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Formulation and Evaluation of Antimicrobial Topical Ointment from Acacia Nilotica (L.,) Extract

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Abstract: The use of topical antimicrobial agents for management of inconsequential skin infections is a clinical strategy that is commonly accomplished in the majorcommunities.The aim of present study was to formulate & evaluate herbal ointment of Acacia nilotica(L.) to treat recurrent microbial skin infections. Herbal ointments offer several advantages over other conventional ointments. Method used to prepare herbal ointment was very modest. Firstly, oil phase was prepared, the mixture of stearyl alcohol and white petroleum were melted together at about 75℃. Secondly, aqueous phase was prepared, mixture of methanolic and aqueous extract of Acacia nilotica(L.), sodium lauryl sulphate, propylene glycol and purified water heated at 75℃. The oil phase was added to the aqueous phase with continuous stirring.The prepared herbal ointment was evaluated through physical parameters such as pH, homogeneity, appearance, color andspreadability. The herbal formulation showed good consistency, good spreadability, homogeneity, pH, washability, easy to apply, non-irritant, non-greasy and no coarse particles found and no evidence of phase separation. The prepared batch formulation was evaluated for antimicrobial activity and shown promising results with a new hope in Indian Market.

Keywords: Acacia nilotica(L.) bark, methanolic extract, water extract, phytochemical screening, ointment, anti-bacterial activity

1. INTRODUCTION

Medicinal plants and products obtained therefrom have served as sources of relief for ailments, promoting healing and maintaining good health for as long as time and existence itself. Plant compounds like other natural products are increasingly being used as non-toxic and potent substitutes for synthetically manufactured products (Banso, 2009). The plant Acacia nilotica(L.)has received a lot of recognition because of its ethno-medicinal claims, some of which have been justified by scientific studies.

Plants and plants extract used for medicinal purposes and play an important role in developing countries since; they are inexpensive, effective and have a natural origin. In traditional medicine, Acacia niloticais widely used. Almost all their parts used in medication including root, bark, leaves, flower, gum, and pods (Said, 1997). This plant has anti-microbial, anticancer, anti-mutagenic and antioxidant activity and used for treatment of against cold, cough, diarrhea, tuberculosis, piles, hepatitis C virus, burns, and scalds(Rasha, 2018).

Acacia first described in 1773 by the Swedish botanist Carl Linnaeus. Acacia is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae or Leguminosae(MUZ.N, 2014).

Traditional uses of Acacia nilotica: Acacia nilotica is used in many cultures to treat bronchitis, chest pain, colds, diarrhea, dysentery, fever, hemorrhages, leprosy, eye disorders, pneumonia, sore throat (Chhabra, 1991),syphilis (Watt, 1962; Kambizi, 2001) oral thrush, fungal skin infections (Lev, 2002;

Srinivasan, 2001), malaria and toothache (Jain, 2005; Kubmarawa, 2007). The ancient Egyptians used it as a dewormed against internal bleeding, diarrhea and dermatological problems (Ndiaye, 2016). In Africa, it is used as a hemostatic, healing ulcers, calming coughs (Vassal, 1998), anti-diarrheal and anti-dysenteric infants, in mouth ulcers and gingivitis and also against eye inflammation(Guinko, 1997). Several species of Acacia are often used as reserve fodder in arid areas and for his forage value (Benbrahim, 2014).In veterinary breeders used A.nilotica to treat foot and mouth disease syndrome (Houndje, 2016). It is also used, mixed with sodium bicarbonate, in racehorses suffering from tendinitis.

2. MATERIAL AND METHODS

Plant drug collection and authentication: The barks of Acacianiloticawere collected from local area of village Mota-Goniyasar, Tal:Mandvi- Kutch, Gujarat, India and authenticated by Dr.Pankaj N. Joshi, Program Director, Biodiversity Conservation Sahjeevan, Bhuj- Kachchh. Drugs were collected, washed using distilled water, dried for 24 h and ground into fine powder and used for research study.

