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Research Paper

Phytochemical And Antibacterial Study of Citrullus Lanatus Seed Extract

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ABSTRACT

Watermelon (Citrullus lanatus) seeds are rich in bioactive compounds that hold promise for nutritional and therapeutic uses. This study aimed to assess the phytochemical components and antibacterial properties of extracts from watermelon seeds, utilizing solvents with varying polarities. Fresh seeds were gathered, dried in the shade, ground into powder, and then subjected to Soxhlet extraction with petroleum ether, chloroform, and methanol. The resulting extracts were concentrated and analyzed for their extraction yield, phytochemical content, and antimicrobial effectiveness against Escherichia coli using the agar well diffusion technique. Among the three extracts, the methanolic extract yielded the highest percentage at 3.529% w/w, with chloroform and petroleum ether following at 2.032% w/w and 1.417% w/w, respectively. Phytochemical analysis identified alkaloids, carbohydrates, flavonoids, and amino acids in all extracts, while tannins, terpenoids, and glycosides were not detected. Antibacterial activity was evaluated at 500, 1000, and 1500 µg/mL concentrations and compared to the standard drug amoxycillin. Each extract showed antibacterial effects against E. coli that increased with concentration. The petroleum ether extract was the most effective, achieving a 28 mm inhibition zone at 1500 µg/mL, followed by the chloroform extract at 26 mm and the methanolic extract at 25 mm. These findings suggest that watermelon seeds possess phytoconstituents with the potential to inhibit bacterial growth. The study suggests that Citrullus lanatus seeds may serve as a promising natural source of antimicrobial agents and could be further explored for the development of phytopharmaceutical formulations and value-added medicinal products.

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INTRODUCTION

1.1 Plant description:

Watermelon, a tropical species, flourishes in warm and sunny environments. It requires good drainage and fertile soil with a slightly acidic pH level. Watermelon is propagated from seeds and is frequently grown in the coastal regions, forest areas, and northern savannahs of Ghana [1]. Watermelons, scientifically referred to as Citrullus lanatus, are named for their significant water content (approximately 93%). The "melon" portion of the name pertains to their large, rounded shape and sweet, juicy flesh. The scientific name has its origins in Greek and Latin. "Citrullus" is derived from the Greek term "citrus," which may be a reference to the fruit itself. "Lanatus," from Latin, translates to "woolly," alluding to the seeds [2]. Watermelons (Figure: 1) are multi-purpose fruits with numerous applications. They can be enjoyed as a healthy snack, a delightful dessert, or a refreshing salad [3]. We can also concoct delectable beverages with the watermelon juice. The quality of various watermelon types is mostly determined by their sweetness and sugar content. Watermelons are extremely healthy, thirst-quenching, and low in calories despite their sweetness [4]. It is commonly known that watermelon seeds are extremely nutritious; they are rich in protein, vitamin B, minerals (including magnesium, potassium, phosphorus, salt, zinc, manganese, and copper), fat, and phytochemicals [5]. Often disregarded, watermelon seeds have enormous economic potential, particularly in impoverished nations. These seeds can be used to make a variety of goods, including sauces, flour, and snacks. Both cooking and cosmetics use the oil that is taken from them. As the market for these goods expands, the production of watermelon seeds [6].

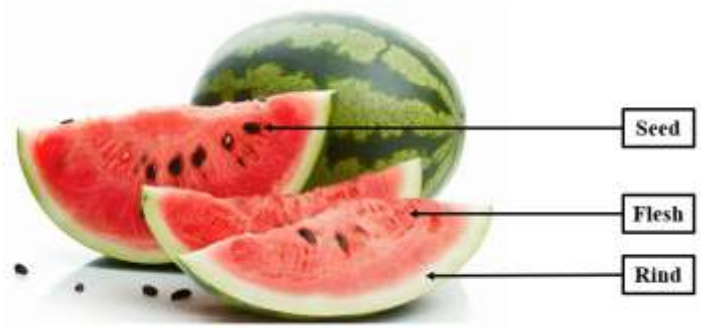

Fig 1: Watermelon Seed

1.2 Taxonomical classification: - [7]

- **Kingdom:** Plantae
- **Class:** Equisetopsida
- **Subclass:** Dilleniidae
- **Order:** Cucurbitales
- **Superorder:** Violence
- **Family:** Cucurbitaceae
- **Genus:** Citrullus
- **Species:** *C. lanatus*

