



Formulation and *in-vitro* Evaluation of Topical Antimicrobial Preparation

Ganesh Bharskar*, Suhas Siddheshwar

Department of Pharmacy, Pravara Rural College of Pharmacy, Pravaranagar, India.

*Corresponding author's E-mail: ganeshb7748@gmail.com

Received: 09-07-2022; Revised: 24-09-2022; Accepted: 30-09-2022; Published on: 15-10-2022.

ABSTRACT

Due to the fact that medicinal plants are the most abundant source of the bioactive compounds utilised in both traditional and modern medicine, plant-derived compounds and herbal medicines have recently received significant interest due to their wide range of applications. The primary objectives of this study are to create an herbal ointment with antibacterial activity using *Adansonia digitata* and *Ocimum sanctum* extracts. With the aid of *A. digitata* leaf extract and *Ocimum sanctum* leaf extract, it is designed as an herbal ointment in this research study. Following formulation, the quality of the ointment was evaluated based on its irritancy, spreadability, consistency of the content, and stability. By employing the Agar cup plate method in an *in vitro* study, the antibacterial efficacy of herbal ointments containing extracts from *Adansonia digitata* and *Ocimum sanctum* against bacteria like *Staphylococcus aureus* was determined. The findings of the zone of inhibition provided by the various extract ratios in ointment on *Staphylococcus aureus* were then compared to determine the most efficient combination. The goal of the current study is to formulate an herbal ointment and evaluate it utilising *Adansonia digitata* and *Ocimum sanctum* extracts.

Keywords: *Adansonia digitata*, Herbal, *Ocimum sanctum*, Ointment, *Staphylococcus aureus*, UV-Spectroscopy.

QUICK RESPONSE CODE →

DOI:

10.47583/ijpsrr.2022.v76i02.004



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2022.v76i02.004>

INTRODUCTION

Herbal products are largely preferred to synthetic drugs due to their widespread availability as well as the vast empirical and accessible data regarding to their traditional use. However, modern scientific methods should be applied to validate the claims about the therapeutic effects of the plants, resulting in confirmation the traditional system of medicine.¹ Along with other dosage forms, herbal drugs are also formulated in the form of ointment. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base.² *Adansonia digitata* is a native deciduous tree of African savannas belongs to Bombacaceae family, the bombax or kapok family. It is used in the treatment of bronchial asthma, dermatitis, sickle cell anemia, diuretic, anti-diabetic, diarrhoea, dysentery, laxative, hiccough in children, anti-oxidant, anti-inflammatory, antidote for poison, anti-trypanosome uses.³

Tulsi is an aromatic shrub in the basil family Lamiaceae (tribe ocimeae) that is thought to have originated in north central India and now grows native throughout the eastern world tropics.⁴ The medicinal properties of tulsi have been studied in hundreds of scientific studies including *in vitro*, animal and human experiments. These studies reveal that Tulsi has a unique combination of actions that include:

Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), mosquito repellent, anti-diarrheal, anti-oxidant, anti-cataract, anti-inflammatory, chemo preventive, radioprotective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic, analgesic, anti-pyretic, anti-allergic, immunomodulatory, central nervous system depressant, memory enhancement, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritis, adaptogenic, anti-stress, anti-cataract, anti-leukodermal and anti-coagulant activities.^{5,6}

MATERIALS AND METHODS

Collection and authentication of Plant material

Leaves of *Adansonia digitata* Linn. (Family: Malvaceae) were collected from Medicinal Garden, Pravara Rural College of Pharmacy, Pravaranagar. The plant was authenticated by Department of Botany and Research centre, PVP College Loni with reference number PVPC/Bot/2021-22/121-1.

The ethanolic extracts of *Ocimum sanctum* were collected from the Amsar Private Ltd., Indore, India.

Preparation of *A. digitata* leaves extract

The *Adansonia digitata* leaves were dried under shed and grinded into fine powder, using pestle and mortar. Then a 100g of the grinded powder was dissolved in 400 mL ethanol (70%), and incubated for 48 hours at room temperature. The extract was then filtered using maceration Method, and the supernatant was then boiled



to evaporation. Finally, ethanolic extract was collected and concentrated. The extract was stored in the airtight container at cool and dark place.

