



(RESEARCH ARTICLE)



Formulation and evaluation of herbal ointment using leaves of *Acacia nilotica* (L.) Delile extract

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Abstract

Diseases have co-existed with life, the study of diseases and their treatment is important part of our ancient plant worldwide. Herbal plants are wonderful origin of traditional & modern medicine, useful for primary health care system. Herbal medicine prepare various part of plant are used. Instead off an herbal drug is design as the alternative formulation for the external use in the form of ointment. For the medicinal use the herbal ointment apply externally on human body. The main aims of this research are preparation of herbal ointment from the *Acacia nilotica* (L.) Delile plants the antibacterial activity. Under this research work, it is design as herbal ointment with the help of ethanolic extract of *Acacia nilotica* (L.) Delile determines the anti-bacterial capacity on it. *In vitro* study the antibacterial activity of the ethanolic extracts of dried leaves of *Acacia nilotica* (L.) Delile are determine by using the Agar cup plate method versus different bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* etc. By blending the ethanolic extract of *Acacia nilotica* (L.) Delile (10 % w/w) into aqueous cream we formulated herbal ointment. The emulsifying agent and simple ointment bases are added in above herbal ointment for increase the antibacterial effectiveness during *in vitro* evaluation. *Acacia nilotica* (Family Leguminosae) are broadly used in traditional system of medicine throughout different part of India, Africa & America. It is used in the treatment of diarrhea, dysentery, cough, tuberculosis and leprosy. The current study deals with the determination of formulation and evaluation of herbal ointment using *Acacia nilotica* (L.) leave extract.

Keywords: *Acacia nilotica*; Herbal ointment; *Staphylococcus aureu*; *Escherichia coli*; *Leguminosae*; Babool

1. Introduction

In current days herbal ointment is more popular formulation use for external application. The conveying of drugs through the skin are encouraging concept because easy of access, large surface area, vast exposure to the circulatory and lymphatic networks and protective nature of the treatment [1]. Instead of the alternative formulation like herbal medicine may also be prepared in the form of ointment. These ointment mention a viscous semisolid preparation applied externally on body surfaces area such as the skin, mucus membranes of the eye, vagina, anus, and nose etc. These ointments have medical properties. The medicated ointments contain a medicinal ingredient mixed, suspended or emulsified in the ointment base. Herbal ointment applied externally such as antipruritic, keratolytics, protectants, antiseptics, emollients and astringents. Ointment bases are mainly free from water and generally contain one or more chemical in suspension or solution or dispersion form. Hence Ointment bases may be different types like absorption bases, dehydrating hydrocarbon water soluble type [2].

Herbal plants have ability for the formation of secondary metabolites such as steroids, phenolic substances, flavonoids, alkaloids, etc. These secondary metabolites are used to treatment of many diseases. *Acacia nilotica* belonging to the family Leguminosae brought its importance for its different traditional uses throughout India. On folklore study *Acacia*

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nilotica have more therapeutic importance. There are about 1200 species found in tropical and subtropical region of Asia, South America, Africa and Australia. *Acacia nilotica* contains many chemical constituents like- gallic acid, arabic acid, chlorogenic acid. [3]. *Acacia nilotica* is useful against multidrug resistant of bacteria and fungus causing nosocomial and community acquired infections. Phytochemical analysis of different parts of *Acacia nilotica* shows different classes of secondary metabolites which have therapeutic potential [4]. It is known as Babool, Kikar (Hindi), Babul (Marathi & Bengali), Babaria (Gujrati), Babli (Kannada) Nallatamma (Telugu). It is small to medium sized tree. Its bark has dark brown to almost black. Leaves are evergreen with 4-8 pinnate and flowers in spherical heads on the new growth. Root part used in treatment of tuberculosis, it is also used in cancerous tumour of (eye & ear). Leaf part used to treat diarrhea & alzheimer"s disease, it is also used as emulsifying agent, suspending agent. Stem part is used as antioxidant, astringent & emollient. Bark contains tannins, saponins, terpenoids, gallic acid, and leaf part contain cresol (antiseptic). The other chemical constituent of Acacia is arabic acid which on hydrolysis gives L-arabinose, L-rhamnose, D-galactose, D-glucuronic acid. Others species of babool are *Vachellia nilotica*, *Acacia arabica*. [5]