1. MATERIALS AND METHODS:

2.1 Sample Collection:

Watermelon (*Citrullus lanatus*) seeds used for this study were obtained fresh from retailed fruit sellers from Barasat Market, north 24 pargana, West Bengal state.

2.2 Preparation of extract:

Fresh watermelon seeds were collected, washed thoroughly with distilled water after washing shade-dried at room temperature for

approximately 10–15 days. The dried seeds were then pulverized using a grinder to obtain a coarse powder. Extraction was carried out using a Soxhlet apparatus with solvents of increasing polarity petroleum ether, chloroform, and ethanol. Initially, 80 g of the powdered material was packed in a thimble made of filter paper and placed inside the Soxhlet extractor. Petroleum ether (60–80°C) was used as the first extraction solvent. Approximately 500 mL of petroleum ether was added to a round-bottom flask attached to the Soxhlet apparatus [8]. The extraction process was continued for 6–8 hours. After completion of petroleum ether extraction, the marc (residue) was removed, air-dried to eliminate residual solvent, and subjected to further extraction with chloroform. About 500 mL of chloroform was used, and extraction was continued for 6–8 hours under similar conditions. The residue obtained after chloroform extraction was again dried and subsequently extracted with 500 mL of ethanol using the Soxhlet apparatus for 8–10 hours. Ethanol extraction enabled the isolation of polar compounds such as phenolics, flavonoids, glycosides, tannins, and other bioactive constituents. The obtained petroleum ether, chloroform, and ethanol extracts were separately concentrated under reduced pressure using a rotary evaporator and further dried on a water bath to obtain semisolid masses [9]. The dried extracts were weighed to determine percentage yield and stored in airtight containers at 4°C for subsequent phytochemical and pharmacological studies. The extraction process is shown in figure no 2.

2.3 Phytochemical screening: -

Phytochemical analysis of the extracts were carried qualitatively laboratory techniques. Tests on the presence of alkaloid, flavonoids, glycosides, saponins and tannin were conducted accordingly [10].

2.3.1 Test For Alkaloid: -

2.3.1.1 Mayer's test: - Take 2ml of plant extract in a test tube. Add 1ml of potassium mercuric iodide solution (Mayer's reagent) to this test tube. Shake gently to mix correctly. Observe the formation of precipitates in the test tube. The test result is shown in the fig: 3.

Figure 3

2.3.1.2 Wagner's test: - Take 2ml of the extract and add Wagner's reagent contains Iodine-Potassium iodide solution. The reaction gives reddish-brown precipitate which confirms the

presence of alkaloids. The test result is shown in the fig: 4.

Figure 4

2.3.1.3 Dragendorff test: - Take 2ml of extract and add few drops of Dragendorff's reagent contains potassium bismuth iodide solution, Alkaloid forms a salt with bismuth and the reddish-brown precipitate forms that confirms the presence of alkaloids. The test result is shown in the fig: 5.

Figure 5

2.3.2 Test For Carbohydrate: -

2.3.2.1 Benedict Test: - 2 mL of extract solution. Add 2 mL of Benedict's reagents over the sample. Place the test tube over a boiling water bath and heat for 3–5 minutes or directly heat over a flame. Observe for colour change. The test result is shown in the fig: 6.

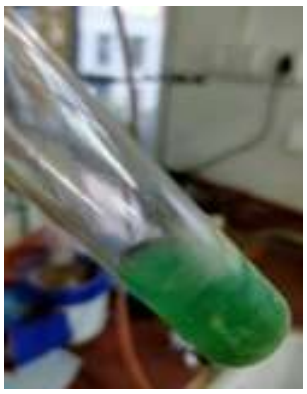

Figure 6

2.3.2.2 Fehling's Test: Take 1ml of given sample solution in a clean test tube. Add 1 ml of Fehling's solution A and Fehling's solution B to it. Keep the solution in a boiling water bath for about 10 minutes. the formation of red precipitate then the presence of carbohydrate is confirmed. The test result is shown in the fig: 7.