Formulation of Ointment

Formulation of ointment base:

Table 1: Formula for ointment base

Sr. No.	Name of Ingredient	Quantity to be taken
1.	Wool fat	0.5g
2.	Hard paraffin	0.5g
3.	Cetostearyl alcohol	0.5g
4.	White soft paraffin	8.5g

Formulation of Herbal ointment:

Table 2: Formula for Herbal ointment

Formulation code	Prepared <i>A. digitata</i> leaves extract (g)	<i>Ocimum sanctum</i> Leaves extracts (g)	Ointment base q.s. (g)
F1	1	0.5	10
F2	0.5	1	10
F3	1	1	10

Procedure for preparation of herbal ointment

a) Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath at 70°C. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.

b) Herbal ointment was prepared by mixing *A. digitata* leaves and *Ocimum sanctum* Leaves extract to the ointment base by levigation method to prepare a smooth paste with 2 or 3 times its weight of base, gradually incorporating base until to form homogeneous ointment, finally transferred in a suitable container.



Figure 1: Ointment Formulation

Evaluation

1. Colour and Odour

Physical parameters like colour and odour were examined by visual examination.

2. Consistency

Smooth and greediness is observed.

3. PH

2 gm ointment formulations sample of each batch was taken in 100 ml dry beaker, 50 ml water was added to it. Beaker was heated on water bath maintained at about 60°C to 70°C for 10 minutes, cooled to room temperature. The pH measurements were done by using a digital type pH meter by dipping the glass electrode into the ointment formulation. PH was determined in triplicate for the solution and average value was calculated.

4. Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed 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

$$S = M \times L / T$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

5. Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds.

6. Diffusion study

The diffusion study was carried out by preparing agar nutrient medium. A hole board at the center of medium and ointment was by placed in it. The time taken by ointment to get diffused through was noted. (After 60 minutes)

7. Washability

Ointment formulations were applied on the skin and then ease extend of washing with water was checked. Washability was checked by keeping applied skin area under the tap water for about 10 min.

8. Non irritancy Test

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

9. Viscosity

The measurement of viscosity of prepared ointments was carried out with Brookfield Viscometer. The values of each ointment formulation were done in triplicate.

10. Antimicrobial activity

The extracts of plants were taken in different ratios were carried out for anti-microbial activity using cup plate method. Nutrient agar medium was prepared, sterilized and used as growth medium for bacterial culture. 25 ml of sterilized medium was poured into each petri plates, covered semi half and allowed it to solidify. Then the test microorganism *Staphylococcus aureus* was inoculated into the petri plates. Then different formulations were poured inside the plates were incubated at 37°C overnight for observation. The presence of zone of inhibition was noted after 24 hrs. The susceptibility of the test to the tested plant extracts was determined by observing the zone of inhibition around each well.

11. Content Uniformity

10mg of the ointment was taken and dissolved in distilled water. Then absorbance was measured at 405 nm⁷ and 410 using UV-Visible spectrophotometer.

12. Stability study

Physical stability test of the herbal ointment F3 was carried out for Two Months at various temperature conditions like 20°C, 25°C and 37°C. The herbal ointment was found to be physically stable at different temperature i.e., 20°C, 25°C, 37°C within four weeks.

RESULTS AND DISCUSSION

Table 3: Evaluation Results for Colour, Odour, Consistency

Formulation	Colour	Odour	Consistency
F1	Green	Characteristic	Smooth
F2	Green	Characteristic	Smooth
F3	Green	Characteristic	Smooth

Table 4: Physicochemical data of formulations

Formulation	pH	Spreadability (gm.cm/sec)	Extrudability	Diffusion study	Viscosity
F1	6.66	8.51	0.28gm	0.7 cm	33.32
F2	6.68	9.2	0.26 gm	0.8 cm	33.44
F3	6.68	8.32	0.28 gm	0.8 cm	33.26

Table 5: Evaluation Results for Washability, non-irritancy Test

Formulation	Washability	Non irritancy Test
F1	Good	Non irritant
F2	Good	Non irritant
F3	Good	Non irritant

Antimicrobial activity

Zone of inhibition of F1, F2 and F3

1. Formulation F1: 14.4 mm
2. Formulation F2: 16.8 mm

3. Formulation F3: 19.6 mm

4. Control (Gentamycin): 22.7 mm

Content Uniformity

Drug content of formulations was found to be in between 96% to 98.7 %.

