



FORMULATION AND EVALUATION OF HERBAL OINTMENT USING *NERIUM OLEANDER. LINN* (FLOWER) AND EVALUATION OF ANTI-MICROBIAL ACTIVITY.

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ABSTRACT

Nerium oleander (Fam. Apocyanaceae) has been used as medicine in ancient times and has long been known to have antibacterial and anti-fungal properties. The aim of present study to formulate the *Nerium oleander* ointment and evaluate its anti-microbial activity. The formulations were prepared by fusion method and evaluated for its physiochemical parameters like colour, odour, pH, spreadability, irritancy, stability study. The invitro anti-bacterial activity of the *Nerium oleander* ointment was determined against *proteus vulguris* and *Enterococcus faecalis* by using the nutrient agar and invitro anti-fungal activity of the *Nerium oleander* ointment was determined against *candida albicans* by using the sabouraud dextrose agar by cup plate method. This study shows the *Nerium oleander* ointment has anti-microbial activity when compared to other synthetic ointment.

KEY WORDS

Anti-microbial activity, *Nerium oleander* ointment, *proteus vulguris* and *Enterococcus faecalis*, *candida albicans*.

INTRODUCTION

The increasing mortality rate of infectious diseases is one of the most challenging public health problems faced by different countries worldwide. Numerous synthetic antibiotic agents have been used for the management of infectious diseases. Medicinal plants have been used for many years in the treatment of a vast number of human diseases by the community, specifically in traditional medicine. They are considered the main source of new natural and safe drugs to be utilizing in managing diseases as an effective and harmless alternative medicine ¹. *Nerium oleander* flowers grow in clusters at the end of each branch; they are white, pink to red, 2.5–5 cm (1–2 inches) diameter, with a deeply 5-lobed fringed corolla round the central corolla tube.

Nerium oleander extract inhibits the growth of Gram positive and Gram-negative bacteria, such as *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Pseudomonas*, *Shigella*, *Salmonella*, *Proteus*, and *Helicobacter pylori*^{2,3} and inhibits the growth of anti-fungal activity. It is an efficient useful treatment in conditions like snake bites, ulcers, cardiac diseases, asthma, renal and vesicle calculi, chronic stomach diseases, skin related problems, joint pains, leprosy, cancer, etc. It is good fungicide, insecticide, larvicide, parasiticide, rodenticide.

MATERIALS AND METHODS

Collection of Plant Material

The flower part of *Nerium oleander* was collected from the local market in Madurai. Wool fat, ceto stearyl alcohol, hard paraffin and white soft paraffin were purchased from SD fine chem limited.

Method of Extraction

The 60g of shade dried and grinded bulb of garlic was filled a soxhlet apparatus and poured chloroform over a bulb. Then allow the extraction for about 72 hours, after extraction completed, the collected chloroform extract that should be removed using distillation. After distillation the *Nerium oleander* powder was collected for further experimental process ⁴.

Preparation of *Nerium oleander* ointment

Table 1

S. No.	Name of Ingredient	Quantity to be taken
1	Wool fat	0.5g
2	Cetostearyl alcohol	0.5g
3	Hard paraffin	0.5g
4	White soft paraffin	8.5g
5	<i>Nerium oleander</i> extract	0.1 mg

Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in china dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting

and mixing homogenously followed by cooling of ointment base. To this added accurately weighed *Nerium oleander* extract to the ointment base by fusion method to form a smooth paste ⁵.

Evaluation of *Nerium oleander* ointment ⁶

Organoleptic Characters:

The *Nerium oleander* ointment was formulated and evaluated for its organoleptic characters (color, state, odour). The appearance of the *Nerium oleander* ointment was analyzed by its color and roughness visually and by touch.

PH determination

The pH of the formulations was determined using digital pH meter. About 0.5 g of the ointment was weighed and dissolved in 50 ml of DMSO, the measurement of pH of formulation was done in triplicate and average values were calculated.

Spreadability

Spreadability of the ointment was done by using two sets of glass slides of standard dimensions. The *Nerium oleander* ointment formulation (3 g) was placed over one of the slides, the other slide was placed on the top of the ointment, such that the ointment was sandwiched between the two slides. Weight was placed upon the upper slides so that the ointment between the two slides was pressed uniformly to form a thin layer.

