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Formulation And Evaluation Of Herbal Ointment By Using Adhatoda Vasica Extract

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Abstract: Background: *Adhatoda vasica* Nees also known as "Justicia Adhatoda L., vasaka, adosa, Malabar nut tree" belongs to the family of Acanthaceae is considered the most significant plant in the world. This plant has been used in the indigenous medicinal system of India for more than 2000 years. Vasicine is the main chemical constituent present in this plant which possess various medicinal properties like antispasmodic, sedative, expectorant, antitussive, anti-bacterial, anti-microbial, anti-diabetic, antiasthma, wound healing, abortifacient and anti-pyorrhea.

Objective: The main objective of the study was to formulate and evaluate the antimicrobial properties herbal ointment from the local medicinal plants.

Methods: The extract was obtained by the decoction method. Simple ointment IP was use as ointment base for the dispersion of the plant extract. The ointment was formulated and evaluated using standard parameters. The antimicrobial activity of the extract in different concentration were investigated by using one gram-positive bacteria (Staphylococcus aureus) and one gram-negative bacteria (Escherichia coli) through disc-diffusion method.

Results: The antimicrobial potential of the formulated ointment was studied. The ointment also showed significant antimicrobial activity against all the tested organism. This activity was maintained when the extract was incorporated into the ointment base.
Conclusion: In future, the extract can be used for the commercial production of *Adhatoda vasica* ointment. Similarly, this ointment can be tested for skin related bacterial infection.

KEYWORDS: *Adhatoda vasica*; decoction; anti-microbial; disc-diffusion method; irritancy; spreadability; stability

INTRODUCTION

Antimicrobial activity is the ability of a substance to inhibit or kill bacteria cells. Microorganisms such as bacteria can cause many types of skin- related diseases such as skin rashes, acne, eczema, psoriasis, dermatitis and etc. Staphylococcus aureus and Escherichia coli are the main pathogen that causes these skin infections. Topical ointment containing antimicrobial properties can be used in the treatment and prevention of this kind of bacteria causes the infection. Most of the available topical drugs used to treat skin-related diseases in the market are obtained by various synthetic processes which are using chemicals and have some kinds of side effect. The differences between the antibiotic drug and herbal antibiotic is that they are not just limited to the effect of one chemical but they have several constituents with the variety of healing properties and produce more synergistic effect to fight to bacteria. Topical ointment containing extract of medicinal plant is one alternative to treat the skin infection caused by bacteria and prevent the use of oral antibiotic which then can develop bacterial-resistant[1].

A bacterial skin infection, also known as a skin and soft tissue infection (SSTI) or acute bacterial skin and skin structure infection (ABSSSI). It occurs when bacteria enter the skin, either from an outside source or because they are present on the skin. They can enter into the skin through a hair follicle or after a wound. Staphylococcus and Escherichia Coli are the most common bacteria causing bacterial skin infection. Symptoms can include redness, swelling, pain, warmth, pus and sometimes fever[2].

"Herb" it consists of entire plant or any part of the plant. "Herbal Drug" it consists of plant or any part of plant, usually in unprocessed or crude forms (crude drugs) which have medicinal value. "Herbal Drug Preparation" they are processed form of herb. They are derived from herbal drugs by various techniques like extraction, purification, concentration, fermentation and may be the form of powder, extracts, tincture, fixed oil, volatile oil, resin gum etc[3].

OINTMENT:

The word ointment comes from the Latin ungere meaning anoint with oil. An ointment is a viscous semisolid preparation used topically on variety of body Surfaces. An ointment is homogenous, viscous semisolid preparation, most commonly a greasy, oily (Oil- 80%, water- 20 %) with high viscosity that is intended for external application to skin or mucous membranes. Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membrane of the eye (an eye ointment), chest, vulva, anus and nose. In the case of protective ointment, it serves to protect the skin against moisture, air, sun rays and other external factors.

An ointment may or may not be medicated. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Anti-microbial herbal ointment is an ointment that are used to inhibit the growth of bacteria or kill the bacteria cells. There are five classes or types of ointment bases which are differentiated on the basis of their physical composition.

