



FORMULATION AND EVALUATION OF ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF NEEM HERBAL CREAM

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ABSTRACT

In this article we are going to formulate and evaluate the antimicrobial and antifungal activity of neem herbal cream. Neem is a medicinal plant that has lot of pharmacological activities and therapeutical use. It has major use in herbal industries and many pharmaceutical formulations. The neem herbal cream was formulated and the formulated cream was evaluated for antibacterial and antifungal activity by using Agar well diffusion method. Neem is found to be effective against microbes. Our evaluation in this article will give us an insight about the potency of neem herbal cream against microbes.

KEYWORDS: Neem, Herbal cream, Antifungal, Antibacterial.

INTRODUCTION

Neem is a medicinal tree and a sacred gift of nature. Neem tree is mainly cultivated in the India. Neem is a member of the mahogany family, *Meliaceae*. Today it is known by the botanical name *Azadirachta indica*. Neem has been used extensively by humankind to treat various diseases has been recorded from the beginning of history. Since prehistoric times, neem has been used by humankind.

The latinized name of neem, *Azadirachta indica*, is derived from the Persian. Azad means “free”; dirakht means “tree”; i-Hind means “of Indian origin”. Hence it literally means “the free tree of India”. The neem tree is an incredible plant that has been declared the “Tree of the 21st century” by the United Nations. The US National Academy of Science published a report in 1992 entitled “Neem: A tree for solving global problems”.

In the annals of the ancient Siddha system of medicine, the first medicinal plant mentioned as well as found a place, in ancient Tamil literature is neem or margosa. Neem has been used by humankind from time immemorial as a deterrent for smallpox and other infectious diseases and is also considered to possess powers to ward off evil spirits. The earliest mention about the uses of medicinal plants were found in Thirumular Thirumanthiram-Ennayiram, Tholkappiam and the ancient Tamil works of Sangam literature, which were believed to have been written thousands of years ago.

The roots of Siddha medical system, which has been practiced in South India, go far beyond the historical boundaries. It has existed as a separate system of medicine thousands of years before the dawn of history.

All parts of neem tree are commonly used in traditional Indian medicine for household remedy against various human diseases. The definition of Siddha medicine is conquest of death: “that which ensures preventive against mortality”. Siddha medicine originated from Tamil Nadu, South India. The system was very popular in ancient India. Believed to be more than 10000 years old, the Siddha system of medicine is considered to be one of the most antiquated traditional medical systems. The Siddha system of medicine is prevalent in the southern states of India, Sri Lanka, Malaysia, and Singapore. The first medicinal plant mentioned in the Siddha medical system is neem.

The uniqueness of Siddha system is evident by its continuous service to the humanity for more than 5000 years in combating diseases and also in maintaining its physical, mental and moral health while many of its contemporaries had become extinct long ago.

Siddhars were spiritual adepts who possessed the Ashta Siddhis, or the eight supernatural powers. The word Siddha came from the word siddhi, which means the perfection of heavenly bliss. Sage Agathiyar is considered the guru of all Sidhars, and the Siddha system is believed to have been handed over to him by Lord Muruga.

Typically, siddhars were saints, doctors, alchemists and mysticists all at once. They wrote their findings, in the form of poems in Tamil language, on palm leaves which are collected and stored in what are known today as palm leaf manuscript, today still owned by private families in Tamil Nadu and handed down through the generations, as well as public institutions such as universities all over the world (India, Germany, Great Britain and USA).

Herbal cream

Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external application. It is applied on outer part of the skin and its main ability is to remain for a longer period of time at the site of application. The function of a skin cream is to protect the skin against different environmental condition, microbial infections and gives soothing effect to the skin. There are different types of creams like cleansing, cold, foundation, vanishing, night, massage, hand and body creams. The cream we formulated is a Neem herbal cream which produces both antibacterial and antifungal activity.

MATERIALS AND METHODS

Collection of *azadirachta indica a. juss* leaves

The *Azadirachta indica A.juss* leaves were collected from in and around Perambalur. Then the leaves cleaned properly and shade dried at room temperature.

Soxhlet extraction of neem

Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction (also referred to as leaching or Lixivation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pre-treatment.

Conventional Soxhlet extraction remains as one of the most relevant techniques in the environmental extraction field.

Materials required

Ethanol was purchased from Merk, USA. Whatman No.1 filter paper was purchased from Millipore, USA.

Procedure

1. Test sample can be fresh (*Neem- 29012*) or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area.
2. The test sample should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble).
3. All equipment should be too assembled. Build a rig using stands and clamps to support the extraction apparatus.
4. Following this, the ethanol is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle.
5. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor.
6. The side arm is lagged with glass wool.
7. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser.
8. The condensate then drips into the reservoir containing the thimble.
9. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again.
10. The process should run for a total of 4 hours.
11. Once the extraction set up, it can be left to run without direct supervision.
12. It is not advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, so a technician or other lab user should be made aware.
13. The equipment can be turned off.



