ISSN 1996-3351

Asian Journal of Biological Sciences

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OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.583.588

Research Article
Formulation and Evaluation of Hydrophilic Ointments Containing Dry Methanolic Extract of *Acacia nilotica* Pods

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Abstract

Background and Objective: *Acacia nilotica* is a species of nilotica in the Fabaceae family. Phytoconstituents in the pods extract may be responsible for the antimicrobial activity of the plant. The purpose of this study was to formulate and evaluate hydrophilic ointment of Pods extract microbiologically. **Materials and Methods:** Medicated Ointment was formulated as 5% dry methanolic extract of 50 g of powdered material of *Acacia nilotica* pod, hydrophilic bases were used. Commercially available Fucidin ointment was used as a standard for this study. Six formulae were prepared and evaluated for various pharmaceutical parameters such as rheological properties, stability, pH and external characters as well as anti-microbial property against different pathogenic micro-organism. Inhibition zone (in mm) of different ointments was also measured. Two-way ANOVA was used to analyze the obtained data. **Results:** Each of ointment mixture of extract and citric acid and ointment of extract alone were better than each of ointments of citric acid, standard fucidin ointment and non-medicated one. **Conclusion:** *Acacia nilotica* pods have satisfactory results pharmaceutically as well as biologically and promote good antimicrobials properties.

Key words: Extract, herbal, fucidin, *Acacia nilotica* pods, citric acid

Received: December 14, 2018 Accepted: February 01, 2019 Published: June 15, 2019

Citation: Mohammed Khalil, Elnazeer I. Hamedelniel, Mustafa H. Mustafa and Abdelmonem M. Abdellah, 2019. Formulation and Evaluation of hydrophilic ointments containing dry methanolic extract of *Acacia nilotica* pods. Asian J. Biol. Sci., 12: 583-588.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acacia nilotica is a proverbial, medium sized tree and is broadly scattered in most part of Sudan. It has an inspiring range of medicinal uses with potential anti-oxidant activity. This plant contributes a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes. *A. nilotica* is a medicinal plant acknowledged to be rich in phenolics consisting of condensed tannin.

In most countries of Africa and Asia, herbal medicine is the most commonly used alternative wound treatments. These ethnomedicines are affordable and rarely cause hypersensitive reactions[1-2]. They also encourage blood clotting, fight infection and accelerate tissue regeneration[3]. Because of their long history of use in the treatment of different human diseases, most of the herbal remedies are believed to be safer and effective than synthetic drugs. According to WHO[4], herbal medicines serve the health needs of about 80% of the world population.

Wound is a physical trauma which occurs when the integrity of any tissue is compromised[5]. Wound healing is a dynamic physiological process that involves a series of phases and mediates through the interaction of a complex cascade of a cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in phases including inflammation, wound contraction, re-epithelialization, tissue remodeling and formation of granulation tissue with angiogenesis. If the phases of wound healing do not proceed in this way, healing may progress inappropriately to a chronic wound[6]. The main goals of researches in wound healing are to evaluate the influence of various measures in wound management programs on healing and to screen drugs that encourage healing process efficiently[7].

Several medicinal plants have been used since time immemorial for treatment of wounds and showed promising effects[8]. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body. These constituents include various phytochemical groups like alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and other various phenolic compounds[9]. In Sudan, folk medicine, the use of the pods of *Acacia nilotica* is well-known to be effective against a variety of bacterial diseases. Therefore, this study is conducted to formulate and evaluate hydrophilic ointment of pods extract microbiologically.

MATERIALS AND METHODS

Plant collection and identification: The pods of *Acacia nilotica* are collected from Shendi and authenticated by the taxonomist in the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research, Khartoum, Sudan. The plant pods were collected, washed three times using distilled water, dried on hot air oven at 40°C for 24 h and ground into fine powder.

Preparation of crude extract: About 50 g of powdered material of plant was taken in flat-bottomed glass container and soaked in 200 mL of methanol. The container with its content was sealed and kept for a period of 7 days with occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate (methanolic extract of the pods) was evaporated to dryness by Rotavap under reduced pressure about 70 mmHg and low temperature (40°C). The percentage yield of methanol extract was then calculated, following the equation:

$$\text{Yield (\%)} = \frac{\text{Weight of dry extract}}{\text{Weight of ground plant material}} \times 100$$

Determination of phytochemical constituents: Preliminary phytochemical analysis was undertaken using standard qualitative methods[10]. The different crude extracts obtained were qualitatively tested for the presence of various phytochemicals constituents using standard protocols[11].

