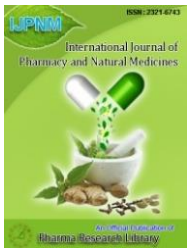

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Evaluation of Anti-Dandruff Activity of *Citrullus Lanatus* (Watermelon) Seeds

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A B S T R A C T

Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about once a month. Usually, scalp sheds dead cells in nearly invisible way, but sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff. Seed extracts have emerged as promising sources of bioactive compounds with notable antioxidant and antibacterial properties, primarily attributed to their rich content of phenolic and polyphenolic components. These bioactive compounds exhibit significant potential as therapeutic agents for managing disorders associated with oxidative stress and combating infectious diseases. The antioxidant capabilities of seed extracts are particularly noteworthy, as oxidative stress plays a pivotal role in the pathogenesis of various health conditions. Phenolic and polyphenolic compounds present in these extracts have demonstrated the ability to neutralize reactive oxygen species (ROS) and free radicals, which are implicated in cellular damage and the progression of oxidative stress- related disorders. The bioactive chemicals found in the sample have been shown to have pharmacological and physiological properties. These seeds contain significant free radical scavenging activity and consequently antioxidant activity, according to our research. The amount and kind of bioactive compound produced by medicinal plants determine its anti-ailment action and different physiological impacts on the human body system. The seed extracts represent a rich source of bioactive compounds with substantial antioxidant and antibacterial properties, mainly attributed to their phenolic and polyphenolic components. The exploration of these compounds as potential therapeutic agents holds great promise for managing oxidative stress-related disorders and combating infectious diseases. As research in this field continues to advance, the identification, isolation, and mechanistic understanding of seed-derived bioactive compounds will undoubtedly contribute to the development of novel treatments and interventions in the realm of medicine.

Keywords: antioxidant, reactive oxygen species (ROS), polyphenolic, *Citrullus Lanatus*, bioactive compounds

A R T I C L E I N F O

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C o n t e n t s

1. Introduction

Dandruff is a major cosmetic problem that poses very great public health concern both in developed and developing countries. No population in any geographical region would have passed through freely without being affected by dandruff at some stage in their life. Dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when scalp sheds epidermal cells in large clumps. The skin of scalp renews itself about

once a month. Usually, scalp sheds dead cells in nearly invisible way, but sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff. Dry dandruff (Pityriasis sicca) The scales are fine, thin, furfur aceous, white or grayish and dryoronly slightly greasy. The hair is dry and luster less. There is mild to moderate itching. The scales fall freely on the shoulders. This type of dandruff is more common in winter than in summer. It signifies exaggeration of normal exfoliation of the horny layer of the epidermis. It usually affects people with dry integument and scalp. In nutritional disorders, scaliness of the scalp is exaggerated.

Fig 1.Oilydan druff (Pityriasis Steatoides)

Fig 2. Dry dandruff (Pityriasis sicca)

Etiology of dandruff

There could be several etiopathologic pathways with complex mechanisms, which may cause dandruff. Recent technical advances including improved microbial and analytical techniques have provided new insights into the underlying pathology.

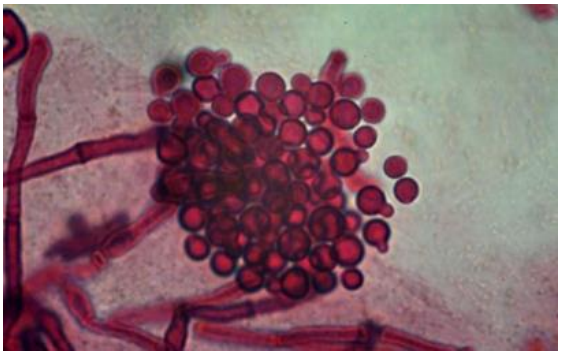

Fig 3. Micrograph of *Malasseziafurfur*

Mechanism of action

Malassezia organism can be found on the skin in 75-90% healthy people. *Malassezia Furfur* is a lipophillic, saprophytic, budding, unipolar, dimorphic, gram positive double walled, oval to round yeast.