Drug and chemicals: The chemicals were procured from Pharmacy laboratories of Veerayatan Institute of Pharmacy, Jakahnia, BhujMandvi Road, Mandvi- Kutch,Gujarat, India. Tetracycline, sodium lauryl sulphate, propylene glycol, stearyl alcohol, white petroleum was used in the research study.

Preparation of Methanolic Extract of Crude Drug: About 50 g of powdered bark 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 was evaporated to dryness by hot plate under low temperature (45^0C). The percentage yield of methanol extract was then calculated.

Fig 1: Filtration of extract

Fig 2: Evaporation of extracts

Preparation of Water Extract of Crude Drug (Mohammed, 2019): About 50 g of powdered bark material of plant was taken in flat-bottomed glass container and soaked in 200 mL of water. 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 was evaporated to dryness by hot plate under low temperature (45^0C). The percentage yield of methanol extract was then calculated.

Preliminary phytochemical screening (Khandelwal, 2008): Dried methanol and water extracts were subjected to various chemical tests to detect the presence of various phytoconstituents like carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids and saponins.

Detection of Carbohydrates

1. Molish test–To 2-3 ml extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids Indicates the test as positive.
2. Fehling's test – Mix 1ml Fehling's A and 1ml Fehling's B solutions, boil for one minute. Add equal volume of test solution. Heat it in boiling water bath for 5-10 min. First a yellow, then brick red precipitates is observed Indicates the test as positive.
3. Benedict's test – Mix the equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.
4. Barfoed's test – Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min in boiling water bath and cool it. Red precipitateswere observed indicates the test as positive.

Detection of Alkaloids

1. Dragendorff's test – To 2-3 ml filtrate, add few drops Dragendorff's reagent. Orange brown precipitates were formed indicates the test as positive.
2. Mayer's test – 2-3 ml filtrate with few drops Mayer's reagent gives precipitates is indicates the test as positive.
3. Hager's test – 2-3 ml filtrate with Hager's reagent gives yellow precipitates is indicates the test as positive.
4. Wagner's test – 2-3 ml filtrate with few drops Wagner's reagent gives reddish brown precipitates is indicates the test as positive.

Detection of Tannins and Phenols

1. 5% $FeCl_3$Solution – To 2-3 ml of aqueous and methanolic extract, add few drops of 5% $FeCl_3$ solution, deep blue- black color indicates the test as positive.
2. Lead acetate solution – To 2-3 ml of aqueous and methanolic extract, add few drops of Lead acetate solution, white precipitates observed indicates the test as positive.
3. Gelatin Solution – To 2-3 ml of aqueous and methanolic extract, add few drops of gelatin solution, white precipitates observed indicates the test as positive.
4. Bromine water – To 2-3 ml of aqueous and methanolic extract, add few drops of bromine water, decoloration of bromine water indicates the test as positive.
5. Acetic acid solution – To 2-3 ml of aqueous and methanolic extract, add few drops of acetic acid solution, red color solution indicates the test as positive.
6. Potassium dichromate – To 2-3 ml of aqueous and methanolic extract, add few drops of potassium dichromate, red precipitates indicate the test as positive.
7. Dilute iodine solution – To 2-3 ml of aqueous and methanolic extract, add few drops of dilute iodine solution, transient red color indicates the test as positive.
8. Dilute HNO_3 – To 2-3 ml of aqueous and methanolic extract, add few drops of dilute HNO_3 solution, reddish to yellow color indicates the test as positive.
9. Dil.Potassium permanganate solution – To 2-3 ml of aqueous and methanolic extract, add few drops of dil. Potassium permanganate solution, decoloration indicates the test as positive.

Detection of Saponin Glycosides

Foam test – Shake the dry powder vigorously with water. Persistent foam observed indicates the test as positive.

Detection of Glycosides

Borntrager'stest for anthraquinones glycosides– To 3 ml extract, add dil.H2SO4. Boil and filter it. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red indicates the test as positive.