Ointment formulation: Four types hydrophilic ointment were formulated and compared by Fucidin ointment Company Astra Zeneca Pharmaceuticals. A mixture of 1% sodium lauryl sulphate, 12% propylene glycol, 25% stearyl alcohol, 25% white petrolatum and 37% purified water was prepared. Stearyl alcohol and white petrolatum were melted together at about 75°C to form oleaginous phase. The other agents including extracts were dissolved in purified water and heated at the same temperature. Then, the oleaginous phase was added to the aqueous phase with continuous stirring until the two phases were mixed properly. Sodium lauryl sulphate acts as emulsifying agent while stearyl alcohol and white petrolatum, comprising the oleaginous phase of emulsion and the other ingredients in aqueous phase[12].

Evaluation of ointments: Preliminary evaluation of formulations was carried out as follows:

Colour and odour: Color and odour of the prepared ointments were visually examined[13].

pH: The pH of various formulations was determined by using digital pH meter. About 1 g of ointment was dissolved in 100 mL of distilled water and stored for 2 h. The measurement of pH of each formulation was done[13] after 2 h.

Uniformity of weight: A total of 10 collapsible tubes were selected randomly and weighed, ointments were removed from each collapsible tube and each empty collapsible tube was washed with methanol. The collapsible tubes were dried and their weight was taken. The different between two weights was calculated as net weight of the ointment of 10 collapsible tube was noted:

$$\text{Average collapsible tube content} = \frac{\text{Total content of collapsible tubes}}{\text{No. of collapsible tubes}}$$

Viscosity: The viscosity was determined by Brookfield viscometer. Test sample was taken in a clean and dry 250 mL beaker and the viscosity of the test sample was determined by standard operating procedure using spindle[14] nos 1-4.

Stability study: All the developed formulations were subjected to accelerated stability testing for about 5 weeks. Room temperatures were maintained as per ICH guidelines 1993. The parameter of formulation such as color, skin irritation and viscosity were determined for all formulae.

Anti-bacterial activity of ointments: The antibacterial activity of ointment was evaluated using well diffusion pour plate method

- About 1 g of each ointment was weighed and placed in 5 mL test tube which racked and transferred to water bath at 50°C for melting to easily transfer by micropipette. Using micro pipette, 0.05 mL of melted ointment was transferred to well plate agar surface
- Each ointments sample was put in covered plates, labeled, with organism named and dated
- Plates were inverted and incubated at 35-37°C for 24 h
- Antibacterial activity was checked by measuring the inhibition zone if present
- Experiments were repeated three times and the average was obtained
- Two-way ANOVA was used to analyze the obtained data

RESULTS AND DISCUSSION

Homogeneity and colours: Table 1 showed different formulae of ointments prepared by this study. Ointment formed of extract and citric acid (OEC), ointment with extract only (OE), ointment with citric acid 10% only (OC1), ointment with citric acid 5%, (OC2) placebo ointment (OP). All of these formulae of ointments were homogenous, smooth, opaque and non-greasy. Both OEC and OE ointments were brown while each OC1, OC2 and OP were white.

The percentage yield of methanolic extract was 28.6%.

Weight uniformity: Table 2 showed the uniformity of weight. The obtained results were within the range accordingly to official monograph.

pH measurements: Table 3 showed the measurements of ointments. The pH of all formulae range from 5.68-8.56 except those contained citric which is less 5.00 weak acidic.

Tests of viscosity: Measurements of viscosity are shown in Table 4. Slightly change were observed in ointments during storage for 90 days.

Anti-bacterial activity of ointments: The plates were removed from the incubator, a metric ruler was used, to measure the zone of inhibition which represents the distance between the edge of the wall and edge of bacterial growth, at the widest point (Table 5, Fig.1) showed the results zone inhibition of prepared ointments and Sodium fucidate against different pathogenic bacteria.

Screening of different concentrations of medicated ointment (extract) for the anti-bacterial activity against selected test bacteria showed good inhibition zones. The ointment has the potential to make inhibition zone against *Staphylococcus aureus* (IZ = 23 mm), *Pseudomonas aeruginosa* (IZ = 20 mm), *Escherichia coli* (IZ = 20 mm), (IZ = 18 mm), *Salmonella typhi* B (IZ = 15 mm), *Salmonella paratyphi* B (IZ = 15 mm), *Proteus mirabilis* (IZ = 10 mm) and in anti-fungal activity the inhibition zone against *Candida albicans* is 15 mm. These results showed that the given test ointment has maximum activity against *Staphylococcus aureus* and minimum against *Proteus mirabilis*.