Plant Profile

English : Watermelon Hindi : Tarbooj
Telugu : Puchakaya Tamil : Tarbuṣaṇi Kannada : Kallambu
Malayalam : Kaarbūs
Plant Classification
Kingdom : Plantae (Plants)
Subkingdom :Tracheobionta(Vascular plants)
Class : Magnoliopsida (Dicotyledons)
Order : Cucurbitales
Family : Cucurbitaceae (Gourd family)
Genus : Citrullus
Species : Citrullus lanatus

Fig. 4. *Citrullus lanatus*

Morphology

Plant Morphology:

Water melon plants are annual vines with trailing or climbing habits. The vines produce large, lobed leaves that are deeply cut and have a coarse texture.

Flowers:

- Watermelon plants bear both male and female flowers on the same vine.
- The flowers are typically yellow and have a distinct fragrance.
- Pollinationisessentialforfruit development.

Fruit:

- The watermelon fruit is a large, oval or round berry with smooth, green skin.
- The flesh can vary in color from red and pink to yellow and orange, depending on the cultivar.

Black seeds are common in traditional varieties, while seedless cultivars have gained popularity.

Medicinal uses of Citrulluslanatus seeds

- **Antioxidant Properties:** Watermelon seeds are rich in antioxidants, including phenolic compounds and flavonoids. Antioxidants help neutralize free radicals in the body, which may contribute to preventing oxidative stress and reducing the risk of chronic diseases.
- **Anti-inflammatory Effects:** Some studies suggest that watermelon seed extracts may haveanti-inflammatory properties. Inflammation is associated with various health conditions, and reducing inflammation could potentially have therapeutic benefits.
- **Antidiabetic Potential:** Preliminary research indicates that watermelon seed extracts may have hypoglycemic (blood sugar-lowering) effects. This could be beneficial for individuals with diabetes or those at risk of developing diabetes.
- **Hypolipidemic Activity:** Watermelon seeds may have a role in managing lipid levels in the body. Some studies suggest potential benefits in reducing cholesterol and triglyceride levels.
- **Antimicrobial Properties:** Watermelon seed extracts have demonstrated antimicrobial activity against certain bacteria and fungi. This suggests apotentialrole in fighting infections, but more research is needed to confirm and understand the mechanisms involved.
- **Hepatoprotective Effects:** Some studies suggest that watermelon seed extracts may have hepatoprotective properties, meaning theycould help protect the liver fromdamage. This could be valuable in the context of liver health.
- **Aphrodisiac Properties:** Traditional medicine in some cultures has used watermelon seeds for their purported aphrodisiac effects. While there is anecdotal evidence, scientific research in this area is limited.

2. Methodology

Materials and Equipment:

- Water melon seeds (Citrullus lanatus)
- Ethanol (orother suitable solvent)
- Soxhlet extractor
- Condenser
- Round-bottom flask
- Heating mantleorhot plate
- Distillation apparatus
- Filter paper
- Rotary evaporator (optional, for solvent removal)

Procedure:

Seed Preparation:

- Clean and dry the watermelon seeds thoroughly to remove any impurities.
- Grind the seeds into a fine powder using a suitable grinder.

Extraction Setup:

Set up the Soxhlet extraction apparatus with a round-bottom flask at the bottom, a sample thimble containing the powdered watermelon seeds, and a condenser at the top.

Solvent Selection:

Fill the Soxhlet extract or with ethanol or another suitable solvent. The choice of solvent depends on the type of compounds you want to extract.

Extraction Process:

- Start the extraction process by heating the solvent.
- The solvent dissolves the compounds from the seeds and carries them back to the flask.
- The solvent vapor rises, condenses in the condenser, and drips on to the powdered seeds in the thimble.

Extraction Duration:

- Allow the extraction process to continue for several hours (typically 6-8hr) to ensure thorough extraction of the target compounds.

Solvent Evaporation:

- After the extraction is complete, remove the round-bottom flask containing the extracted solution.
- Use a rotary evaporator or another suitable method to evaporate the solvent and concentrate the extract.
- **Preliminary phytochemical studies**
- Alkaloids (Mayer's Test):
  Procedure: Mix the plant extract with Mayer's reagent (potassium mercuric iodide). A yellow or cream precipitate indicates the presence of alkaloids.
  End point: Formation of a yellow or cream-colored precipitate.
- **Flavonoids (Shinoda Test):**
  Procedure: Add magnesium ribbon and a few drops of concentrated hydrochloric acid to the plant extract. A pink or red coloration indicates the presence of flavonoids.
  End point: Development of a pink or red color.
- **Tannins (Ferric Chloride Test):**
  Procedure: Mix the plant extract with a few drops of ferric chloride solution. A dark green or blue-black coloration indicates the presence of tannins.
  Endpoint: Formation of a dark green or blue-black color.
- **Saponins (Froth Test):**
  Procedure: Shake the plant extract vigorously with water. The formation of a stable froth indicates the presence of saponins.
  End point: Persistent frothing.
  Glycosides (Legal's Test):
  Procedure: Treat the plant extract with glacial acetic acid and add a few drops of ferric chloride. A blue or green coloration indicates the presence of glycosides.
  End point: Development of a blue or green color.
- **Steroids/Tri terpenoids (Liebermann-Burchard Test):**