They are:

(1) Oleaginous bases

(2) Absorption bases

(3) Water in oil emulsion bases

(4) Oil in water emulsion bases

(5) Water soluble or water miscible bases[4]

PLANT INTRODUCTION

Adhatoda Vasica whole plant

Adhatoda Vasica leaves

Adhatoda vasica Nees also known as “vasaka, adosa, Malabar nut tree” belongs to the family Acanthaceae. It is also known as Justicia adhatoda synonymously. It is considered the most significant drug in Ayurveda and Unani medicinal systems[5,6]. The plant is small evergreen mainly found in the plains regions of India, Sri Lanka and Burma[7]. This plant has been used for 2000 years to treat respiratory problems and it was also said by ancient Indians, ”No man suffering from phthisis needs despair as long as the Vasaka plant exists”. The leaves of this plant give a stimulant effect on the respiratory system[8]. In Ayurveda medicinal system it is considered a prime herb to cure diseases like cough, symptoms of cold, asthma, bronchitis[9]. This plant is treated like a mother to doctors in the traditional medicinal system of India therefore known as Vaidyamata singhee in Sanskrit[10]. The major constituents reportedly present in the A. vasika plant is Vasicine that is a quinazoline-type alkaloid that is of great medicinal importance[11]. The plant is known to have a rich source of Vitamin C, carotene and essential oil, phenolics, flavonoids, sterols[12,13]. In Ayurveda medicinal system, it is used to cure various diseases like Shwasa (dyspnea), Ksaya (phthisis), Kasa (cough), Raktapitta (hemorrhagic disease), Kamala (jaundice) and Kushtha (skin disease)[14]. The source of the therapeutic drug ‘Vasaka’ is considered for its indigenous system of medicine. It contains various therapeutical properties including cardiovascular protection, antitubercular, antiulcer, antiasthmatic, hepatoprotective, antibacterial, antitussive, antimutagenic, abortifacient[15].

Botanical classification of Adhatoda vasica plant

Table No.:- 1.

Taxonomical Rank	Taxon
Kingdom	Plantae
Division	Angiosperms
Class	Eudicots
Order	Lamiales
Family	Acanthaceae
Genus	Justicia
Species	J. adhatoda[16]
Common name	Adulsa (Vasaka)

PRAPARATION OF OINTMENT

Formula for simple ointment

Table No.:- 2.

Sr. No.	Name of Ingredient	F1	F2	F3	F4
1	*Adhatoda vasica* extract	1ml	1.25ml	1.5 ml	**0.8ml**
2	Wool fat	1gm	1gm	1gm	**1gm**
3	Cetostearyl alcohol	1gm	1gm	1gm	**1gm**
4	White soft paraffin	16.00gm	16.00gm	15.75gm	**16.5gm**
5	Hard paraffin	1gm	0,75gm	0.75gm	**0.8gm**

MATERIAL AND METHODS

Chemical and Reagent

The various chemicals used through experimental work are summarized.

Table No. :- 3.

Sr. No.	Chemicals
1.	*Adhatoda Vasica*
2.	Wool Fat
3.	Cetostearyl Alcohol
4.	Hard Paraffin
5.	Soft Paraffin

Collection of Herbal Plant

The leaves of *Adhatoda Vasica* were collected from the B. Pharmacy college, Rampura, Kakanpur, TA: Godhra, DIST: Panchmahal, STATE: Gujarat.

Extraction

Decoction Method

Leaves of *Adhatoda vasica* were collected and washed thoroughly with distilled water and sun dried for 8-10 days. Dried leaves were ground into powder form. 100gm of powder were taken into 1000ml beaker and approx. 1000ml water added into it. The above mixture was boiled until the color of water change (greenish black) and then filtered. The filtrate was gently heated with occasional stirring. The thick extract was obtained.

Extraction process

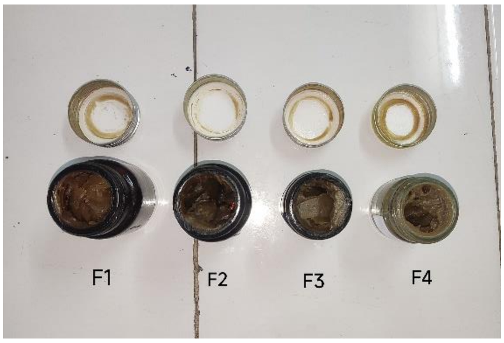
Adhatoda Vasica extract

Procedure

Procedure for Preparation of Herbal Ointment:

a) Initially, prepare the ointment base by weighing accurately grated hard paraffin which is to be place in evaporating dish on water bath. After melting of hard paraffin add remaining ingredients and stir gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Prepare the herbal ointment by mixing accurately weighed Adhatoda extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable container.



Herbal Ointment

EVALUATION TEST

Organoleptic evaluation (color, odor) was done by sensory and visual inspection and compared to the marketed Herbal ointment. The evaluation was carried out on the Herbal ointment by using the following parameters:

- **Color and odour**

  Color and odour of prepared ointment were examined by visual examination.

- **pH**

  The pH of ointment was determined by digital pH meter. 1gm of ointment was dissolved in 50 ml of distilled water and the pH was measured.

- **Skin irritancy**

  Herbal ointment prepared was applied to the skin of human being and observed for the effect.

- **Spreadability**

  The spreadability was determined by placing excess of sample in between two slides which was compared to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability.