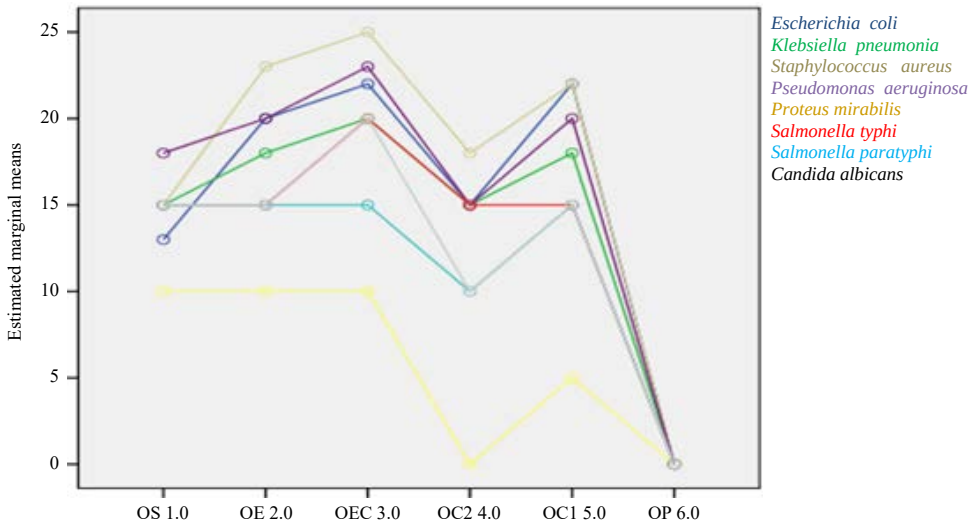

Fig. 1: Anti-microbial potentials of different ointments against selected micro-organisms in terms of inhibition zone (mm)

Table 1: Homogeneity of ointments formulae

Formulation (%)	Color	Homogeneity	Appearance
OEC	Brown	Homogenous	Smooth opaque and non-greasy
OE	Brown	Homogenous	Smooth opaque and non-greasy
OC1	White	Homogenous	Smooth opaque and non-greasy
OC2	White	Homogenous	Smooth opaque and non-greasy
OP	White	Homogenous	Smooth opaque and non-greasy

OEC: Ointment with extract and citric acid, OE: Ointment with extract only, OC1: Ointment with citric acid 10% only, OC2: Ointment with citric acid 5% only, OP: Placebo ointment

Table 2: Uniformity of weight

Bottle number	Bottle 96-103%	√ or x content (mg)
1	1564101.7	√
2	1497	97.3√
3	1536	99.8√
4	1587	103.2√
5	1520	98.8√
6	1545	100.4√
7	1567	101.9√
8	1479	96.0√
9	1505	97.8√
10	1583	102.9√
Average	1538.5	

Table 3: pH of ointments on storage

Formulation	Temperature	Time (day)				
(%)	(C°)	Initial	14	21	28	35
OEC	25	7.2	6.95	6.78	6.57	6.48
OE	25	8.56	8.43	8.03	7.79	7.05
OC1	25	5.46	5.09	4.65	4.49	4.05
OC2	25	6.84	6.65	6.49	6.22	5.68
OP	25	7.46	7.33	7.23	7.04	6.87

OEC: Ointment with extract and citric acid, OE: Ointment with extract only, OC1: Ointment with citric acid 10% only, OC2: Ointment with citric acid 5% only, OP: Placebo ointment

Table 4: Viscosity of ointments formulated with *Acacia nilotica*

Formulation	Temperature	Time (day)	
(%)	(C°)	Initial	90 days (cp)
OEC	25	94.5	94.1
OE	25	93.5	93.2
OC1	25	85.4	85.0
OC2	25	81.2	80.0
OP	25	81.1	80.1

OEC: Ointment with extract and citric acid, OE: Ointment with extract only, OC1: Ointment with citric acid 10% only, OC2: Ointment with citric acid 5% only, OP: placebo ointment

Screening of medicated ointment (extract+citric acid) for the anti-bacterial activity against selected test bacteria showed very good inhibition zone. The ointment extract has the potential to make inhibition zone against *Staphylococcus aureus* (IZ = 25 mm), *Pseudomonas aeruginosa* (IZ = 23 mm), *Escherichia coli* (IZ = 22 mm), *Klebsiella pneumonia* (IZ = 20 mm), *Salmonella typhi* B (IZ = 20 mm) *Salmonella paratyphi* B (IZ = 15 mm), *Proteus mirabilis* (IZ = 10 mm) and in anti-fungal activity the inhibition zone against *Candida albicans* is (IZ = 20 mm). These results showed that the given test ointment have maximum activity against *Staphylococcus aurous* and minimum activity against *Proteus mirabilis*.