Procedure: Mix the plant extract with acetic anhydride, followed by concentrated sulfuric acid. A color change from violet to blue or green indicates the presence of steroids/tri terpenoids.
End point: Violet to blue or green color change.

- **Terpenoids (Salkowski Test):**
  Procedure: Mix the plant extract with chloroform and concentrated sulfuric acid. A reddish-brown coloration at the interface indicates the presence of terpenoids.
  End point: Formation of a reddish-brown color at the inter face.
  Phenols (Ferric Chloride Test): Procedure: Mix the plant extract with a few drops of ferric chloride solution. The development of a bluish-black color indicates the presence of phenols.
  End point: Formation of a bluish-black color.

Materials and Reagents:

- Sabouraud Dextrose Agar (SDA)or modified Dixon's agar
- Petridishes
- Inoculation loops or swabs
- Sterile distilled water
- Incubatorsetto32-37°C(89.6-98.6°F)
- Para film or another sealing material
- pH indicator paper

Procedure:

Preparation of Agar Plates:

Prepare Sabouraud Dextrose Agar or modified Dixon's agar according to the manufacturer's instructions. Sabouraud Dextrose Agar (SDA) is a specialized agar medium used for the isolation and cultivation of fungi. It is named after the French dermatologist Raymond Sabouraud, who developed it. The medium is selective for fungi because it has a low pH (around 5.6) and contains dextrose as the primary carbon source. Here's a basic recipe and procedure for preparing Sabouraud Dextrose Agar:

Ingredients:

- Dextrose:40.0 grams
- Peptone:10.0grams
- Agar:15.0 grams
- Distilled water: 1000 ml

Procedure:

Weighing:

- Weigh the specified amounts of dextrose, peptone, and agar.
- Use a balance to ensure accuracy in measurements.

Mixing:

- Add the dextrose, peptone, and agar to the distilled water in a large flask or container.
- Stir the mixture to dissolve the components

Heating:

Heat the mixture while stirring until it boils. This helps to dissolve the components completely.

Sterilization: Sterilize the medium by autoclaving it at 121°C for15minutes.

- Autoclaving is crucial to eliminate any contaminants and to ensure the medium is sterile for fungal cultivation.

3. Results and Discussion

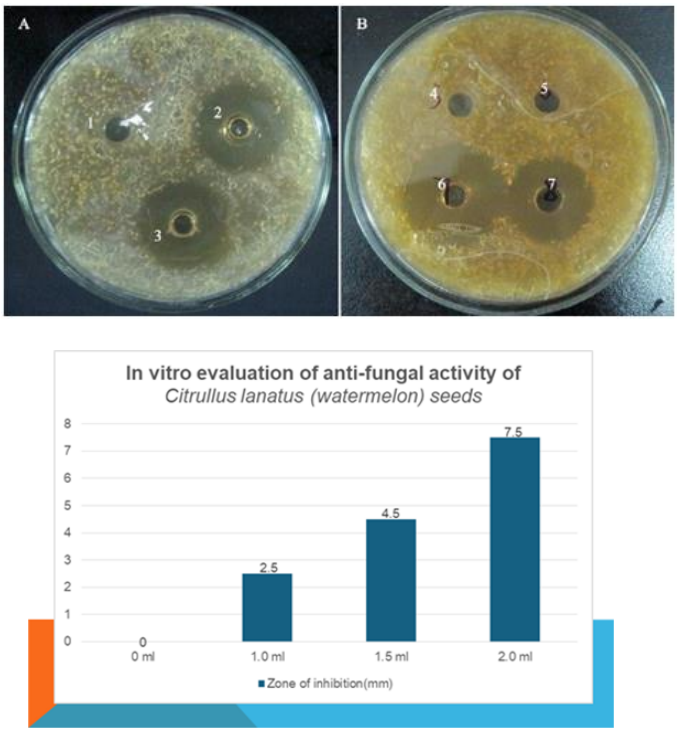

Fig.5. In-vitro evaluation of anti-fungal activity of *Citrullus lanatus* (watermelon) seeds