  Spreadability was calculated by following formula

  $$S=M*L/T$$

  Where, S= Spreadability

  M= Weight tide to upper slide

  L= Length of glass slide

  T= Time taken to separate the slides

- **Washability test**

  Washability was determined by rubbing the little amount of base on hand for test.

- **Test of solubility**

  The content should be soluble in 9 parts of water and I 1.7 parts of hot water.

- **Stability study**

  The stability study was carried out for the prepared ointment at the temperature of 37 c for 15 days.

- **Antimicrobial test**

- **Sterilization of glassware:**

  The glassware that are used in this test procedure were sterilized by using hot air oven. Hot air oven used for sterilizing the glassware. It is also known as forced air circulating oven. Firstly, the glassware were clean with distilled water and then it wrapped with paper. After that the glassware were put in hot air oven for 60 minutes at 170 c[17].

- **Test microorganisms**:

  A panel of common pathogenic microorganisms were used in the study, which includes one- gram positive bacteria (Staphylococcus aureus) and one gram – negative bacteria (Escherichia coli).

- **Preparation of media:**

  1. Take each ingredient or complete dehydrated marketed nutrient agar powder 28g, transfer in a conical flask and make up the volume till the mark of 1000ml with distilled water.
  2. Heat this mixture while stirring to fully dissolve all components.
  3. After proper mixing, pH of media is determined by using pH meter and adjusted the required pH.
  4. Sterilized the dissolved mixture at 121 c temperature, 15 lbs pressure for 15 minutes in autoclave.
  5. Once the nutrient agar has been sterilized, allow it to cool at room temperature but not allowed to solidify the media.
  6. Pour required quantity nutrient agar into petri plate and leave plates on the sterile surface until the agar solidified.
  7. After solidify the nutrient agar media in each petri dish, each petri dish was store in a refrigerator[17].

- **Disc-diffusion method**

  The antimicrobial activity of ointment and extract were evaluated using disc-diffusion method. A suspension of the tested microorganisms was uniformly swabbed on agar plates using sterile cotton swabs. Sterile blank disc were individually impregnated. Ointment was placed onto the inoculated agar plates. The plates were inverted and incubated at 37 c for 24 hr for bacteria. The antimicrobial activity was measured by measuring diameter of the resulting zone of inhibition against the tested organisms.

RESULTS

Table No. :- 4. Quality control evaluation of formulation

Sr. no.	SAMPLE	F1	F2	F3	F4
1.	Color	Brown	Brown	Brown	**Brown**
2.	Odor	Characteristics	Characteristics	Characteristics	**Characteristics**
3.	Texture	Slightly hard	Slightly hard	Smooth	**Smooth**
4.	pH	6.32	5.95	6.59	**6.18**
5.	Skin irritancy	Non irritant	Non irritant	Non irritant	**Non irritant**
6.	Spreadability	35.71mm	41.66mm	48mm	**50mm**
7.	Washability	Washable and slightly sticky	Washable and slightly sticky	Washable and slightly sticky	**Washable and slightly sticky**
8.	Solubility	Soluble in boiling water and ether,	Soluble in boiling water and ether,	Soluble in boiling water and ether,	**Soluble in boiling water and ether,**

		Insoluble in water and ethanol	Insoluble in water and ethanol	Insoluble in water and ethanol	**Insoluble in water and ethanol**
9.	Stability	Stable	Stable	Stable	**Stable**
10.	Antimicrobial activity	Formulation has antimicrobial activity against Gram positive (S. aureus) and Gram negative (E. Coli) bacteria			

DISCUSSION

Adhatoda vasica is believed to have many beneficial effects in the treatment of various diseases such as skin rashes, cellulitis, cough, wound healing, asthma, fever and diabetes mellitus etc. Various phytochemicals presence in the leaves of this plant is responsible for therapeutic effects. The antimicrobial potential of the extract of *Adhatoda vasica* leaves along with formulated ointment were determined by using disc-diffusion method against Staphylococcus aureus and Escherichia coli. The extract of *Adhatoda vasica* leaves showed significant antimicrobial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of phytochemical present in the extract. The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using decoction process to obtain a good yield of extract and there was no any harm to the chemical constituents and their activity.

CONCLUSION

The antimicrobial activity of the extract of *Adhatoda vasica* leaves was evaluated. This activity was maintained when the extract was incorporated into the ointment base for topical use in the treatment of common skin condition. From the study it can be conduct that prepare ointment using *Adhatoda vasica* is suitable for treat or prevent skin infection and so better alternative other than allopathic ointment. Hence reduce the side effect. *Adhatoda vasica* has good antimicrobial properties and use for the treatment of skin infection cause by susceptible organism.

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