Screening of ointment (citric acid 10%) for the anti-bacterial activity against selected test bacteria showed good inhibition zone. The ointment have the potential to make inhibition zone against *Staphylococcus aureus*

Table 5: Anti-bacterial activity of different ointments and sodium fucidate as standard against the same pathogens where used

	Inhibition zone (mm)					
Name of pathogen	OS	OE	OEC	OC2	OC1	{6} OP
Escherichia coli	13	20	22	15	22	0
Klebsiella pneumonia	15	18	20	15	18	0
Staphylococcus aureus	15	23	25	18	22	0
Pseudomonas aerogenosa	18	20	23	15	20	0
Proteus mirabilis	10	10	10	R	5	0
Salmonella typhi B	15	15	20	15	15	0
Salmonella paratyphi B	15	15	15	10	15	0
Candida albicans	15	15	20	10	15	0

OS: Standard ointment, OEC: Ointment with extract and citric acid, OE: Ointment with extract only, OC1: Ointment with citric acid 10% only, OC2: Ointment with citric acid 4% only, OP: Placebo ointment

(IZ = 22 mm), *Pseudomonas aeruginosa* (IZ = 20 mm), *Escherichia coli* (IZ = 22 mm), *Klebsiella pneumonia* (IZ = 18 mm), *Salmonella typhi* B (IZ = 15 mm) *Salmonella paratyphi* B (IZ = 15 mm), *Proteus mirabilis* (IZ = 5 mm) and in anti-fungal activity the inhibition zone against *Candida albicans* is 15 mm. These results showed that the given test ointment has maximum activity against *Staphylococcus aureus*, *Escherichia coli* and minimum activity against *Candida albicans*.

Screening of ointment (citric acid 5%) for the anti-bacterial activity against selected test bacteria showed mild inhibition zone. The ointment has the potential to make inhibition zone against *Staphylococcus aureus* (IZ = 18 mm), *Pseudomonas aeruginosa* (IZ = 15 mm), *Escherichia coli* (IZ = 15 mm), *Klebsiella pneumonia* (IZ = 15 mm), *Salmonella typhi* B (IZ = 15 mm) *Salmonella paratyphi* B (IZ = 10 mm), *Proteus mirabilis* (R) and in anti-fungal activity the inhibition zone against *Candida albicans* is 10 mm. These results showed that the given test ointment has maximum activity against *Staphylococcus aureus* and minimum against *Proteus mirabilis*.

The anti-microbial activity as well as pharmaceutical stability of medicated ointment (extract+citric acid) is more than medicated ointment (extract only) because citrate enhances the antimicrobial properties of polyphenols of plant origin[15]. Also citric acid is reported to function in cosmetics as a chelating agent, pH adjuster or fragrance ingredient skin conditioning agent and buffering agent[16].

Use of ethno-pharmacological knows ledge is one attractive way to reduce empiricism and enhance the probability of success in new drug-finding efforts[17]. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immune suppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[18].

In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. The present results therefore offer a scientific basis for traditional use of medicated hydrophilic ointment (Extract+citric acid) and medicated hydrophilic ointment (Extract).

CONCLUSION AND RECOMMENDATIONS

These results explain that the wide spread of Medicinal Plants in Sudan have potential as antimicrobial against *Staphylococcus aureus*, *Pseudomonas aeruginosa Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* B, *Salmonella paratyphi* B, *Proteus mirabilis* and *Candida albicans*.

Results clearly showed that medicated ointment formulated of dry methanolic extract of *Acacia nilotica* pods has satisfactory results pharmaceutically as well as biologically and promotes good anti-microbials properties compared to each of control, citric acid alone and non-medicated ointments. More investigation of *Acacia nilotica* pods as anti-microbial treatment is of paramount recommended.

SIGNIFICANCE STATEMENT

This study contributes to the existing literature by drawing the attention to the pharmaceutically and biologically use of *Acacia nilotica* pods extract against different strains of bacteria caused wound infection. Moreover, this study contributes to the current body of knowledge pertaining to herbal medicine as alternative wound treatments.

ACKNOWLEDGMENT

The authors acknowledge the assistance of all those who contributed to this study.

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