Today in the modern era, the pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against the infectious diseases. So some of the active compounds prohibit growth of the disease causing microbes either singly or in combinations [6]. For a long period of time plants have been a precious source of natural products which are used for maintain the human health, especially in last decades with more extensive studies for natural treatments. There is a continuous and immediate need to invent the new antimicrobials compounds with the varied chemical structure and innovative mechanisms of action for new and re -appearing infectious diseases. So scientists are increasingly turning their attention to community medicines, looking for new leads to develop better drugs against microbial infections [7]. Considering that extracts of *Acacia nilotica* (L.) Delile show broad spectrum antimicrobial activity. The aim of the study was to show that *Acacia nilotica* (L.) Delile are antibacterial activity and also has high potential as antibacterial agent when synthesized as ointment for topical use ethanolic extract of *Acacia nilotica* (L.) Delile. *Acacia nilotica* (L.) Delile belonging to the family Leguminosae brought its importance for its different traditional uses throughout India. It is used in the treatment of various diseases such as colds, diarrhea, dysentery, bleeding piles and bronchitis [8]. The aim of the current study deal the formulation and evaluation of herbal ointment using *Acacia nilotica* (L.) Delile extract.

2. Material and methods

Fresh leaves parts of *Acacia nilotica* (L.) Delile were collected from fields of Chandeshwar, district of Azamgarh, Uttar Pradesh, India in the month of January 2018 and authenticated by Prof N.K Dubey, Taxonomist, Department of Botany Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India. A voucher specimen has been preserved in Department of Natural Product, Pharmacy college, Azamgarh, Uttar Pradesh, India for future reference (Voucher specimen no.Mimosa.2018/3). The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers. The macroscopic characters were studied as per given procedure in WHO guidelines on quality control methods for medicinal plants materials [9].

3. Macroscopical studies

The leaves of the plant were studied for their macroscopic characters such as color, odour, taste, shape and size of the leaf.

4. Physicochemical studies

The loss of drying [10,12], extractive values (petroleum ether 60-80 °C, ethanol, chloroform, benzene, aqueous) were determined according to the official methods of ayurvedic pharmacopoeia of India [11,13-15], were performed according to the official methods prescribed in Indian Herbal Pharmacopoeia [16]. and WHO guidelines [17].

4.1. Test microorganisms

The microorganisms used for the study were *Staphylococcus aureus* and *Escherichia coli*. In this study, multi drug resistant wound separates bacteria from pathology, Civil Line, Azamgarh were used. The bacterial strains were raised and managed on Mueller Hinton agar at 37 °C.

4.2. Microbiological media

Chemicals and standard drugs Mueller Hinton Agar and Nutrient broth are collect from the Chemical store of the Pharmacy College, Azamgarh. Gentamicin ointment (1mg of Gentamicin in the form of Gentamicin Sulphate), obtain by medical store of Maha Mrityunjay Hospital, Azamgarh.

4.3. Preparation of ethanolic extract of *Acacia nilotica* (L.) Delile (EEAN) leaves

The sun-dried leaves of *Acacia nilotica* (L.) Delile powdered 4g of milled leaves powder are extracted with ethanol by maceration for 48 hr. The extracts are filter and concentrate using evaporator at 35°C to obtain semisolid extract. The extracts are stored in a desiccator. (EEAN) prepare at different concentrations of 500mg/ml, 250mg/ml and 100mg/ml.

