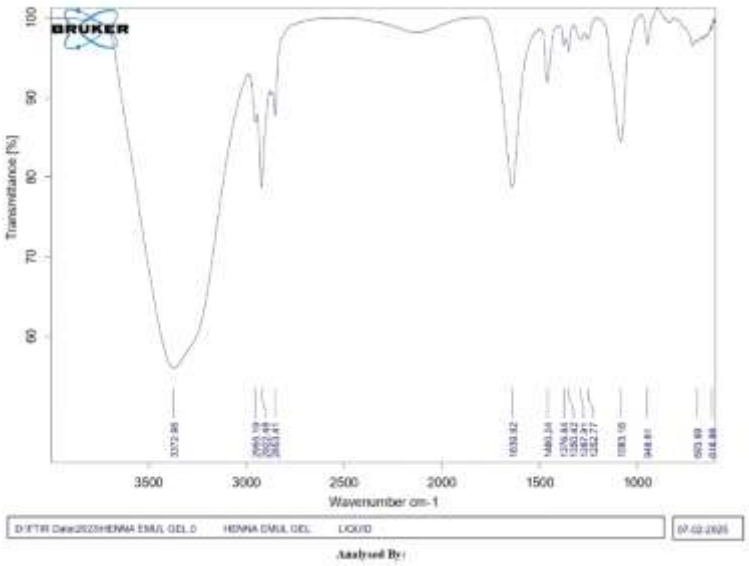
components remained stable within the emulgel matrix. The lack of significant peak shifts or disappearance suggests no adverse chemical interactions between the garlic and henna components or with the excipients. This supports the stability, compatibility, and potential therapeutic effectiveness of the combined garlic and henna emulgel for topical applications.

FTIR Spectra of Henna extract



FTIR Spectra of Henna emulgel



Interpretation of Henna emulgel:

The phenolic group (O-H) stretch appeared at 3372 cm^{-1}. The aromatic C=C stretching frequency appeared at 1639 cm−1, C-H bending vibrations at 1460 cm^{-1} while C-H wagging vibrations of CH_2 are observed at 1376. The C-O stretching vibrations at 1083 cm^{-1} indicating the presence of phenolic and quinone groups characteristic of lawsone were preserved in the emulgel. The retention of these functional groups suggests that the formulation process did not alter the chemical integrity of henna extract.

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Phytochemical Tests:

S. No	Tests	Interference	
		Allium sativum	**Lawsonia inermis**
1.	Carbohydrates	Presence	Presence
2.	Alkaloids	-	Presence
3.	Tannins	Presence	Presence
4.	Flavonoids	Presence	Presence
5.	Terpenoids	Presence	Presence
6.	Glycosides	Presence	Presence
7.	Phytosterols	Presence	Presence
8.	Saponins	Presence	Presence
9.	Sulfur Compounds	Presence	-
10.	Anthraquinones	-	Presence

Organoleptic evaluation of Garlic and Henna

S. No	Properties	Interference	
		Garlic	**Henna**
1.	Colour	Creamy	Green
2.	odour	Vanilla	Vanilla
3.	Appearence	Good	Good

Henna Emulgel

Garlic Emulgel

Physical Evaluation:

S. No	Tests	Parameters	
		Allium sativum	**Lawsonia inermis**
1.	pH	6.50	6.56
2.	Viscosity (CPs)	3990	3190
3.	Spreadability (cm)	7.1	6
4.	Swelling index	Good	Good
5.	Centrifuge test	No Phase separation	No Phase separation
6.	Grittiness	None	None

Skin Patch Test:

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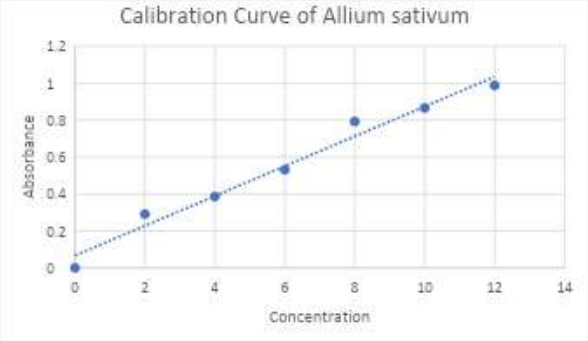
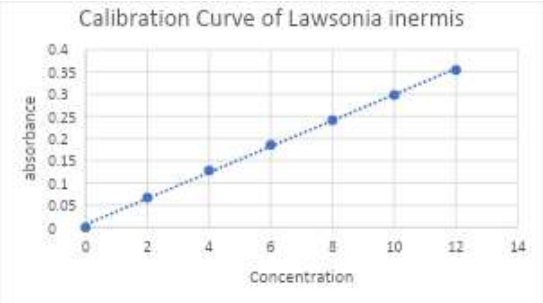
Calibration curve of Allium Sativum:

S.NO	Concentration (µg/ml)	Absorbance(nm)
1.	0	0
2.	2	0.291
3.	4	0.386
4.	6	0.532
5.	8	0.792
6.	10	0.865
7.	12	0.987



Calibration Curve of Lawsonia inermis

S.NO	Concentration (µg/ml)	Absorbance(nm)
1.	0	0
2.	2	0.067
3.	4	0.128
4.	6	0.186
5.	8	0.241
6.	10	0.298
7.	12	0.354



Invitro Diffusion studies:

% Drug Release of Allium sativum

S.NO	Time (min)	% Drug Release of Allium sativum	% Drug Release of Lawsonia inermis
1.	15	20.87±1.23	18.12±1.05
2.	30	29.45±0.82	28.90±0.90
3.	45	40.12±0.25	39.89±0.85
4.	60	58.98±0.85	55.60±1.05
5.	120	73.45±0.35	71.98±1.14
6.	180	84.34±0.45	82.34±0.88
7.	240	92.78±0.49	90.90±1.12

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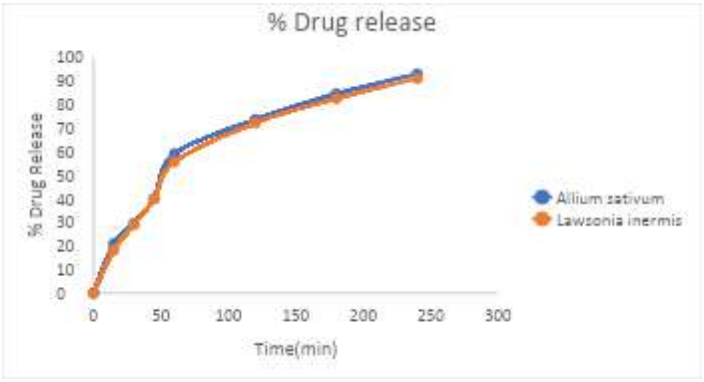

Anti- Microbial Activity:

S.NO	Formulation	Zone of Inhibition(mm) Microrganisms	
		E.Coli	Staphylococcus aureus
1.	Henna emulgel	10.6 ±0.27	11.8 ± 0.34
2.	Garlic Emulgel	13.8 ±0.14	12.4 ± 0.25

Anti-microbial Activity

III. SUMMARY:

- Herbal emulgels were prepared from the extracts of Allium sativum and Lawsonia inermis. The powder of each herb was evaluated for organoleptic, physico chemical evaluation, Phytochemical tests and general powder properties. FTIR analysis was also carried out for the powders and the results exhibited that there are no adverse interactions and ensuring chemical integrity. The results were tabulated in table no:
- Maceration technique was employed to extract the chemical constituents. Emulgels were prepared from the extracts and observed for its color, odour and appearance. The formulated emulgels were good in appearance with pleasant odour and colour.
- Prepared emulgels were subjected to various tests includes grittiness, viscosity, Spredability, Centrifuge test, Swelling index, invitro diffusion studies. Both the formulation has showed all the tests within the limits. More over the emulgels were tested for irritancy on rabbit and found no irritation, redness or swelling on the skin of the rabbit.
- Finally the emulgel is tested for antimicrobial activity using cultures E,coli and Staphylococcus aureus by employing pour plate method. Emulgel containing Allium sativum has shown the best results compared to the emulgel of Lawsonia inermis.

IV. CONCLUSION:

An attempt has been made to develop a plant-based emulgel that ensures effective action microorganisms using Allium sativum and

Lawsonia Inermis extracts. The developed emulgels had a pleasing Odor as well as an attractive appearance. Various phytochemical and physical evaluation tests were performed on the prepared emulgels. The anti-microbial activity tests conducted on these formulations revealed positive results. The test results on rabbit skin exhibit no signs of irritation from these formulations. The study results showed that Allium sativum emulgel demonstrated better antibacterial properties while Lawsonia inermis and Allium sativum emulgels maintained their bioactive components and stable formulation characteristics. The antibacterial and therapeutic properties of these ingredients create promising opportunities for use in topical skin treatments.

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