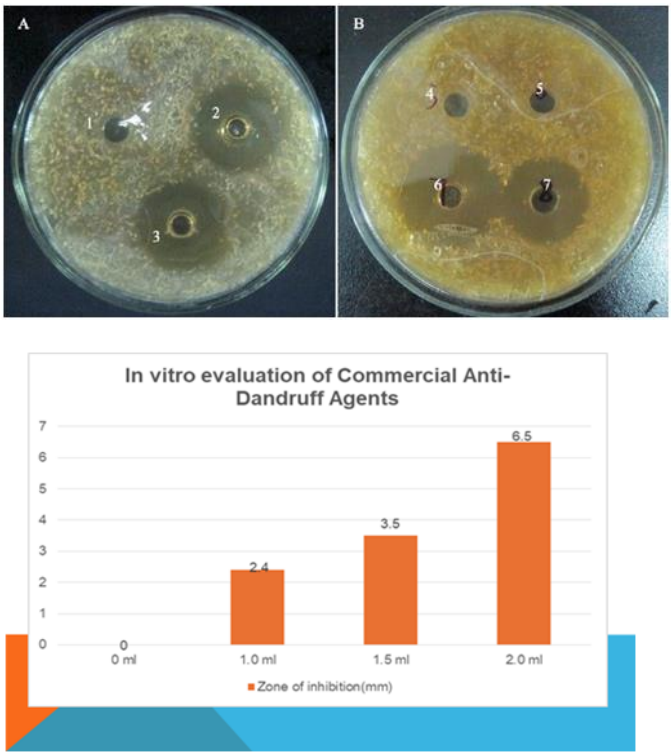

Fig.6. In-vitro evaluation of Commercial Anti-Dandruff Agents

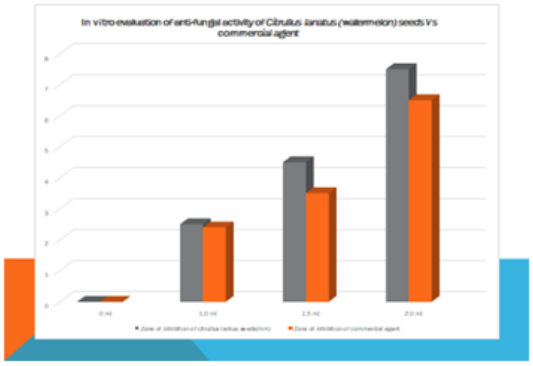

Fig.7. In-vitro evaluation of anti-fungal activity of *Citrullus lanatus* (watermelon) seeds Vs Commercial agents

Discussion

Citrullus lanatus seeds were gathered and analyzed. The presence or absence of secondary metabolites, antioxidant, and antibacterial properties of *Citrullus lanatus* seeds were investigated in this study. Phytochemicals exhibit biological features such as antioxidant activity, antibacterial activity, detoxification enzyme modulation, immune system modulation, and general hormonal activity regulation [3,9,11]. The extraction of phytochemical content is affected by the kind of solvent used and the procedure used to prepare the extract.

Methanolic extract resulted in the highest extraction yield and a more complex phenolic content high secondary metabolite extraction, high flavonoid content, high antioxidant potential, and effective antibacterial activity, according to the findings of this study. Terpenoids, glycosides, steroids, alkaloids, flavonoids, coumarins, and quinones were found in high concentrations in this research, however, phytosterols and anthraquinones were not.

This is consistent with Ali et al., 2012[19], who found that alkaloids and terpenes are extensively dispersed throughout the Citrullus genus for methanol extract of *Citrullus lanatus* seeds, Omoboyowa et al. 2015[20] reported flavonoids (2.310mg/100g), phenol (1.371mg/100g), saponins (1.553mg/100g), alkaloid (33.795mg/100g), and tannins (0.536mg/100g). Gwana, *etal*.2014[21]also found flavonoids 0.01 percent, phenol (GAE) 0.01 percent, saponins 0.09 percent, alkaloid 0.91 percent, and tannins 0.04 percent in the phytochemical percentage composition of the Rosmas type of watermelon seeds.The bioactive chemicals found in the sample have been shown to have pharmacological and physiological properties. These seeds contain significant free radical scavenging activity and consequently antioxidant activity, according to our research. The amount and kind of bioactive compound produced by medicinal plants determine its anti-ailment action and different physiological impacts on the human body system.

