International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 19-06-2024, Accepted: 13-07-2024, Published: 05-08-2024
Volume 9, Issue 3, 2023, Page No. 30-34

Formulation and evaluation of anti-bacterial activity of *Allium Sativum* Emulgel

K Pargunan*, R Priyadharshini, Barish, S Mahalakshmi
Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, India

Abstract
To formulate and evaluate the anti-bacterial activity of *Allium sativum* Emulgel. The garlic emulgel used in the treatment of various bacterial infection in the skin. The different formulation F1, F2, F3, F4, F5 of emulgel was prepared by using carbopol 934 as gelling agent with oil phase such as liquid paraffin and tween 80 and span 80 as a emulsifying agent. The different concentration of extract are used formulation of emulgel. Gel base are prepared separately. Then emulsion phase are prepared by using mixing of oil phase and water phase. After preparation of emulsion, mixing the gel base with emulsion 1: 1 ratio. The formulated gel are evaluated its physical property like appearance, colour, odour, pH, and spreadability of the emulgel also evaluated. Then the formulation study to the anti-bacterial activity of the preparation. The selected F1 and F2 are taken for the ani-bacterial test. The selected 2 bacteria are used in the anti-bacterial activity test. Extract of garlic also tested for bacterial activity. Both F1 and F2 produce better inhibiting activity.

Keywords: Emulgel, anti-bacterial activity, ethanol extract

Introduction
Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, sublingual, rectal, parental, topical, inhalation etc. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders like acne.
Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Topical preparations are applied to the skin for surface, local or systemic effects. In some cases, the base may be used alone for its therapeutic properties, such as emollient, soothing or protective action. Topical drug delivery offers the advantages of ease of delivery, a cooperative patient, increased compliance as well as the avoidance of first-pass metabolism. Disadvantages are the lack of, or reduced rates of absorption and cosmetic considerations.

Garlic (*Allium sativum)*
Garlic (*Allium sativum* L.) has acquired a reputation in different tradition as a prophylactic as well as a therapeutic medicinal plant. Garlic has played important dietary and medicinal roles throughout the history. Some of the earliest references to this medicinal plant were found in Avesta, a collection of Zoroastrian holy writings that was probably complied during the sixth century BC. There is a some evidence that during the earliest olymphics in Greece, garlic was fed to the athletes for increasing the stamina.
Ancient Chinese and Indian medicines recommended garlic to aid respiration and digestion and to treat leprosy and parasitic infestation. Garlic is a useful compound in the treatment of arthritis, toothache, chronic cough, constipation, snake and insect bites, gynecologic diseases, as well as a infectious diseases.

Materials and Method
Collection of *Allium sativum* Bulb
The *Allium Sativum* L Bulb are colleceted from in the local market. The collected bulb are authenticated by the scientist (F) and Head of office of TNAU Campus (Tamil Nadu Agriculture University) Coimbatore. Then the bulb are cleaned properly, washed and shade dried at room temperature.

Cold maceration process of *Allium sativum*
The collected, cleaned, and shade dried leaves are subjected to the size reduction and seived. Then the garlic extract are prepared by the cold maceration process. About 40gm of dry powdered garlic are taken with 250ml of 70% (w/v) Ethanol are maceration for week in a round bottom flask with occasional shaking.
The flask was kept in the dark to avoid effect of the light on the active constituents of the garlic. Then the extract are filtered through a muslin cloth after a week of maceration. The extract are concentrate till dryness. The use of water bath maintain the room temperature the extract are heated for evaporation till the dryness.

Fig 1: Crude Extract of *Allium sativum*

Formulation of *Allium Sativum* L Emulgel
Formulation of gel
Accurately weighed Carbapol 934 was taken and dispersed in beaker containing 300ml of distilled water. The beaker was set aside for half an hour for allowing Carbapol 934 to swell. The Carbapol 934 was stirred continuously until no lumps are found. Then add 5-6 drops of Triethanolamine and methyl paraben sodium to the Carbapol 934 and stir continuously until a clear, transparent gel is formed.