Figure 7

2.3.2.3 Molisch's Test: - To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulfuric acid through sides of test tube, purple to violet colour ring appears at the junction. The test result is shown in the fig: 8.

Figure 8

2.3.3 Tests for cardiac glycosides: -

2.3.3.1 Keller-killiani test :- Take 2ml of the extract add glacial acetic acid and add ferric chloride after that add few drops of conc sulfuric acid. Appear of bluish green color presence of deoxy sugar. The test result is shown in the fig: 9.

Figure 9

2.3.3.2 Bromine water test: Take 2 ml of extract solution and add few ml of bromine solution. Shake the test tube for 5 minutes if the solution colour changes to yellow that determine the presence of deoxy sugar.

2.3.4 Test for the Flavonoids: -

2.3.4.1 Alkaline reagent test: -Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCL, indicating that flavonoids were present. The test result is shown in the fig: 10.

Figure 10

2.3.4.2 Lead Acetate Test: - To 1 mL of Watermelon Solution added 1 mL of 10% lead acetate solution. Yellow precipitate formed, indicates Presence of flavonoids. The test result is shown in the fig: 11.

Figure 11

2.3.5 Test for tannins: -

2.3.5.1 2 mL of Watermelon seed Solution was taken with 2mL of distilled water and stirred followed by addition of a few drops of Ferric chloride solution, green precipitate appeared, showing presence of tannins. The test result is shown in the fig: 12.

Figure 12

2.3.6 Test for terpenoids: - Take 1 mL of Watermelon seed Solution followed by addition of 0.5 mL of acetic anhydride (acetic acid) and then a few drops of concentrated Sulfuric acid. Appearance of Bluish green precipitate confirmed presence of terpenoids. The test result is shown in the fig: 13.

Figure 13

2.3.7 Test For Amino acid: -

2.3.7.1 Ninhydrin test: - Took 1 mL of watermelon seed Solution and treated with few drops of Ninhydrin reagent, development of purple color confirmed presence of amino acids. The test result is shown in the fig: 14.

Figure 14

2.3.7.2 Xanthoproteic Acid Test: - Take one ml solution and add a few drops of marble chips. Now heat the solution with concentrated Nitric Acid. After cooling the solution add a few drops of Sodium Hydroxide. If it shows orange color then it confirms there are aromatic amino acids present. The test result is shown in the fig: 15.

Figure 15

2.3.7.3 Millon's test: - Take a 1ml sample, add a few drops of Millon's reagent, Shake the solution. Then add a few drops of Concentrated Nitric Acid. If red color shows, then it will be tyrosine**.** The test result is shown in the fig: 16.

Figure 16

2.3.7.4 Histidine Test: - First, take a 2ml sample, add a few drops of 5% bromine and 33% Acetic Acid solution and place for 10 minutes. Then add 2 ml of Ammonium Carbonate and boil for 5 minutes. If blue color shows, then it will be histidine. The test result is shown in the fig: 17.

Figure 17

2.4 Antimicrobial Activity of *Citrullus lanatus* seed extract: -

The Antibacterial susceptibility test was performed by agar-well diffusion method. The antimicrobial activity of the *Citrullus lanatus* seed extracts were tested against 1 microorganisms gram negative bacteria: - *Escherichia coli.*

2.4.6 Bacteria slant preparation

Bacterial slants were prepared using Mueller–Hinton agar (MHA) by dissolving the required quantity of medium in distilled water and heating until completely melted. The prepared medium was then dispensed into clean, dry test tubes, which were plugged with cotton and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the tubes were placed in an inclined position to allow the medium to solidify as a slant, providing a larger surface area for microbial growth. Once solidified, the slants were aseptically inoculated with the desired bacterial culture using a sterile inoculating loop and incubated at 35–37°C for 18–24 hours. The prepared bacterial slants were then stored at refrigerated conditions for further experimental use.