Fig. 1: Soxhlet extraction and neem extract.

Preparation of herbal cream**Formulation of neem herbal cream**

S. NO	Ingredients	Quantity
1.	Propylene glycol	10 ml
2.	Stearic acid	17 gm
3.	Carbopol	1.0 gm
4.	Methyl paraben	0.2 ml
5.	Propyl paraben	0.1 ml
6.	Glycerine	1 ml
7.	Triethanolamine	10 ml
8.	Neem extract	2 ml
9.	Purified water	q.s
10.	Rose oil	q.s

Procedure

The Propylene glycol, Stearic acid and Carbopol are melted in water bath and heated upto 70°C. This mixture is known as oil phase. Take a china dish and add water soluble components like glycerine, methyl paraben, triethanolamine are dissolved in water and mixed thoroughly. This solution is heated to 70°C. The phase is

known as aqueous phase. The temperature should be maintained properly at 70°C for the both phase. The accurately weighed neem herbal extract are added in the aqueous phase. Then slowly add this aqueous phase to heated oily phase. The perfume is dissolved and it is added to the cream and mix thoroughly.



Fig. 2: Neem herbal cream.

Evaluation**Physical properties of cream**

The formulated cream is evaluated for its physical properties like color, odor and state. The formulated cream is semi-solid in nature, has a characteristic odor

and light green in color. The texture of cream is smooth and has homogeneity. By visual appearance and touch it is confirmed that the formulation produces a uniform distribution of extract.

S.NO	Specification	Observation
1.	State	Semi solid
2.	Color	Light-green
3.	Odor	characteristic
4	Texture	Smooth

Determination of pH

The pH of the cream was found to be 6.8 which is good for skin pH. The pH of the above prepared cream was determined using pH meter.

Determination of Spreadability

The spreadability of the prepared neem cream are determined by sample was applied between the two slides and was 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 slide was taken as measure of spreadability.

$$\text{Spreadability} = m \times l/t$$

m = Weight tide to upper slide

l = length moved on glass slide

t = time

The spreadability plays a considerable role in patient compliance and ensures uniform application of cream to a larger area of the skin. The low value of spreadability coefficient of the cream was sufficient suggesting easy

spreading. The lower value of spreadability indicates the lesser work required to spread the cream over the skin, which means formulation was easily spreadable by applying small amount of shear. The spreadability test showed that formulation has good spreadable property. The spreadability of the neem herbal cream was found to be 18.3 cm/sec.

Dilution test

The type of emulsion is determined by the dilution test. The type of cream is identified as a O/W type, its determined by dilution test. The prepared cream is diluted either with water or oil. When the prepared cream is diluted with water it remains stable as water is a dispersion medium, the cream is a O/W type of cream, but when it is diluted with oil the cream breaks and oil. So, the prepared cream is identified as a O/W type of emulsion.

Antibacterial activity of neem herbal CREAM

Agar well diffusion method

Principle

The antimicrobials present in the given sample were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials required

(*Bacteroides fragilis* and *Staphylococcus aureus*- 902) was purchased from MTCC, Chandigarh, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Himedia, India. Test

samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

1. Agar- well diffusion method

a. Nutrient Agar Medium

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm Petriplates (25-30ml/plate) while still molten.

b. Nutrient broth

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

1.1. Procedure

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (*Bacteroides fragilis* and *Staphylococcus aureus*- 902) Wells were cut and concentration of sample Neem (29012) (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

RESULTS

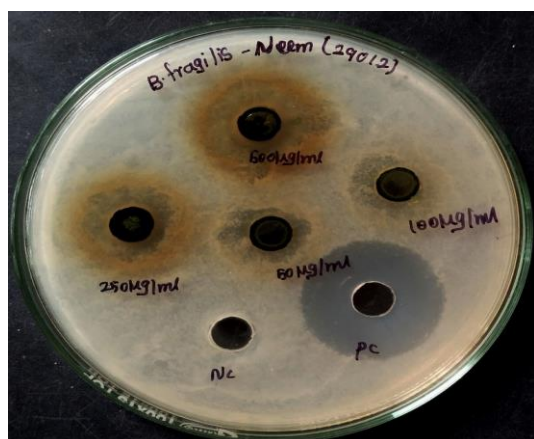
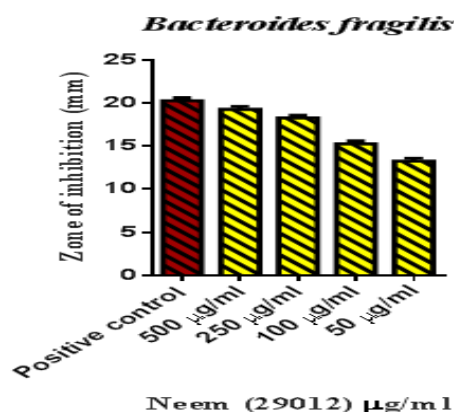


Fig. 3: Effect of sample neem (29012) against *bacteroides fragilis*.