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The activities of watermelon seed constituents such as Dodecenol, 11-Dodecenol, β -Sitosterol, 1-Azabicyclo (3,1,0) hexane, Cyclopropane carboxylic acid, Nonivamide, 8-Nonenoic acid, γ–Sitosterol have been revealed to possess antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistaminic, antieczemic, immunomodulatory and cardioprotective activities. Oxazole has anti-cancerous, anti-viral, anti- diabetic, and antibiotic activity[31]. V.H.A. Enemor et al., 2019 [32]revealed the presence of many other benign chemical compounds in *Citrullus lanatus* seeds beyond the retention time of 60mins. Some noteworthy such compounds are Pyrrole, Isoxazole, Methyl Guanidine, 2-Propenenitrile, Thiirane, Methanesulfonyl fluoride, Silamine, Oxiranecarboxaldehyde,1,6-dichloropyruvicacid, 3-Methyl-1,3-pentadiene, Propiolamide, N-Ethylformamide, Fluoramine, Fomepizole. These are also pharmacologically active compounds found in *Citrullus lanatus. Citrullus lanatus* seeds show antibacterial action against many strains, according to our research, which might be attributable to terpenoids and phenols. *Citrullus lanatus* seeds, it may be established, have great therapeutic potential.

Table.1 In vitro evaluation of Commercial Anti-Dandruff Agents

	Micro organism Inoculum	Anti-Dandruff Agents	Medium	Zone of inhibition (mm)
1	Malassezia species	0	Sabouraud Dextrose Agar	0mm
2	Malassezia species	1ml	Sabouraud Dextrose Agar	2.4±0.7mm
3	Malassezia species	1.5ml	Sabouraud Dextrose Agar	3.5±0.9mm
4	Malassezia species	2ml	Sabouraud Dextrose Agar	6.5±0.2mm

4. Conclusion

The antioxidant capabilities of seed extracts are particularly noteworthy, as oxidative stress plays a pivotalrole in the pathogenesis of various health conditions. Phenolic and polyphenolic compounds present in these extracts have demonstrated the ability to neutralize reactive oxygen species (ROS) and free radicals, which are implicated in cellular damage and the progression of oxidative stress-related disorders. By scavenging these harmful entities, seed extracts may contribute to the prevention and management of conditions such as cardiovascular diseases, neurodegenerative disorders, and certain cancers.

Moreover, the anti-bacterial properties of seed extracts further enhance their therapeutic potential. The phenolic and polyphenolic components exhibit antimicrobial activity against a broad spectrum of bacteria, including both Gram-positive and Gram-negative strains. This antimicrobial action suggests that seed extracts could be explored as natural alternatives or adjuncts to conventional antibiotics in the treatment of bacterial infections. The rise of antibiotic resistance has underscored the need for novel antimicrobial agents, and seed extracts present a promising avenue for research in this regard.

To harness the full therapeutic potential of seed extracts, it is essential to delve into the specific phenolic and polyphenolic compounds present in different seed varieties. The identification and isolation of these bioactive constituents can pave the way for the development of targeted therapies and pharmaceutical formulations. Advances in analytical techniques, such as chromatography and mass spectrometry, enable researchers to unravel the complex composition of seed extracts and elucidate the individual contributions of various compounds to their antioxidant and antibacterial activities.

Furthermore, understanding the mechanisms through which seed-derived compounds exert their therapeutic effects is crucial for optimizing their applications in medicine. Research endeavors focused on elucidating the signaling pathways and molecular targets influenced by these compounds can provide valuable insights into their mode of action. This knowledge is instrumental in designing targeted interventions for specific health conditions and tailoring therapeutic strategies based on the unique properties of seed extracts.

In conclusion, seed extracts represent a rich source of bioactive compounds with substantial antioxidant and antibacterial properties, mainly attributed to their phenolic and polyphenolic components. The exploration of these compounds as potential therapeutic agents holds great promise formanaging oxidative stress-related disorders and combating infectious diseases. As research in this field continues to advance, the identification, isolation, and mechanistic understanding of seed-derived bioactive compounds will undoubtedly contribute to the development of novel treatments and interventions in the realm of medicine.

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