Explore–Journal of Research
Peer Reviewed Journal
ISSN 2278 – 0297 **(Print)**
ISSN 2278 – 6414 **(Online)**

https://www.patnawomenscollege.in/journal

Screening of Plant Extracts Against Opportunistic Pathogens Causing Dermatitis After Prolonged Pad/Diaper Rash and Preparing a Gel

• **Piyush Kumar Rai** • **Shreya Suman** • **Shailja Sharan**
• **Deepika Kumari**

Received : December, 2023
Accepted : January, 2024
Corresponding Author : Piyush Kumar Rai

Abstract : *In the contemporary world, women's menstrual pads and children's diapers are widely used, yet the prevalent issue of severe rashes often goes unnoticed. These rashes, potentially leading to contact dermatitis, result from bacteria like Staphylococcusaureus and fungi such as Candidaalbicans exacerbated by humid conditions and friction in the thigh regions. This study focuses on developing a topical gel using plant extracts-neem leaves, curry leaves, lemon peels, and pomegranate peels to address the opportunistic pathogens causing pad and diaper rashes. Ethanolic extracts of these plants were prepared using a Soxhlet apparatus, and an antimicrobial susceptibility test (AST) was conducted through a disc diffusion method on muller hinton agar (MHA) plates. Results revealed the effectiveness of all plant extracts in suppressing microbial growth. Pomegranate exhibited the maximum inhibition range against Candida albicans, Azadirachta indica was most effective against Staphylococcus aureus. Subsequently, gel containing plant extracts at 50% and 100% concentrations was prepared, and anti-microbial susceptibility test (AST), using the agar well diffusion method indicated a greater inhibition range at the higher concentration, particularly against Staphylococcus aureus.*

Keywords: *Antimicrobial, opportunistic pathogens, dermatitis, anti-microbial susceptibility test (AST), ethanolic extracts*

Piyush Kumar Rai
Assistant Professor, Department of Botany
Patna Women's College (Autonomous),
Bailey Road, Patna–800 001, Bihar, India
E-mail: raipiyush518@gmail.com

Shreya Suman
B.Sc. III year, Botany (Hons.), Session: 2021-2024,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Shailja Sharan
B.Sc. III year, Botany (Hons.), Session: 2021-2024,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Deepika Kumari
B.Sc. III year, Botany (Hons.), Session: 2021-2024,
Patna Women's College (Autonomous),
Patna University, Patna, Bihar, India

Introduction:

Both pads and diapers play crucial roles in ensuring the health and cleanliness of women and children at different stages of life. Pads are essential for women's menstrual hygiene, offering protection against leaks and facilitating comfortable menstrual flow management. Access to high-quality pads enhances women's overall well-being, allowing them to engage fully in daily activities without hindrance. Despite the widespread use of adult and child pads and diapers, certain discomforts like infections and rashes often go unnoticed. Prolonged exposure, inadequate cleanliness, and subpar materials can lead to inflammatory reactions, resulting in rashes and skin irritation. Diapers, carefully crafted with various materials, including super absorbent polymers, may

cause rashes and potential infections, such as those from opportunistic pathogens like *Candida albicans* and *Staphylococcus aureus.*

To address these issues, this project explores the use of plant extracts, including neem leaves, curry leaves, lemon peels, and pomegranate peels, to create a topical gel with antibacterial and antifungal properties. These extracts have been selected based on their unique qualities and proven antibacterial effects against specific microorganisms. The study aims to provide a natural remedy for pad and diaper rashes, addressing the growing interest in plant-based solutions as opposed to chemical alternatives. The selection of components for the topical gel is based on their unique qualities, as researchers have demonstrated the antibacterial effects of plant extracts. Ethanolic extracts from *P. granatum* peels, at doses of 30 to 50 µg/ml, exhibit potential against *Micrococcus luteus, S. aureus,Bacillus megaterium*, and Gram-negative bacteria like *E. coli* and *P. aeruginosa* (Duman *et al*., 2009; Dey *et al*., 2012). Additionally, the antimicrobial activity of ethanolic *Punica granatum* extract and its fractions show significant antibacterial effects against Gram-positive *(S. aureus* and *B. cereus*) and Gramnegative bacteria (*E. coli* and *S. typhi*) (Alzoreky, 2009; Mahboubi *et al*., 2015; Mostafa, AA., 2018). Pomegranate peels possess potent anti-inflammatory properties, soothing irritated skin, and aiding conditions like eczema. Neem leaves exhibit antibacterial activity against *S. aureus*, including multi drug-resistant strains (MRSA), starting at 50% concentration (Disegha*et al*., 2014). The leaves of *Murraya koenigii* in Ayurvedic medicine have antidiabetic, antioxidant, antimicrobial, and anti-inflammatory actions (Doddanna *et al*., 2013). Lemon peel extract from *Citrus limon* shows high potential for antimicrobial activity against *Escherichia coli* (Henderson AH *et al*., 2018) and maximum antifungal activity against *Malassezia* species (Pingili*et al*., 2016) (Ali *et al*., 2017).

