Formulation And Characterization Of Root Extract Of *Punica Granatum L.* Containing Ointment Against Bacterial Pathogens

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ABSTRACT
The potential antimicrobial activity and physicochemical characteristics of a ointment containing roots of *Punica granatum L.* were examined. The antimicrobial activity was carried out by performing agar well diffusion method. Antimicrobial efficacy of aqueous and ethylacetate solvent extract of roots of *Punica granatum L* was evaluated against *E.coli* and *Staphylococcus aureus*. The extracts which showed better results were chosen for formulation of ointment. We created an ointment by mixing 10% w/w aqueous extract of *Punica granatum* into an aqueous cream. The homogeneity, spreadability, extrudability, pH, viscosity, the ointment formulation were all evaluated. These results imply that a herbal ointment derived from *Punica granatum* is a potent topical formulation that combines contemporary pharmaceutical technology with traditional herbal medicine to treat skin infections.

Introduction

All parts found in *P. granatum* – contains chemical constituents have the best medicinal history to cure many ailments and disorders. The fruit is a rich source of medical ailments because of its high anti-oxidant, anti-inflammatory, and anti-microbial activities. *Punica granatum* roots have not been thoroughly studied. This research was conducted to treat a variety of illnesses, infections, and conditions in the female reproductive organs. In addition to being used as medicine to treat numerous immortalities, pomegranates has come to represent life, femininity, fertility, etc [1,2]

Pomegranates are a plant rich in flavonoids, with their roots containing a variety of elastannins, such as punicalin, punicalgin, and several periperidine alkaloids. Chemical groups found in *Punica granatum* are bioactive compounds with therapeutic qualities that can interfere with the host's defenses against harmful and infectious pathogens. It is crucial to assess plants with these qualities in order for the pharmacological and medical sectors to create new drugs. Therefore, the goal of the current study was to ascertain *Punica granatum* L. antimicrobial potential extracts from roots.

Methodology:

Punica granatum roots were collected from the Kothagiri and Ooty Hills rural regions. The samples were approved by the Botanical Survey of India (BSI) in Coimbatore.

Extraction of Sample

The roots were brought to the laboratory within 24 hours, cleaned with tap water, and allowed to dry at room temperature for 4 hours. They were then coarsely ground, and 20 grams of the sample were combined with ethyl acetate solvent and the extraction was carried out by maceration method. The aqueous extract was prepared by boiling method. The extracts were store for further studies.

ANTIMICROBIAL STUDIES

Microbial Strains

To evaluate the antibacterial activity of the crude extract, Gram-negative – *E.coli* ATCC 25922), and Gram Positive ATCC culture (*Staphylococcus aureus* ATCC25923) was chosen.

Antibacterial activity

The antibacterial activity of crude extract was carried out by agar well diffusion method [(3).] Muller-Hinton

Agar plates were used for the test. Pathogenic bacterial strain (1 × 105 CFU/ mL) was uniformly swabbed on the MHA petri plates. Using cork borer four wells of size approximately 6 mm were made on prepared plates. 100 µl of Crude extract of each solvent, positive control standard antibiotic gentamycin 1mg/ml negative control was loaded into the wells. The plates were incubated for 24 h at 37 °C, after 24 h the plates were examined for zone of inhibhition in mm.

Formulation of Ointment:

The ointment base was first made by precisely weighing grated hard paraffin and placing it in an evaporating dish on a water bath. Following the melting of hard paraffin, the remaining ingredients were added and vigorously stirred to facilitate the melting and homogeneous mixing [4,5,6,7,8]. The ointment base was then allowed to cool. The herbal ointment was made by combining precisely weighed aqueous extract with the ointment base using the levigation method. This created a smooth paste that was two or three times the weight of the base. More base was added gradually until the ointment was homogenous, and it was then transferred to an appropriate container.

Evaluation of ointment

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

- **Colour and odour**: Colour and odour of ointment were visually examined.
- **Loss on drying**: Loss on drying was evaluated by placing 1 g of ointment in the Petridish and heated at 105 °C in waterbath.
- **pH**: The pH was determined after dissolving 1 g of ointment in 50 ml of distilled water.
- **Diffusion study**: Agar nutrient medium of any concentration was prepared in order to conduct the diffusion study. It was filled with Petri dishes. Ointment was inserted into a hole that had been bored in the middle. It was noted how long it took for the ointment to diffuse.
- **Stability study**: For two months, the prepared ointment is subjected to stability tests at 37°C.

Results and Discussion

The aqueous extract of *Punica granatum* roots demonstrated outstanding antimicrobial activity against *Staphylococcus aureus and E.coli* in the initial in vitro tests. All of the tested microorganisms were significantly inhibited by the aqueous extract of *Punica granatum*. This observation suggests that the extract's high variety of phyto constitutes is what causes the activity. Therefore, the extract's active ingredients are what give the ointment its observed antibacterial properties, which the ointment also possesses. This was a positive indication that more research should be done on it before it can be used as a commercial ointment to treat bacterial infections. A review of the literature revealed that the plant has a number of pharmacological and traditional uses. *Punica granatum* extract was used in the formulation and assessment of a herbal ointment. Physical constant values, such as extractive value and loss on drying, were calculated. The extract's color and extractive value were examined.