Formulation of emulsion

The oil phase of the emulsion was prepared by dissolving Span 80 in Light liquid paraffin and the extract of garlic was added and heated to 70°C. The aqueous phase was prepared by dissolving Tween 80 in purified water and heated to 80°C. The oil phase was slowly added to aqueous phase with constant stirring until stable emulsion was formed.

Formulation of emulgel

The gel and the emulsion containing the extract of Tridax procumbens was mixed in 1:1 ratio and gently stirred to form the emulgel.

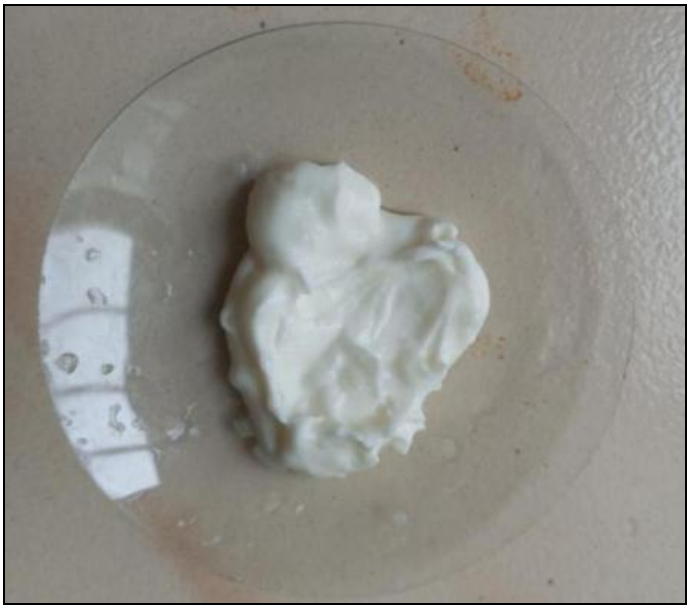

Fig 2: Garlic Emulgel

Table 1: Formulations of Emulgel

S.no	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Garlic extract (gm)	1gm	2gm	1.5gm	2.5gm	1gm
2	Carbopol 934(gm)	1.2gm	1.2gm	1.2gm	1.2gm	1.2gm
3	Span 80 (ml)	3ml	3ml	3ml	3ml	2ml
4	Tween80 (ml)	3ml	3ml	3ml	3ml	2ml
5	Liquid paraffin (ml)	20ml	20ml	20ml	20ml	20ml
6	Triethanolamine	5-6 drops	5-6 drops	5-6 drops	5-6 drops	5-6 drops
7	Methyl paraben (mg)	1mg	1mg	1mg	1mg	1mg
8	Distilled Water	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of emulgel

Physical properties of emulgel

The formulated emulgel are evaluated for its physical properties like colour, state and odor. The appearance of emulgel was analysed by its colour and are examined by visually.

Determination of pH

The pH of the prepared garlic emulgel are examined by use of digital pH meter. Before the measurement of pH, the pH meter are calibrated by using the standard buffer solution. About accurately 1gm of emulgel are weighed and dissolved in 100ml of distilled water. The pH of each formulation was measured.

Determination of spreadability

The spreadability of the prepared garlic emugel are determined by the sample are applied between the two slides and compressed to uniform thickness by placing 100gm of weight for 5mins. Weight was added to the pan. The time required to separate the two slides, the time in which the upper glass slide moved over the lower side was taken measures the spreadability.

Spreadability = m **x** l/t

m = Weight tide to upper slide

l = length moved on glass slide

t = time taken to separate

Determination of viscosity

Viscosity measures the flow characteristics of emulgel formulation. Change in the viscosity of the product is indicative change in stabilty and effectiveness of product

The viscosity of the emulgel was determined by using Brookfield Viscometer.

Determination of homogeneity

All formulated emulgel are tested for homogeneity by visual insepection. They were tested for their appearance and presence of any aggregates.