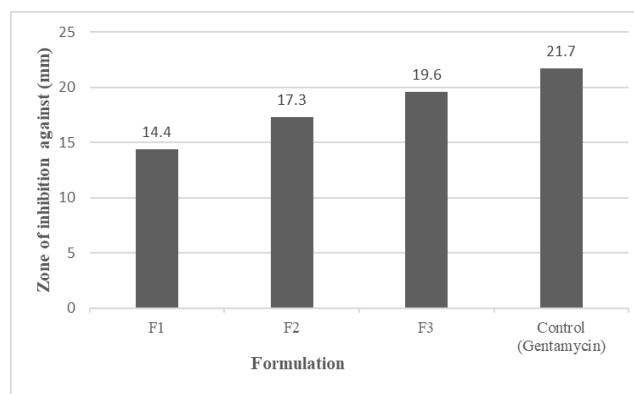


Figure 2: Comparative Study of Antimicrobial activity F1, F2 and F3

Stability study of F3 Formulation for 8 Weeks

Table 6: Formulations were found to be stable at different temperature i.e., 20°C, 25°C, 37°C.

Temperature	Colour	Odour	Consistency	Non irritancy Test
20°C	Green	Characteristic	Smooth	Non irritant
25 °C	Green	Characteristic	Smooth	Non irritant
37 °C	Green	Characteristic	Smooth	Non irritant

CONCLUSION

Ocimum sanctum has been utilised as an antibacterial since ancient times. *Ocimum sanctum* and *A. digitata* leaves both have additional therapeutic effects. The current experimental work showed that herbal ointments containing *A. digitata* leaf extract and *Ocimum sanctum* leaf extracts may be developed and tested for anti-microbial activity. When prepared as an ointment for topical application, this could account for the reported efficacy of the plant's traditional use in the treatment of common skin ailments. The ability of this herbal ointment to kill *Staphylococcus aureus* could be used to control the infection that is thought to be the primary cause of boils, carbuncles, infantile impetigo, and wounds. The final product readily spread on skin surface, showed no irritant effect, diffused well and stable at different temperature.

REFERENCES

1. Fahimi, S., Mortazavi, S. A., Abdollahi, M., & Hajimehdipoor, H., Formulation of a Traditionally Used Polyherbal Product for Burn Healing and HPTLC Fingerprinting of Its Phenolic Contents. *Iranian journal of pharmaceutical research: IJPR*, 2016;15(1): 95–105, PMID: 27610150
2. Awad El-Gied, A. A., Abdelkareem, A. M., & Hamedelniei, E. I., Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* extract. *Journal of advanced pharmaceutical technology & research*, 2015;6(2):53–57, PMID: 25878974
3. Ganesh R Bharskar. A Review on *Adansonia digitata*: An African Tree. *Research Journal of Science and Technology*, 2022; 14(2):127-132, DOI:10.52711/2349-2988.2022.00021
4. Cohen M. M., Tulsi - *Ocimum sanctum*: A herb for all reasons. *Journal of Ayurveda and integrative medicine*, 2014;5(4):51–259, PMID: 25624701
5. Pattanayak, P., Behera, P., Das, D., & Panda, S. K., *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy reviews*, 2010;4(7):95, PMID: 22228948
6. Mahajan, N., Rawal, S., Verma, M., Poddar, M., & Alok, S., A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctum*. *Biomedicine & Preventive Nutrition*, 2013;3(2):185-192, DOI: 10.1016/j.bionut.2012.08.002
7. Sharma S, R HC., Fluorescence analysis, UV-Vis analysis and HPTLC study of *Adansonia digitata*, *World Journal of Pharmaceutical Science & Technology Journal homepage*. 2020;(5):29-41.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: globalresearchonline@rediffmail.com
 New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