4.4. Evaluation of antibacterial activity of Extract

The antibacterial activity of the ethanolic extract of the leaves of *Acacia nilotica* (L.) Delile (EEAN) at concentrations of 100mg/ml, 250mg/ml and 500mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*E. coli* and *S. aureus*) containing approximately 10^8 cfu / ml in a sterile petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the ethanolic extract of the leaves of *Acacia nilotica* (L.) Delile (EEAN). The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Streptomycin and Gentamycin were used as positive and negative controls respectively. The *in vitro* bacterial response to the extract are evaluate using the diameter of the zones of inhibition as follows; resistant: 10mm and below, intermediate: 11-15mm and susceptible: 16mm and above. [18]

4.5. Preparation of Ointments

Three topical ointment bases of varying degrees of aqueous/anhydrous character namely simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepare by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment done by incorporating 10 g of the semisolid ethanolic extract of *Acacia nilotica* (into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Acacia nilotica* extract [19]. The prepared herbal ointments were put in ointment tube, labeled and were stored at room temperature.

4.6. Evaluation of ointment

The evaluations were carried out on the ointment by using the following parameters.

4.6.1. Colour and odour

Colour and odour of ointment, examine by visual examination.

4.6.2. Loss on drying

1 g of ointment was placed in the Petridis and heated in the water bath at 105 °C every 30 min until it get constant weight.

4.6.3. pH

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

4.6.4. Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into Petridis. A hole bored at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

4.6.5. Stability study

The stability studies are carried out for the prepared ointment at temperature of 37°C for 2 months.

5. Results and discussion

The preliminary *in vitro* antimicrobial activity of the ethanolic extract of *Acacia nilotica* (L.) Delile (EEAN) presented showed excellent activity against *Staphylococcus aureus*. The *in vitro* antimicrobial activity of the ethanolic extract of *Acacia nilotica* (L.) Delile (EEAN) based herbal ointments. In various case history show that most of the infections are caused by the gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Less common cause by the gram-negative bacteria such as *Escherichia coli*. The ethanolic extract of *Acacia nilotica* (L.) Delile (EEAN) leaves showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstitunets present in the extract. Hence the observed antibacterial activities of the ointment are due to the presence of active constituents of the extract and the activity also possess as ointment. This was good sign to do further studies on that to make it as one of the commercial ointment for the treatment of bacterial infections. In literature survey it was found that the plant possesses several traditional and pharmacological uses. The formulation and evaluation of herbal ointment study of the leaves extract of *Acacia nilotica* (L.) Delile were done. The leaves were ever green in color. The values of the physical constant like loss on drying, extractive value were determined. Extractive value and color of extract was investigated (Table-2).

Table 1 Macroscopical evaluation of *Acacia nilotica* (L.) leaves

S. No.	Feature	Observation
1.	Color	Green
2.	Odour	Characteristics
3.	Taste	Astringent
4.	Size	Avg. 4-20 mm long

Table 2 Physicochemical Analysis of *Acacia nilotica* (L.) Leaves

S. No.	Solvent	Wt. of Plant material (gm)	%age of yield	Color of extract
1.	Chloroform	4	5%	Greenish Brown
2.	Pet. Ether	4	1.80%	Greenish Brown
3.	Ethanol	4	7.8%	Greenish
4.	Benzene	4	7.5%	Pale green
5.	Aqueous	4	4.75%	Yellowish brown

Table 3 Composition of the ethanolic extract of *Acacia nilotica* (L.) Delile leaves ointment

S. No.	Components	Amount(gm)
1.	Ethanolic extract of leaves Plant <i>Acacia nilotica</i> (L.) Delile	2
2.	Emulsifying wax	28
3.	White soft paraffin	50
4.	Liquid paraffin	20

Table 4 Physicochemical parameters of *Acacia nilotica* (L.) Delile herbal ointment formulation.