Skin Irritancy test:

This test was performed on 3 albino rats and weighing between 150 – 200 g. The animal skin was shaved and cleaned. After cleaning the animal skin, the 30 mg of *Nerium oleander* ointment was applied. Aqueous solution of 0.8% formalin was used as standard irritant. The animals were observed for 7 days for any sign of odema and erythema.

Accelerated stability studies

An accelerated stability study was performed on the formulation by maintaining at room temperature for 20 days with constant time interval. During the stability studies the parameters pH and physical changes were studied.

Table 2

Physicochemical parameters of garlic ointment formulation

S. No	Physicochemical parameters	Observation
1	Colour	Pale white
2	Odour	No odour
3	pH	7.98 ± SD
4	Spreadability study	30 mg .cm/sec
5	Skin irritancy test	No irritancy
6	Stability study	Stable with pH 6.4 and 6.3

***In vitro* Anti-microbial activity**

***Invitro* Anti-bacterial activity by using cup-plate method:**

The Anti-bacterial activity of *Nerium oleander* ointment (*Nerium oleander* ointment diluted by di methyl sulphoxide) diluted solution was carried out by cup-plate method by using test organism *proteus vulguris* and *Enterococcus faecalis*. The sterilized nutrient agar plates were inoculated using spread plate technique in cup plate method. The experimental solution and standard solution were added to the petridish. The concentration of a diluted ointment and standard solution contain 50µl. The petri plates were placed in an incubator at 37°C for 48hrs⁷.

***In vitro* Anti-fungal activity by using cup-plate method:**

The Anti-fungal activity of *Nerium oleander* ointment (*Nerium oleander* ointment diluted by di methyl sulphoxide) diluted solution was carried out by cup-plate method by using test organism *candida albicans*. The sterilized sabouraud dextrose agar plates were inoculated using spread plate technique in cup plate method. The experimental solution and standard solution were added to the petridish. The concentration of a diluted ointment and standard solution contain 50µl. The plates were placed in an incubator at 37°C for 48hrs.

Measurement of Zone of Inhibition

After incubation period is over, the diameter of zone of inhibition was measured in Millimeter side of the ruler. The measurement was taken with ruler from the center of cup to edge of zone of inhibition. The diameter of zone of inhibition was determined by multiplying radius of zone of inhibition with 2. Finally, we get the diameter of zone of inhibition^{8,9}.

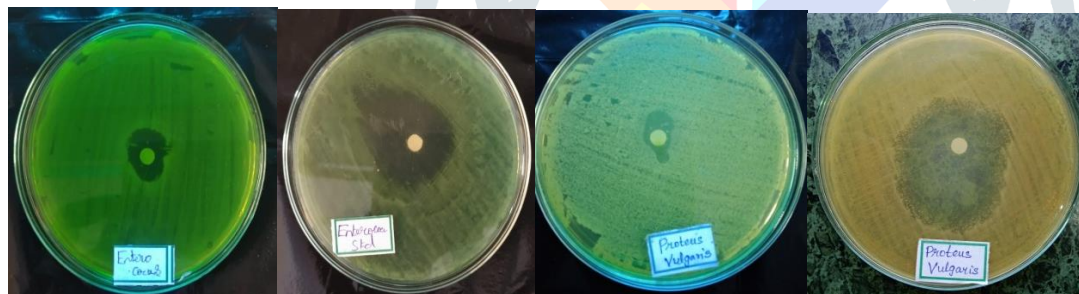


Fig.1

Fig.2

Fig.3

Fig.4

Figure 1 *Enterococcus faecalis* (gram +ve) - *Nerium oleander*.L. ointment(sample) **Figure 2:** *Enterococcus faecalis* (gram +ve) – Ampicillin solution (standard) **Figure 3:** *Proteus vulgaris* (gram –ve) - *Nerium oleander*.L. ointment(sample) **Figure 4:** *Proteus vulgaris* (gram –ve) – Amoxicillin solution (Standard)

Measurement of zone of inhibition:**Table 3**

Micro organism		Zone of inhibition (diameter/mm)
<i>Proteus vulgaris</i>	M.plate	44
	E.plate	16
<i>Enterococcus faecalis</i>	M.plate	36
	E.plate	15