Determination of grittiness

All the formulations were evaluated microscopically for the presence of particles. Hence the emulgel preparetion is free from any particulate matter and from grittiness as desired for any topical preparation.

Determination of Antibacterial Activity

Test microorganisms

The following bacterial and were used for the screening of antibacterial activity. Bacterial cultures such as *Entrococcus faecallis, Pseudomonas aeruginosa* were obtained from Eumic analytical Lab and Research institute, Tiruchirappalli. Bacterial strains were maintained on nutrient agar medium.
(Hi media) at 4^0C.

Preparation of test and standard solution

The test sample are prepare by dissolving the prepared gel in ethanol (95% v/v) the prepared cream and herbal extract are taken as test sample. 10mg of sample are taken and dissolved with 1ml of ethanol and taken 100 micro liter as final volume. The Gentamicin are used as standard 10mg are dissolved in 1ml ethanol and final volume taken as 100 micro liter. Ethanol (0.1 ml) was used as solvent control as it was used for the extraction process, so that we confirm that the antibacterial activity was not due to solvent.

Inoculum preparation

Bacterial culture were subcultured in liquid medium (Nutrient broth) at 37^0C for 8hrs and further used for the test (10^5-10^6CFU/ml). These suspension were prepared immediately before the test was caried out.

Preparation of culture media

Nutrient agar media

Nutrient agar media is one of the most commonly used medium fot several routine bacteriological purpose.

Table 2: Ingredients for preparation of culture media

S.No.	Ingredients	Grams/Liter
1.	Peptone	5gms
2.	Beef extract	3gms
3.	Agar	15gms
4.	Sodium chloride	5gms
5.	Yeast extract	1.5gms

After adding all the ingredients into distilled water it is boiled to dissolve the medium completely and sterlized by autoclaving at 15 Ib psi pressure (121^0C) for 15mins.

Nutrient broth

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water its boiled to dissolve completely and sterilized by autoclaving at 15 Ib psi pressure ($121^{0=}$C) for 15mins.

Microbial inoculum preparation

The nutrient broth were prepared, then identified bacterial and fungal colonies were inoculated into the broth culture were used for antimicrobial activity.

Kirby agar well diffusion assay

The *in-vitro* antimicrobial activity was conducted by agar well diffusion method. This method is based on diffusion of antimicrobial component from the reservoir hole to the surrounding inoculated agar medium, so that the growth of microbe is inhibited as zone around the hole. The nutrient agar medium was prepared and sterilized by autoclaving at 121^0C 15 Ibs pressure for 15 mins then aseptically poured the medium into the sterile petriplates and allowed to solidify the bacterial broth culture was swabbed on each petriplates using a sterile buds. Then wells were made by well cutter at 10mm diameter in the agar media spread with microorganisim. The test and standard samples are added to the each well aseptically.
This procedure was repeated for each petri plates then the petri plates were incubated at 37^0C for 24hrs. The presence of definite zones of inhibition around the cup indicated antibacterial act.

Results and discussion

The five different formulations of Emulgels (F1, F2, F3, F4, and F5) were formulated using different concentration of garlic extract and excipients. The formulated emulgels were evaluated using evaluation parameters like Homogenity, Viscosity, Spreadability, pH, Physical appearance and Grittiness.

Physical appearance

The formulated Emulgel was evaluated for its organoleptic properties like colour, odour and state. The formulation F1, F2, F3, F4, and F5 was found to be semi-solid in nature, aromatic odour and milk white in colour. All the formuation were homogeneous and free of grittiness.

Determination of ph

The pH values of the formulated emulgels (F1, F2, F3, F4 and F5) was found to be between 6 to 7 which is in the skin pH range. The pH range is considerable to avoid the skin irritation after application to the skin.

Determination of spreadability

The spreadability diameter for different formulation F1, F2, F3, F4, F5 show good spreadability. i.e, emulgel is easily spreadable. The spreadability plays an important role in patient compliance and ensures uniform applicattion of emulgel to the skin surface.