Objectives:

1. Preparation of plant extract from selected plants.
2. Collecting clinical samples of selected pathogens.
3. Testing of antimicrobial activity of the extracts prepared against selected pathogens.
4. Preparation of the gel for topical application from the potential extracts.

Hypothesis:

This study aims to test the hypothesis that a gel made from medicinal plant extracts evaluated in vitro can successfully treat opportunistic pathogen-induced dermatitis following a protracted pad or diaper rash. Through in vitro screening, particular plant extracts like neem leaves, curry leaves, pomegranate peels, and lemon peels with strong antimicrobial activity against the opportunistic pathogens causing dermatitis will be identified. With these potent plant extracts in the gel composition, it is anticipated that the skin will recover more quickly, experience less irritation, and be protected from infections.

Materials and Methods:

To carry out our project we followed the given steps-

Sample collection: Fresh leaves of *Azadirachata indica* (neem), *Murraya koenigii* (curry) leaves were collected from garden of Patna Women's College, Patna in a sterile bag, *Punica granatum* (pomegranate) and *Citrus limon* (lemon) peels were collected from Boring Road. Colonies of *Staphylococcus aureus* and *Candida albicans* were collected from Mahavir Cancer Sansthan, Patna in sterilized viables and brought to the laboratory.

Preparation of ethanolic plant extract: The leaves and peels of collected plant species were cleaned, dried and were grounded separately using clean mortar and pestle. Ethanolic extract of plants were prepared using the Soxhlet apparatus. 8 grams of the powdered leaves and peels were filled inside four thimbles separately, each thimble was covered with cotton and then it was placed inside the extractor of the Soxhlet apparatus. 80 ml of ethanol was poured in the round flask. This measurement was taken to fulfil the ratio of 1:10. The cycle of evaporation and condensation was repeated till the colour of the ethanol present in the thimble chamber became colourless (Redfern *et al*., 2014). After extraction all these extracts were poured in sterilized glass bottles and were labelled.

MHA plate preparation: Muller hinton agar was used to test anti-microbial susceptibility test. 38g of MHA powder was taken and dissolved in 1 litre distilled water then it was autoclaved at 121 for 20 minutes. After autoclaving it was cooled down a bit and then was poured inside the petri plate under laminar air flow. It was then stored in freeze at 4 for future use (Murray *et al.*, 2018).

Antimicrobial susceptibility test (AST) of prepared ethanolic plant extract: Whatman filter paper was sterilized under UV light for 20 minutes. Around 10 ml of extracts prepared from different plant parts were taken in different petri dishes and was kept in hot air oven at 50. The round disc of filter paper was kept in the petri dishes for half an hour. The MHA plates were swabbed with *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungus) with the help of swab sticks. Few colonies of *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungus) were taken from their NA and SDA plates respectively and was mixed with 6ml distilled water inside a test tube using inoculating loop and was labelled. Both of these tubes were kept in incubator for 2 hours and after incubation, swabbing was done on MHA plates. After swabbing, with the help of sterile forceps, the discs were placed on the plates. Each plant extract was used twice that is on both bacteria and fungus to see their inhibition. Plates of ethanol was used as control. All the plates were incubated at 26°C for 2-3 days and the growth were observed regularly. Diameter of zone of inhibition were measured and recorded.

Gel preparation: 1 gm of Carbopol 934 as soaked in 10 ml of distilled water and was kept overnight. The mixture was the homogenized using a homogenizer. Then all the ethanol was evaporated from the extract and only the left portion of extracts were used 10 ml of each extract was taken in petri plates and was kept in hot air oven at 40°C until the ethanol got evaporated. Around 2mg of each extract that is neem leaf, curry leaf, pomegranate peel, and lemon peel was used and was mixed with 5 ml PEG4000, 15ml glycerine, 10mg vitamin E in another beaker. 50% concentration of gel was prepared in the same way but here only 1mg of each extract was used and all other ingredients were in the same amount. Then this mixture was added to carbopol 934 mixture. Then 1ml of triethanolamine was added to this mixture. The topical gel was prepared (Dange *et al.*, 2019).

Antimicrobial susceptibility test of prepared gel using agar well diffusion method: Antimicrobial susceptibility test of gel was done at two concentrations that is at 50% concentration and 100 % concentration. One MHA plate was swabbed using *Staphylococcus aureus* and other was swabbed using *Candida albicans.* Cork screw was sterilized and was used to create well in the MHA plate. Two wells were created in each plate to see the inhibition at different concentration of gel.A small quantity of different concentration of gel was filled in the specific well created and then it was incubated at 27 for 2-3 days and inhibition range was observed and measured (Balouiri*et al.*, 2016).