Detection of Flavonoids

NaOH test– Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration, which was decolorized after addition of acid.

Preparation of Ointment
The Methanolic ointment was prepared according to formula:

Ingredient	Quantity to prepare 20g
Sodium lauryl sulphate	0.2 gm/ml
Propylene glycol	2.5 gm/ml
Stearyl alcohol	5 gm/ml
White petroleum	5 gm/ml
Extract (methanolic)	1 gm/ml
Purified water	6.3 gm/ml

Stearyl alcohol and white petroleum were melted together at about 750C to form oleaginous phase. The other agents including extracts (methanolic) 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 act as emulsifying agent with stearyl alcohol and white petroleum comprising the oleaginous phase of emulsion and other ingredients,as shown in aqueous phase (Sumitra, 2016).

Figure 3: Methanolic extract containing Ointment

The herbal ointment (20 gm) was prepared according to formula mentioned in below table-1:

Ingredient	**Quantity (gm)**
Sodium lauryl sulphate	0.2
Propylene glycol	2.5
Stearyl alcohol	5
White petroleum	5
Herbal Extract (Water/ Methanol)	1
Purified water	6.3

Table: 1 Formulation of Herbal ointment

Stearyl alcohol and white petroleum were melted together at about 75^0C to form oleaginous phase. The other agents including extracts (water) 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 sulphateis act as an emulsifying agent with stearyl alcohol and white petroleum, comprising the oleaginous phase of emulsion and the other ingredients in aqueous phase (Sumitra, 2016).

Evaluation of herbal ointment

Physicochemical parameters: The formulation was evaluated for its color and odour visually examined(Rajashree, 2012)
Consistency determination: Smooth and no greediness were observed (Aravinda, 2017).
pH determination: pH of prepared herbal ointment was measured by using pH paper.
Solubility testing: Soluble was determined in water, alcohol and chloroform (Aravinda, 2017).

Washability: Formulation was applied on the skin and then ease extend of washing with water was checked (Aravinda, 2017).
Non irritancy Test: Prepared herbal ointment was applied to the skin of human being and observed for the effect (Aravinda, 2017).
Spreadbility: The spreadability is a very important factor of any semi-solid formulation to determine the covered area per specific weight of the formula. The spreadability of ointment was evaluated by measuring the spreading area of the ointment(1 g) between two glass plates (20 cm × 20 cm) after 1 minute of applying standard weight of 50 g (Suliman, 2020).

Antimicrobial activity (Mounyr, 2016): Disc diffusion method: The in-vitro antimicrobial activity was carried out using the disc diffusion method. The diffusion technique is suitable for testing aqueous suspensions of plant extracts, since the presence of suspended particles in the sample being tested is less likely to interfere with the diffusion of the antimicrobial substance into the agar than other methods, such as the pour plate method and cylinder plate systems.
Take 500 ml of beaker and add about 5 g of Nutrient Agar (NA) is dissolve in 100 ml of purified water and then boil on the burner until the clear solution appear and poured into the plate; and allowed to harden. The surface of the NA plate was then be inoculated with a sterile swab of the selected microorganism namely Escherichia coli. The disc containing anti-microbial agent namely tetracycline(20mg/ml) were used as controls. Discs were loaded with 2.5%, 5%, 7.5%, 10% of various concentrations of methanolic and water extract solutions of acacia bark and arranged on the surface of the inoculated plates in such a way so as to be at least 20 mm apart and incubated at 350C-370C for 24 hours. After 24 hours, zone of inhibition was checked to determine the antimicrobial activity of prepared ointment.

Figure 4: Various concentration of methanolic extract and tetracycline

3. RESULTS AND DISCUSSION

The plant drug was successfully authenticated and used in research study. The water and methanolic extract from bark of A. nilotica was obtained in 4%w/w and 10%w/w respectively. The preliminary phytochemical analysis of these extracts was carried out for the purpose of detection of presence of important class of phytoconstituents like alkaloids, tannins, saponins, flavonoids, glycosides; carbohydrates are being depicted in table 1.