S. No	Physicochemical parameters	Observation
1.	Colour	Dark greenish
2.	Odour and taste	Characteristic
3.	Loss of drying	0.18%
4.	pH	6.2
5.	Diffusion study	2.1cm in 1 min
6.	Stability study	Stable with pH 6.1

Table 5 zone of inhibition of the ethanolic extract of *Acacia nilotica* (L.) Delile (EEAN) leaves

S. No	Concentration(mg/mL) of MEEO	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1.	100	12.67 ± 3.06	11.00 ± 2.00
2.	250	14.67 ± 1.53	13.00 ± 2.00
3.	500	17.00 ± 2.00	16.33 ± 3.05
4.	Ciprofloxacin (5 µg)	29.33 ± 3.06	27.00 ± 3.00

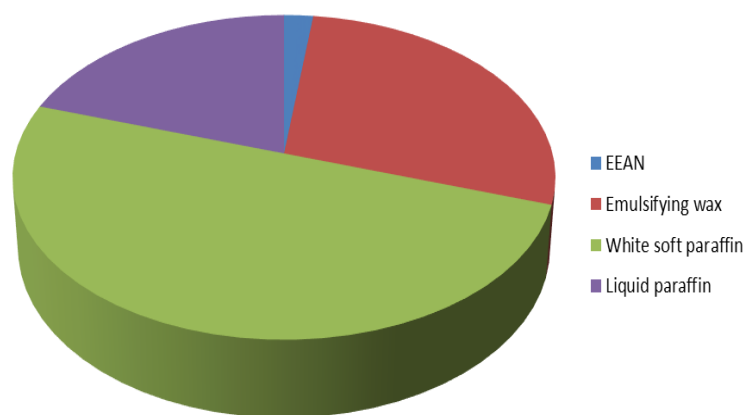


Figure 1 Composition of the ethanolic extract of *Acacia nilotica* (L.) Delile leaves ointment

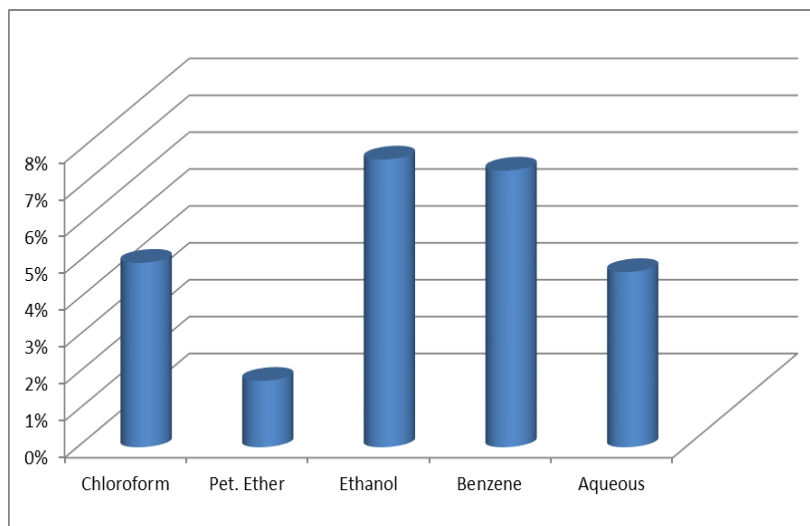


Figure 2 Physiochemical Analysis of *Acacia nilotica* (L.) Leaves

6. Conclusion

In concluded that the present investigation comes out with the fact that *Acacia nilotica* (L.) Delile essential oil are required so that better, safe and cost effective drugs for treating *S. aureus* causing diseases. This study shows that *Acacia nilotica* (L.) Delile are antibacterial activity and have high potential as antibacterial agent. When formulated as 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. The potency of the *Acacia nilotica* (L.) Delile herbal ointment against *Staphylococcus aureus* could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile- impetigo and wound. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature. It also require to research on phytochemical and pharmacological aspect. However research going on it would be easier to develop new formulation.

Compliance with ethical standards

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