Results and discussion:

After conducting antimicrobial susceptibility test (AST) on ethanolic plant extracts and the prepared gel, we gained the following observations:

Antimicrobial activity of ethanolic plant extract: The results revealed that all four plant extracts were potentially effective in suppressing microbial growth using disc diffusion method. The growth of *Candida albicans* was inhibited mostly by ethanolic extract of pomegranate followed by lemon and neem. The curry leaves showed the least inhibition activity. The growth of *S. aureus* was inhibited mostly by ethanolic extract of neem leaves followed by pomegranate peels and curry leaves. The lemon peels showed the least inhibition activity here. Given below are table 1 and table 2 which contains the data showing inhibition range in cm by different plant extracts on *Candida albicans* and *Staphylococcus aureus*.

The data showing antimicrobial screening test of different plant's ethanolic extract on *Candida albicans* (Table 1).

Table 1. Inhibition range in cm by different plant extract on *Candida albicans*

Observation	Inhibition range in cm by different plant extract on *Candida albicans*				
	Ethanol control	Pomegranate peels	Curry leaves	Neem leaves	Lemon peels
Observation: I	1cm	2.5cm	0.8cm	1.2cm	1.4cm
Observation: II	1cm	2cm	1cm	1.2cm	1cm
Observation: III	1.6cm	2.8cm	1cm	1.6cm	1.4cm
Average	1.2cm	2.4cm	0.9cm	1.3cm	1.2cm

The data showing antimicrobial screening test of different plant's ethanolic extract on *Staphylococcus aureus* (Table 2).

Table 2. Inhibition range in cm by different plant extract on *Staphylococcus aureus*

Observation	Inhibition range in cm by different plant extract on *Staphylococcus aureus*				
	Ethanol control	Pomegranate peels	Curry leaves	Neem leaves	Lemon peels
Observation: I	0.7cm	3.5cm	3cm	4cm	2.5cm
Observation: II	1cm	3cm	3cm	4.2cm	3cm
Observation: III	0.6cm	3.6cm	3.5cm	4.4cm	2.8cm
Average	0.7cm	3.3cm	3.1cm	4.2cm	2.7cm

Antimicrobial activity of gel prepared from the plant extract: It was observed that the antimicrobial activity of the prepared gel from the extracts of the for plants was concentration dependent. The gel showed more inhibition range at 100% concentration than at 50% concentration. Also, the prepared gel was more effective against *S. aureus* than *C. Albicans.* Given below is table 3 which contains the data showing inhibition range in cm by gel prepared from different plant extracts on *Candida albicans* and *Staphylococcus aureus* at 100% and 50% concentration. The data showing antimicrobial screening test of prepared gel at 50% and 100% concentration on *Staphylococcus aureus* and *Candida albicans* (Table 3).

Table 3. Antimicrobial susceptibility test of prepared gel at 50% and 100% concentration on *Staphylococcus aureus* and *Candida albicans.*

Observation	Inhibition range in cm by topical gel against *Candida albicans* at different concentration		Inhibition range in cm by topical gel against *Staphylococcus aureus* at different concentration	
	At 50% concentration	At 100% concentration	At 50% concentration	At 100% concentration
Observation I	2cm	3.5cm	2.8cm	4cm
Observation II	2.2cm	2.5cm	2.5cm	4.3cm
Observation III	2.2cm	3.8cm	2cm	4cm
Average	2.1cm	3.2cm	2.4cm	4.1cm

Discussion:

Staphylococcus aureus and *Candida albicans*constitute components of the microbiota residing on mammalian skin. Dermatitis induced by these pathogens is a prevalent issue affecting many individuals and given the possible adverse effects of chemical products, there is a current imperative to embrace natural and organic alternatives. This research proposes the utilization of ethanolic extracts from *Azadirachta indica* (neem), *Murraya koenigii* (curry) leaves*, Punica granatum* (pomegranate), and *Citrus limon* (lemon) peels as a viable treatment against *S. aureus* and *C. albicans* as findings reveal that the ethanolic extracts from these plant parts effectively inhibit the growth of both *Staphylococcus aureus* and *Candida albicans*. In the case of *Staphylococcusaureus*, the neem leaf extract exhibits the highest effectiveness, followed by pomegranate peel and curry leaf extracts, while lemon peel extract is the least effective. This high effectivity of neem leaves and pomegranate peels against *S. aureus* is similar to the results observed in previous works (Mostafa*et al.*, 2018) (Aslam *et al.*, 2009). Regarding *Candida albicans*, pomegranate peel was observed to be the most effective, followed by neem leaf and lemon peel extracts, with curry leaf extract being the least effective. This significant anti-fungal property od pomegranate is in line with previous work done (Gowda *et al.* 2023). Furthermore, the gel formulated from these extracts demonstrates greater efficacy against *Staphylococcus aureus* compared to *Candida albicans.*