M.plate indicate Marketed plate, E.plate indicate experiment plate

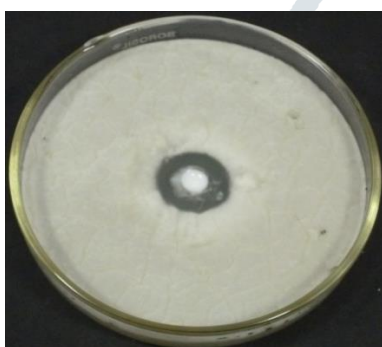
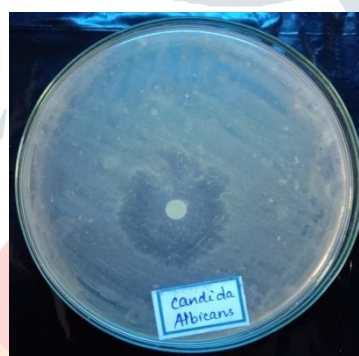
Invitro anti fungal activity**Fig 5****Fig 6**

Figure 5- *candida albicans* *Nerium oleander* ointment (sample) **Figure 6-** *candida albicans* clotrimazole solution (standard)

Measurement of zone of inhibition:**Table 4**

Micro organism		Zone of inhibition (diameter/mm)
<i>Candida albicans</i>	M.plate	35
	E.plate	40

M.plate indicate Marketed plate, E.plate indicate experiment plate

RESULTS&DISCUSSION**Preparation of *Nerium oleander* ointment**

The ointment from *Nerium oleander* prepared by using the fusion method. The prepared ointment filled into the tube and stored at room temperature.

Evaluation of *Nerium oleander* ointment

The evaluation of ointment such as organoleptic parameters, spreadability, pH and stability studies are shown in Table 2.

Invitro Anti-microbial activity:

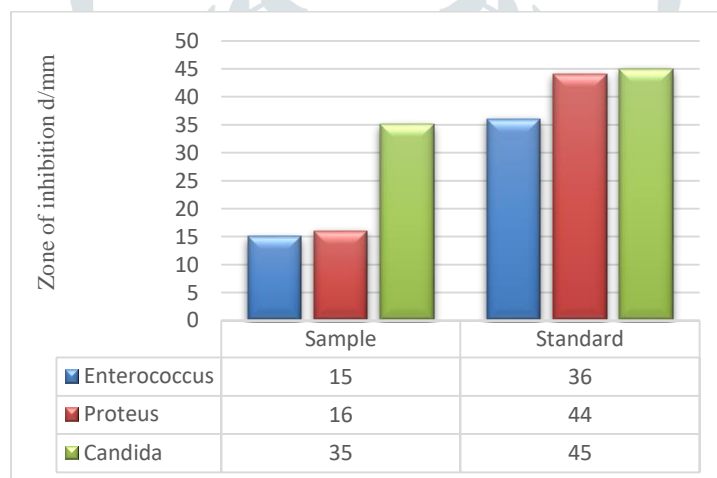
Prepared formulation has shown good activity against both *proteus vulgaris* and *Enterococcus faecalis*. It was found that the formulation of *Nerium oleander* ointment was showing zone of inhibition. The result of anti-bacterial activity of extracts reported in figure 1 and figure 3. Finally *Nerium oleander* ointment against *proteus vulgaris* shows significantly greater Anti-bacterial activity then *Enterococcus faecalis*. The result of anti-fungal activity of *Nerium oleander* ointment reported in figure 5. Finally, *Nerium oleander* ointment against shows significantly Anti-bacterial activity and anti-fungal activity.

Comparative study for anti-bacterial & antifungal activity of *neriumoleander.l.* ointment

Finally based on the above-mentioned report comparative study for anti-bacterial & antifungal activity of *Nerium oleander.l.* ointment. The *Nerium oleander.L.* ointment is more efficiency on antifungal activity than compared to antibacterial activity.

Comparative between antibacterial & antifungal activity

Graph 1



CONCLUSION

According to obtained results, *Nerium oleander.L.* has potential to be used for topical anti-microbial ointment can be a suitable alternative in medicine for prevention and treatment of many diseases. The herbal ointment is required so that better, safe and cost-effective drugs for causing diseases. When formulated as herbal ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. In experimental plate compared to the marketed plate against some microorganisms. The future work is being extended to perform the various activities.

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