Determination of viscosity

All the fomulations of emulgel were subjected to Brookfield viscometer used to measure the viscosity (in cps) by dropping a cone attached to a holding rod from distance of 10cm in such a way that, it should fall on center of the glass cup filled with emulgel.

Table 3: Summary of Evalution of Emugel

Formulation Code	F1	F2	F3	F4	F5
Homogeneity	++	+++	++	++	+++
Grittiness	_	_	_	_	_
Spreadability	11	13.3	18.6	13.1	11.9
Physical appearance	Clear	Clear	Clear	Clear	Clear
Viscosity	3190	3593	3459	3356	3268
pH	6.6	6.3	6.4	6.8	6.5

++ (good)
+++ (excellent)
- (absence of particulate matter)

Screening of anti-bacterial activity of emulgel containing *allium sativum* extract

The prepared herbal emulgel of various concentration and alcoholic extract of *Allium sativum* are exhibited for antibacterial activity against various microorganism such as Gram-negative bacteria are *Pseudomonas aeruginosa* Gram-positive bacteria are *Entrococcus faecallis.*

Antibacterial Activity of Extract

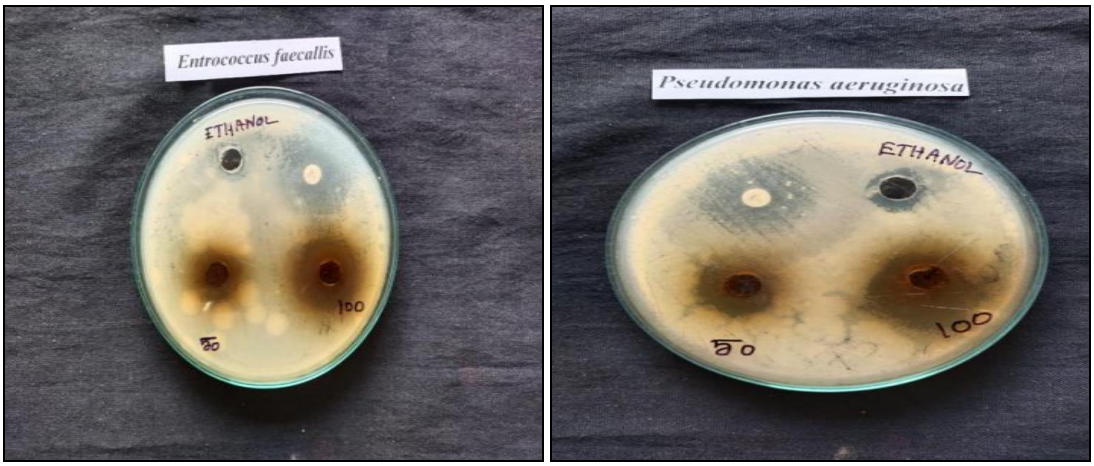

Fig 3: Antibacterial Activity of Extract

Antibacterial Activity of F1

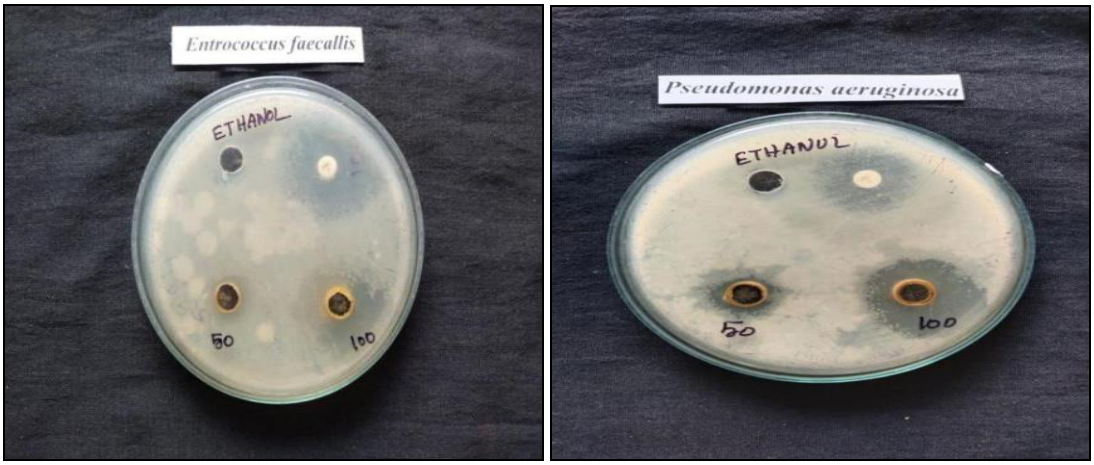

Fig 4: Antibacterial activity of F1

Antibacterial Activity of F2

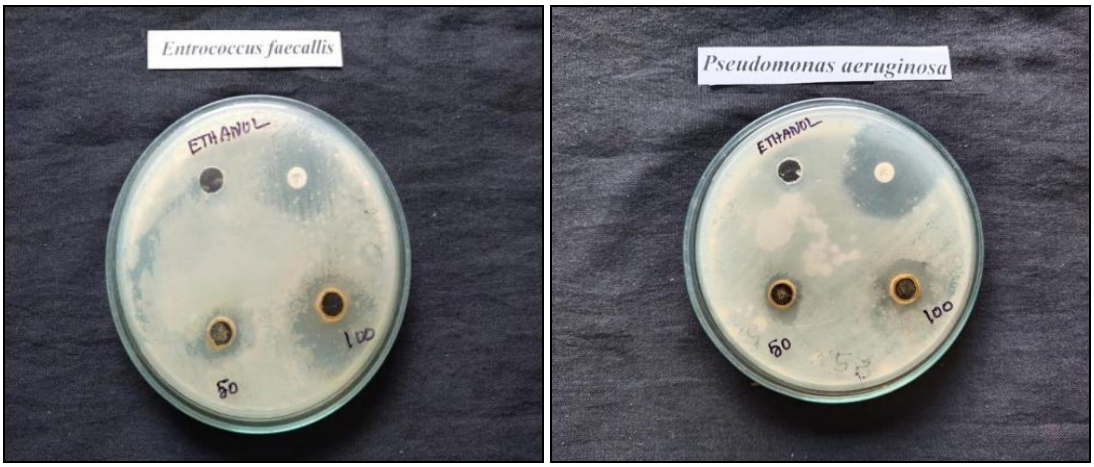

Fig 5: Antibacterial Activity of F2

Antibacterial activity extract and F1 & F2 Formulation

Table 4: Antibacterial Activity of Extract and F1, F2