2.4.7 Bacteria plate preparation

Bacterial culture plates were created using Mueller–Hinton agar (MHA) by mixing the necessary amount of the agar medium with distilled water in a conical flask and heating it until fully dissolved. The flask was then sealed with cotton, covered in aluminum foil, and sterilized through autoclaving at 121°C for 15 minutes. After the sterilization process, the molten agar was allowed to cool to approximately 45–50°C to avoid condensation and contamination. In aseptic conditions, the sterile medium was poured into sterile Petri dishes and permitted to solidify at room temperature. Then solidified the petri dishes. The prepared bacterial plates were then stored at refrigerated conditions for further experimental use.

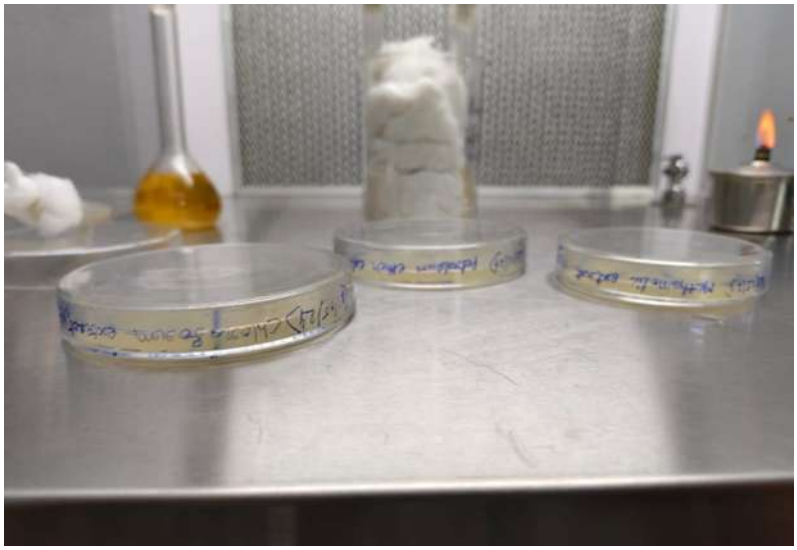

Figure 18: Bacteria plate preparation

2.4.8 Sample solution and standard solution preparation: -

In this we have to prepare the *Citrullus lanatus* seed extracts solution in 3 different concentration (500, 1000, 1500 µg/mL). *Citrullus lanatus* seed extracts were first prepared as sample solutions by dissolving 10 mg of each dried in 10 mL of dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL. Then measure 5 mg of extract and dissolved in 10 ml of DMSO solution to obtained the concentration 0.5 mg/mL. Again measure 15 mg of the extract and dissolved in 10 ml of DMSO to obtained the concentration 1.5 mg/ml.

Amoxycillin is used as a standard in this test. Firstly, we have to weight 10 mg of amoxycillin and dissolved in 10 ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL (1000 µg/mL) (Stock solution). Then take 1 ml from the stock solution and dissolved in 10 ml of dimethyl sulfoxide (DMSO) to obtain a concentration of 100 µg/mL.

2.4.9 Bacterial Transfer and Inoculation on Agar Plates

The transfer of bacteria onto agar plates was carried out under strict aseptic conditions to prevent contamination. A sterile inoculating loop was first flamed until red hot and allowed to cool. A small amount of the bacterial culture was then picked from a previously prepared slant. The sterile Mueller–Hinton agar (MHA) plates were slightly opened near a flame or inside a laminar airflow chamber, and the bacterial culture was gently transferred onto the surface of the agar. For uniform distribution, the inoculum was spread evenly over the surface using a sterile cotton swab (spread plate method) or streaked using an inoculating loop (streak plate method), depending on the experimental requirement. Care was taken to cover the entire surface to obtain a uniform lawn of bacterial growth, especially for antimicrobial studies [11].