Table 2: Phytochemical Analysis of methanolic and water extracts of ointment

Sr. no.	Phytoconstituents		Methanolic Extract	Water Extract
1	Carbohydrates			
	A.	Molish's test	-	+
	B.	Fehling's test	-	-
	C.	Benedict's test	-	-

	D.	Barfoed's test	-	-
2	Alkaloids			
	A.	Dragendorff's test	+	-
	B.	Mayer's test	+	-
	C.	Hager's test	-	-
	D.	Wagner's test	+	-
3	Tannins & Phenols			
	A.	5 % FeCl3 solution	+	+
	B.	Lead acetate solution	+	+
	C.	Gelatin solution	+	+
	D.	Bromine water	+	+
	E.	Acetic acid solution	+	+
	F.	Potassium dichromate	+	+
	G.	Dilute Iodine solution	+	+
	H.	Dilute HNO3	+	+
	I.	Dil. Potassium permanganate solution	+	+
4	Anthraquinone Glycosides			
	A.	Borntrager's test	+	-
5	Saponin Glycosides			
	A.	Foam test	+	+
6	Flavonoids			
	A.	NaOH test	+	+

+= Present; - = absent

Table 3: Evaluation Parameters.

Evaluation parameters	**Methanolicextract containing ointment**	**Water extract containing ointment**
Color	Brown	Brown
Odour	Characteristic	Characteristic
Consistency	Smooth	Smooth
pH	7.2	5.5
Solubility	Soluble in water, alcohol and chloroform.	Soluble in water, alcohol and chloroform.
Washability	Good	Good
Non irritancy	Non irritant	Non irritant

Spreadability: The spreadability was determined by measuring average diameter of covered area formethanolicextract containing ointment and water extract containing ointment and it was found 2.5cm and 3.2cm respectively.

Antimicrobial activity:

Table: 4. Anti-microbial activity of methanol & water extracts from Acacianilotica bark

Microorganism used	Inhibition zone (mm) Methanolic extract (Conc.)				Inhibition zone (mm) Water extract (Conc.)			
	2.5%	5%	7.5%	10%	2.5%	5%	7.5%	10%
Staphylococcus aureus	5.6	10.8	20.6	24.4	2.6	5	6.5	6.6
Listeria monocytogenes	4.2	8.1	13.4	20.5	2.2	4.2	5.8	5.8
Bacillus subtilis	4.8	9.5	15.1	23.4	2.1	4.4	5.7	5.9
Clostridium perfringenes	4.5	8.8	15.3	22.8	2.3	4.1	6.2	6.2
Escherichia coli	6	13	18	20	1.6	2.4	4	4.1

Tetracycline (20 mg/ml) was used as Positive control and zone of inhibition was found 26 mm.

4. CONCLUSION

The herbal ointment showed acceptable physical properties and were compatible with the skin properties. The results of this study revealed the presence of saponins, flavonoids, alkaloids and tannins in methanol bark extract of A.nilotica. Thus, extract could be used as sources of agents for the treatment of infections caused by the tested microorganisms. Hence, formulation was safe and efficient carriers, with potent antimicrobial activity.

Future Scope

Conflict of Interest: Authors have declared that no competinginterests exist.