Table 1: Composition of the aqueous extract of *Punica granatum root* ointment

S.NO.	Components	Amount(gm)
1.	Aqueous extract of *Punica granatum root*	1
2.	Emulsifying wax	25
3.	White soft Paraffin	40
4.	Liquid Paraffin	15

Table 2: Physicochemical parameters of the aqueous extract of *Punica granatum root* ointment

S.NO.	Physicochemical Parameters	Observation
1.	Color	Light Brown
2.	Odour	Characteristic
3.	Loss of drying	0.15%
4.	pH	6.7
5.	Diffusion study	2cm in 1 min
6.	Stability study	Stable with pH 6.4

Table 3: Zone of Inhibhition of the aqueous extract of *Punica granatum root* ointment

S.NO.	Pathogens	Aqueous extract	Solvent Extract
1.	Staphylococcus aureus	16mm	14mm
2.	*E.coli*	15mm	10mm

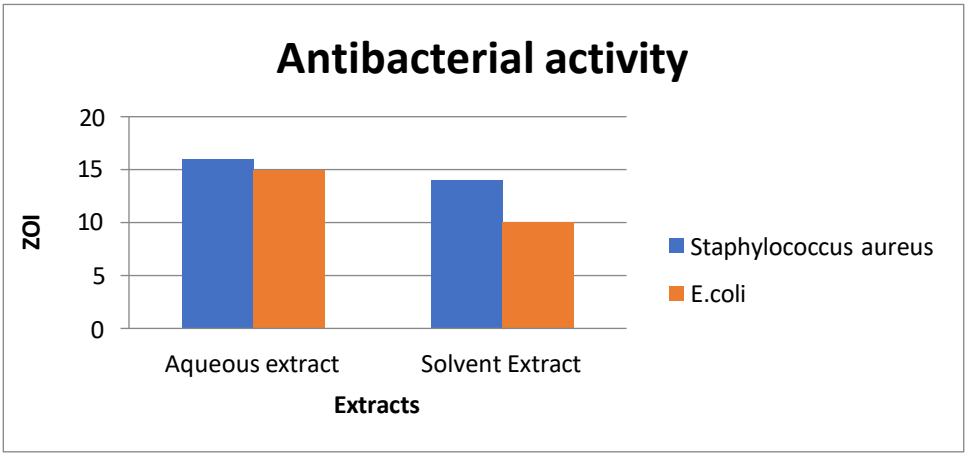

Figure 1: Zone of Inhibhition (ZOI)

Figure 2: Photoplate of antimicrobial activity of extracts against selected microbes

Conclusion:

The current study concludes that *Punica granatum root* is necessary to produce safer, more effective, and more affordable medications to treat diseases caused by S. aureus and E.coli. This study demonstrates the antibacterial activity and high potential of *Punica granatum root* as an antibacterial agent. When prepared as a topical ointment, which could account for the plant's alleged success in treating common skin conditions in folklore. *Punica granatum root* herbal ointment's ability to effectively combat *Staphylococcus aureus*, the most common causative agent of boils, carbuncles, infantile impetigo, and wounds, could be used to contain the organism. The finished product diffused well, was stable at various temperatures, and spread easily on the skin's surface without causing irritation. Research on pharmacological and phytochemical aspects is also necessary.

However, it would be simpler to create a new formulation if research were conducted.

References:

1. Francesca Tozzi, Damaris Nunez-Gomez, Pilar Legua, Massimo Del Bubba, Edgardo Giordani, Pablo Melgarejo. Qualitative and varietal characterization of pomegranate peel: High-value co-product or waste of production. (2022). Scientia Horticulturae. ; 291(3): 110601, DOI:10.1016/j.scienta.2021.110601
2. Hamid Reza Rahimi, Mohammad Arastoo, and Seyed Nasser Ostada. A Comprehensive Review of Punica granatum (Pomegranate) Properties in Toxicological. (2012) Pharmacological, Cellular and Molecular Biology Researches, Iran J Pharm Res. 11(2): 385–400. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3832175/
3. Pandey A, Jagtap JV, Patil AA, Joshi RN, Kuchekar BS Formulation and evaluation of anti-bacterial and anti-fungal activity of herbal ointment containing Aloe vera, Azadirachta indica and Curcuma lona. Journal of Chemical and Pharmaceutical Research. 2010;2(3):182-86.
4. Rajasree PH, Vishwanad V, Cherian M, Eldhose J, Singh R. Formulation and evaluation of antiseptic polyherbal ointment. International Journal of Pharmacy and life sciences. 2012; 3(10):2021-31
5. Yamini K, Onesimus T. Preparation and evaluation of Herbal Antiacne Gel, International Journal of Pharma and Biosciences. 2013;4(2):956-960.
6. Niyogi P, Raju NJ, Reddy PG, Rao BG. Formulation and Evaluation of Antiinflammatory Activity of Solanum Pubescens Wild Extracts Gel on Albino Wistar Rats. International Journal of Pharmacy. 2012; 2(3):484-490.
7. Vandana D, Shweta Pawar, Formulation and evaluation of topical herbal gel containing inclusion complex of curcumin, Vol-12, 2019, 196-201.
8. Deepak PA, Prashanth BS. Formulation and evaluation of herbal gel containing Lanata camara leaves extract. Asian J Pharm Clini Res. 6 (3); 2013: 122-124.