Conclusion:

Pathogenic microorganisms such as *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungus) are well-known for their role as opportunistic pathogens, leading to secondary infections in pad and diaper rashes. Taking the drawbacks of long chemical-based treatments into consideration, herbal formulations, which offer benefits such as minimal or no adverse effects, user-friendly application, and cost-effectiveness are preferred. Plants like *Azadirachta indica* (neem), *Murraya koenigii* (curry), *Punica granatum* (pomegranate), *and Citrus limon* (lemon), have demonstrated efficacy against the growth of microorganisms like *S. aureus* and *C. albicans.* Future research could delve into identifying the active molecules within these plant extracts responsible for their antibacterial and antifungal activities with evaluation of their toxicity.

References:

Ali, J., Das, B., Saikia, T. (2017). Antimicrobial activity of lemon peel (*Citruslimon*) extract. International Journal of Current Pharmaceutical Research, 9(4), 80-82.

Al-Zoreky N. S. (2009). Antimicrobial activity of pomegranate (*Punica granatumL.*) fruit peels. International journal of food microbiology, 134(3), 244–248.

Aslam, F., Rehman, K., Asghar, M. Sarwar, M. (2009). Antibacterial activity of various phyto constituents of neem. Pak. J. Agri. Sci.,46(3), 209-213.

Balouiri, M., Sadiki, M., &Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 6(2), 71–79.

Dange, V.N., Shid, S.J., Patil, S B., Vambhurkarz G.B., Bhutkar, M.A. (2019). Formulation and Evaluation of Novel Herbal Gel by using Lemongrass Oil. Res. J. Pharma. Dosage Forms and Tech, 11(2), 67-70.

Dey, D., Debnath, S., Hazra, S., Ghosh, S., Ray, R., & Hazra, B. (2012). Pomegranate pericarp extract enhances the antibacterial activity of ciprofloxacin against extendedspectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing Gram-negative bacilli. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 50(12), 4302–4309.

Disegha, G.C., Izionworu, V.O. (2014). Antifungal activities of curry leaves (*Murrayakoenigii*) extract on some selected fungi. Chemistry and Materials Research, 6(11), 1-14.

Doddanna, S. J., Patel, S., Sundarrao, M. A., & Veerabhadrappa, R. S. (2013). Antimicrobial activity of plant extracts on *Candidaalbicans*: an in vitro study. Indian journal of dental research: official

publication of Indian Society for Dental Research, 24(4), 401–405.

Duman, A. D., Ozgen, M., Dayisoylu, K. S., Erbil, N., &Durgac, C. (2009). Antimicrobial activity of six pomegranates (*Punica granatumL.*) varieties and their relation to some of their pomological and phytonutrient characteristics. Molecules (Basel, Switzerland), 14(5), 1808–1817.

Gowda, Gagana V., Mamatha, G. P., Usha, V A., Thimmasetty, J., Rajeshwari, M R., Annigeri, Rajeshwari G., (2023). Efficacy of *Punica Granatum* (Pomegranate) in the management of oral candidiasis – A randomized controlled study. Journal of Indian Academy of Oral Medicine & Radiology, 35(1),15-20.

Henderson, A. H., Fachrial, E., & Lister, I. N. E. (2018). Antimicrobial Activity of Lemon (*Citruslimon*) Peel Extract Against *Escherichiacoli*. American Scientific Research Journal for Engineering, Technology, and Sciences, 39(1), 268–273.

Mahboubi, A., Asgarpanah, J., Sadaghiyani, P.N. et al. (2015) Total phenolic and flavonoid content and antibacterial activity of *Punica granatumL*. var. pleniflora flowers (Golnar) against bacterial strains causing foodborne diseases. BMC Complement Altern Med 15, 366.

Mostafa, AA. Al-Askar, AA. Almaary, KS.Dawoud, TM. Sholkamy, EN. Bakri, MM. (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi Journal of Biological Sciences, 25(2), 361-366.

Murray, P. R., &Zeitinger, J. R. (1983). Evaluation of Mueller-Hinton agar for disk diffusion susceptibility tests. Journal of clinical microbiology, 18(5), 1269–1271.

Pingili, M., Vanga, S., Raparla, RK. (2016). Antifungal activity of plant extracts against dandruff causing organism *Malasse ziafurfur*. International Journal of Bioassays, 5(11), 5047.

Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. Journal of microbiology & biology education, 15(1), 45–46.