2.4.10 Incubation Process

In this process we have to add the sample solution (*Citrullus lanatus* seed extracts**)** and the standard solution (Amoxycillin) in to the plates, then the agar plates were allowed to stand for a few minutes to ensure proper absorption of the inoculum into the medium. The inoculated plates were incubated in an incubator at a temperature of 35–37°C for 18–24 hours, depending on the growth requirements of the bacterial species. During

incubation, proper environmental conditions such as temperature and humidity were maintained to promote optimal bacterial growth. After the incubation period, the plates were observed for bacterial growth, colony formation, or zones of inhibition in case of antimicrobial activity testing. The antimicrobial activity of the *Citrullus lanatus* seed extracts was shown in the table no 3.

2. RESULT AND DISCUSSION: -

3.1 Extraction value result: -

In the process of extraction of the *Citrullus lanatus* seed powder with three solvents (Petroleum ether, Chloroform and ethanol). The extraction yield result shown in the Table no 1. By this table we have to conclude that the ethanolic extract has show better percentage of Yield.

Table no 1: - Extraction value of *Citrullus lanatus* seed

Sr. No.	Solvent	Colour	Consistency	% Yield w/w
1.	Petroleum ether	White	Greasy	1.417
2.	Chloroform	Yellowish white	Greasy	2.032
3.	Ethanol	Yellowish dark white	Greasy	3.529

3.2 Phytochemical tests result: -

The phytochemical analysis revealed that the seeds of Helianthus annuus extract contain carbohydrates, alkaloids, flavonoids, tannins, saponins, phytosterol, steroids, and fixed oils and fats [Igbinosa *et al.,* 2009]. The diverse phytochemical compounds found in the seed extract, including flavonoids, alkaloids, and saponins, have been documented to exhibit biological activity against microorganisms [Sidambaram *et al.,* 2011]. The phytochemical tests results shown in the table no 2.

Table no 2: - Phytochemical test result of *Citrullus lanatus* seed

Test for	Methanol	Chloroform	Petroleum ether
Alkaloids	(+)	(+)	(+)
Carbohydrate	(+)	(+)	(+)
Flavonoids	(+)	(+)	(+)
Tannins	(-)	(-)	(-)
Terpenoids	(-)	(-)	(-)
Glycosides	(-)	(-)	(-)
Amino acids	(+)	(+)	(+)

3.3 Anti- microbial activity result: -

By evaluating the zone of inhibition at various doses, the antibacterial activity of the standard and solvent extracts against *Escherichia coli* was assessed (Table 3). At a dose of 100 µl, the standard medication demonstrated good antibacterial activity, producing inhibitory zones of 20 mm (CH), 22 mm (PE), and 25 mm (ME). The activity of the chloroform extract increased in a concentration-dependent manner. The inhibitory

zone was 12 mm at 500 µg/ml, 18 mm at 1000 µg/ml, and 26 mm at 1500 µg/ml. The chloroform extract's maximum activity was marginally higher than that of the conventional methanolic control. Also, the antibacterial activity of the petroleum ether extract increased with concentration. Out of all the extracts examined, the zones of inhibition were the strongest, measuring 14 mm at 500 µg/ml, 19 mm at 1000 µg/ml, and 28 mm at 1500 µg/ml. With inhibitory zones of 15 mm, 17 mm, and 25 mm at 500, 1000, and 1500 µg/ml, respectively, the methanol extract also showed moderate to high antibacterial activity. The petroleum ether extract at 1500 µg/ml showed the greatest zone of inhibition (28 mm), but all extracts showed dose-dependent antibacterial activity against *E. coli*.