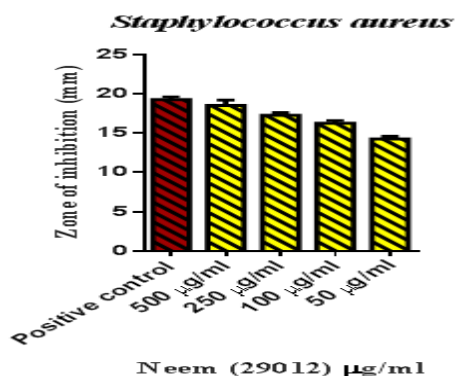


Fig. 4: Effect of sample neem (29012) against *staphylococcus aureus*.

Table 1: SD± Means of zone of inhibition obtained by sample Neem (29012) against *bacteroides Fragilis* and *Staphylococcus aureus*.

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>Bacteroides fragilis</i>	Neem (29012)	19.25±0.35	18.25±0.35	15.25±0.35	13.25±0.35	20.25±0.35
2.	<i>Staphylococcus aureus</i>		18.5±0.7	17.25±0.35	16.25±0.35	14.25±0.35	19.5±0.7

SD – Standard Deviation, *Significance - $p < 0.05$

Antifungal activity of Neem Herbal cream

Agar well diffusion method

Principle

The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

Potato dextrose agar medium, Amphotericin B antimycotic solution, test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

1. Agar- well diffusion method

a. Potato dextrose agar medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato infusion, 2 gm of dextrose

and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

Procedure

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain (*Aspergillus niger* and *Candida albicans*) wells were cut and different concentration of sample Neem (29012) (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

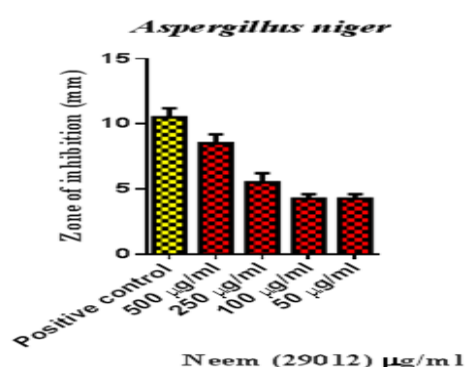


Fig. 5: Effect of sample neem (29012) against *aspergillus niger*.

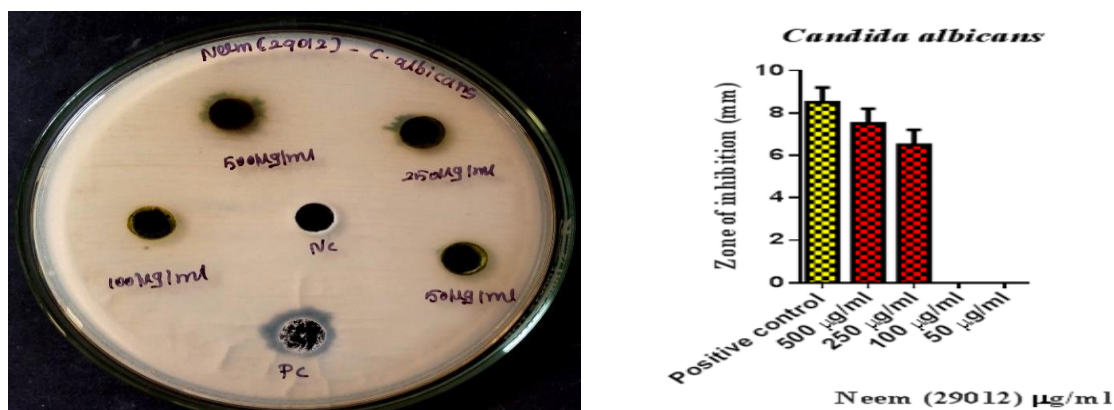


Fig. 6: Effect of sample Neem (29012) against *Candida albicans*.

Table 1: SD± Means of zone of inhibition obtained by sample Neem (29012) against *Aspergillus niger* and *Candida albicans*.

S. NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>Aspergillus niger</i>	Neem (29012)	8.5±0.7	5.5±0.7	4.25±0.35	4.25±0.35	10.5±0.7
2.	<i>Candida albicans</i>		7.5±0.7	6.5±0.7	0	0	8.5±0.7

SD – Standard Deviation, *Significance - $p < 0.05$

CONCLUSION

Formulation of herbal cream was successfully developed that met the relevant pharmaceutical characteristics. The prepared herbal cream has best properties and having nutritional values using less chemicals which protect the skin from the various skin problems. The prepared cream is O/W type emulsion. The prepared formulation contains both antifungal and antibacterial activity, comparatively it produces better antibacterial activity than antifungal activity.

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