Sample	Plant extract		F1		F2		Negative Control	Positive Control
	50µl	100µl	50µl	100µl	0µl	100µl		
Enterococcus facalis	23	32	21	28	16	24	0	30
Pseudomonas aeruginosa	35	38	21	36	20	23	0	30

The antibacterial activity of F1, F2 and Herbal extract are evaluated. The herbal extract are produce a better inhibition activity than prepared formulation, but prepared formulation produce a better activity in minimum concentration of extract. The comparision F1 & F2. F1 produces a better inhibition activity than f2 against both gram-negative and gram-positive bacteria.

Conclusion

The objective of the present work is formulate and evaluate herbal emulgel from the alcoholic extract of the Allium sativum.

To develop a topical formulation of alcoholic extract of garlic bulb for effective treatment of bacterial skin infection. Then the process of collection, drying, size reduction of garlic are done, and extraction of garlic are done by cold maceration process ethanol are used as a solvent. Then the extract are loaded to gel base with eight different concentration. Then the formulation are evaluated its physical parameters, like pH, colour, appearance, odor, viscosity and spreadability etc.

The selected formulation F1 and F2 are taken for antibacterial activity test.

The comparision F1 and F2. F1 produces a better inhibition activity than F2 against both gram-negative and gram-positive bacteria.

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