Table 3: - Antimicrobial activity result

Bacteria used in zone of inhibition	**Standard Conc (mg/ml)**			**Chloroform Conc (mg/ml)**			**Petroleum ether Conc (mg/ml)**			**Methanol Conc(mg/ml)**		
Dose (µl)	**0.1**	**0.1**	**0.1**	**0.5**	**1**	**1.5**	**0.5**	**1**	**1.5**	**0.5**	**1**	**1.5**
Escherichia coli	**20 mm**	**22 mm**	**25 mm**	**12 mm**	**18 mm**	**26 mm**	**14 mm**	**19 mm**	**28 mm**	**15 mm**	**17 mm**	**25 mm**

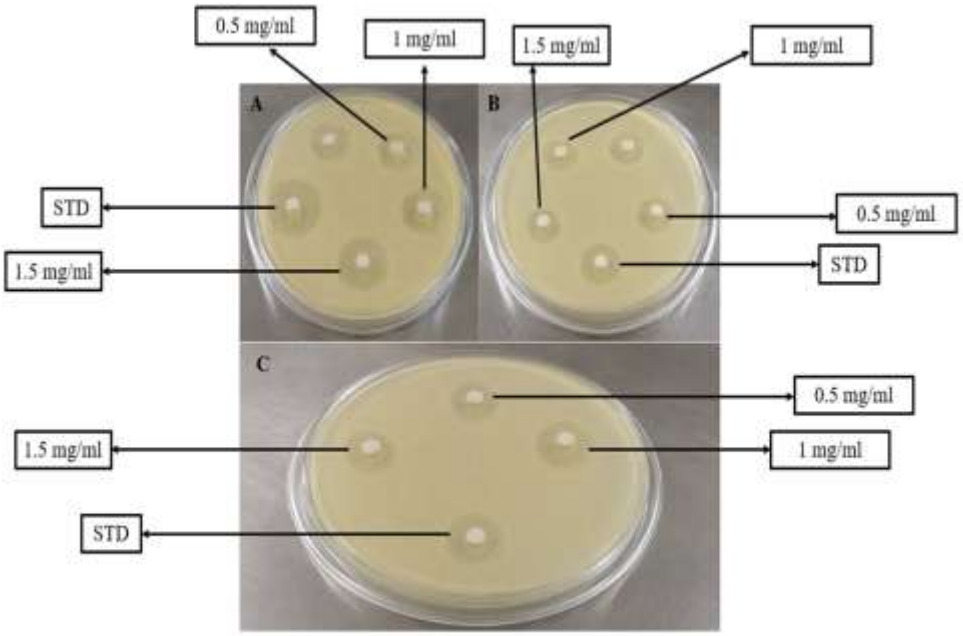

Fig 19: Antimicrobial activity result of Citrullus lanatus seed extracts
A) Methanol extract. B) Chloroform extract and C) Petroleum ether extract

CONCLUSION

The current research indicated that the seeds of Citrullus lanatus (watermelon) contain notable phytochemical components and exhibit considerable antibacterial properties, underscoring their potential as a valuable natural source of bioactive substances. Utilizing sequential Soxhlet extraction with petroleum ether, chloroform, and methanol, it was found that the methanolic extract yielded the highest extraction percentage, suggesting a more effective recovery of polar phytochemicals. Qualitative phytochemical analysis confirmed the presence of alkaloids, carbohydrates, flavonoids, and amino acids across all extracts, while tannins, terpenoids, and glycosides were not detected. These phytoconstituents are recognized for contributing to a range of biological activities, including antimicrobial and antioxidant effects, thereby reinforcing the medicinal significance of watermelon seeds. The antibacterial assessment against Escherichia coli revealed concentration-dependent effectiveness for all extracts tested. Among the extracts evaluated, the petroleum ether extract demonstrated the strongest antibacterial properties, achieving a maximum zone of inhibition of 28 mm at a concentration of 1500 µg/mL, closely followed by the chloroform and methanolic extracts. These results suggest that watermelon seeds, which are typically regarded as agricultural waste, can be an effective source of natural antimicrobial substances. In summary, the research emphasizes the potential pharmaceutical and nutraceutical uses of Citrullus lanatus seeds. Nonetheless, further studies focusing on the isolation and characterization of the active compounds, toxicity assessments, and in vivo pharmacological investigations are advised to confirm their safety, effectiveness, and therapeutic potential for upcoming clinical and industrial uses.

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