5. REFERENCES

1. AravindaNalla, Krishna Mohan Chinnala (2017). Formulation and evaluation of herbal ointment for antimicrobial activity. World journal of pharmaceutical and medical research. 3(7), 113-117.
2. Banso A (2009). Phytochemical and antibacterial investigation of bark extract of Acacia nilotica. J Med Plants Res.; 3(2): 82-85.
3. Benbrahim KF, Berrada H, El Ghachtouli N, Ismaili M. (2014). Acacia: Promising Nitrigen fixing trees for sustainable development in arid and semi-arid areas. International Journal of innovation and Applied studies; 8:46.
4. Chhabra SC, Uiso FC (1991). Antibacterial activity of some Tanzanian plants used in traditional medicine. Fitoterapia; 62:499-503.
5. Guinko S (1997). Role des Acacias dans le developpement rural au Burkina Faso et au Niger, Afrique de l'Ouesr. L'hommeet Le Milieu Vegetal Dans Le Bassin Du Lac Tchad ORSTOM, Paris, 35-51.
6. Houndje EMB, Ogni CA, Noudeke N, Farougou S, Youssao AKI, Kpodekon TM (2016). Recettesethano-veterinaire a base de plantesmedicinalesutilisees pour le trattement de la fievreaphteuse au Benin. International Journal of Biological and Chemical Sciences; 10:2090-2107.
7. Jain A, Katewa SS, Galav PK, Sharma P (2005). Medicinal plant diversity of sitamara wildlife sanctuary, Rajasthan, India. Journal of Ethanopharmacology; 102:143-157.
8. Kambizi L, Afolayan AJ (2001). An ethanobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. Journal of Ethanopharnacology, 77:5-9
9. Khandelwal K.R (2008). Practical Pharmacognosy techniques and experiments. Niraliprakashan, 149-153.

10. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology; 6:1690-1696.
11. Lev E, Amar Z (2002). Ethanopharmacological survey of traditional drugs sold in the Kingdom of Jordan. Journal of Ethanopharmacology; 82:131-145.
12. Khalil M, Hamedelniel, Mustafa MH and Abdel-lahAM (2019). Formulation and Evaluation of Hydrophilic Ointments Containing Dry Methanolic Extract ofAcacia nilotica Pods. Asian Journal of Biological sciences, 12: 583-588.
13. MounyrBalouiri, MoulaySadiki, SaadKoraichiIbnsouda (2016). J Pharm Anal.; 6(2): 71–79.
14. MUZ.N Farzana, I. Al Tharique, Arshiya sultana (2014). A review of ethnomedicine, phytochemical and pharmacological activities of Acacia nilotica (Linn) willd, Journal of Pharmacognosy and phytochemistry, 3 (1), 84-90.
15. Ndiaye A (2016). Contribution a l'etudepharmacologique de deuxplantesmedicinales de la pharmacopeetraditionnellesenegakaise: Moringaoleifeira Lam. Et Acacia nilotica L. Delile, These de Doctorat en Pharmacologie, UniversiteCheikh Anta Diop de Dakar, 140.
16. Rajashree, P.H., V. Vishwanad, M. Cherian, J. Eldhose and R. Singh (2012). Formulation and evaluation of antiseptic polyherbal ointment. Int. J. Pharm. Life Sci., 3: 2021-2031.
17. RashaJame (2018). Phytochemical and pharmacological uses of Acacia nilotica. International journal of Bioorganic Chemistry. Vol. 3(2), 6-10.
18. Said HM (1997). Hamdard pharmacopeia of Eastern Medicine. Ed 2, New Delhi srisatguru publications, 353.
19. Srinivasan D, Nathan S, Suresh T, Perumalsamy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of ethanopharmacology; 74:217-220.
20. Suliman RS, Ali H, Alamer A, Aleid N, Abu-jafal A, Abdulgadir R, Omer M (2020). In vitro Anti-microbial activity and wound healing evaluation of Acacia gum Arabia Aqueous cream. Natural Products Chemistry & Research.8:369. DOI: 10.35248/2329-6836.20.8.369.
21. Sumitra Singh, Nidhi Sharma (2014). Evaluation of wound healing activity of Acacia Auriculiformis A. Cunn. Stem Bark. Asian journal of pharmaceutical and clinical research.Vol 7, Issue 2, ISSN- 0974-2441.
22. Vassal J (1998). Les acacias au Senegal: taxonomie, ecologie, principaux interest, p. 15-33.
23. Watt JM, Breyer-Brandwijk MG (1962). The medicinal and poisonous plants of southern and eastern Africa Livingstone Ltd., Edinburgh, 546.