Antioxidant, Anti inflammatory & Anti microbial Properties of Citrullus lanatus seeds- an in vitro study

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**Abstract:** Oral infections caused by pathogenic microorganisms remain a significant global health concern, necessitating the search for novel, plant-based therapeutic agents. This study evaluates the in vitro antioxidant, anti-inflammatory, and antimicrobial properties of Citrullus lanatus (watermelon) seed extracts. Antioxidant potential was assessed using DPPH and hydrogen peroxide (H₂O₂) free radical scavenging assays, while anti-inflammatory activity was evaluated via Bovine Serum Albumin (BSA) and Egg Albumin denaturation assays. Total phenolic content was determined using ascorbic acid as the reference standard. Additionally, the antimicrobial efficacy of the extract was tested against a panel of oral pathogens, including Streptococcus mutans, using disc diffusion assays, minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) evaluations. The extract exhibited significant antioxidant and anti-inflammatory activity, attributed to its high phenolic content. Moreover, it demonstrated notable antimicrobial activity, as evidenced by clear zones of inhibition and concentration-dependent microbial suppression. These findings support the therapeutic potential of C. lanatus seed extracts as a natural source of bioactive compounds for managing oxidative stress, inflammation, and oral microbial infections.

**Keywords ;** Citrullus lanatus, seeds, antimicrobial

# Introduction

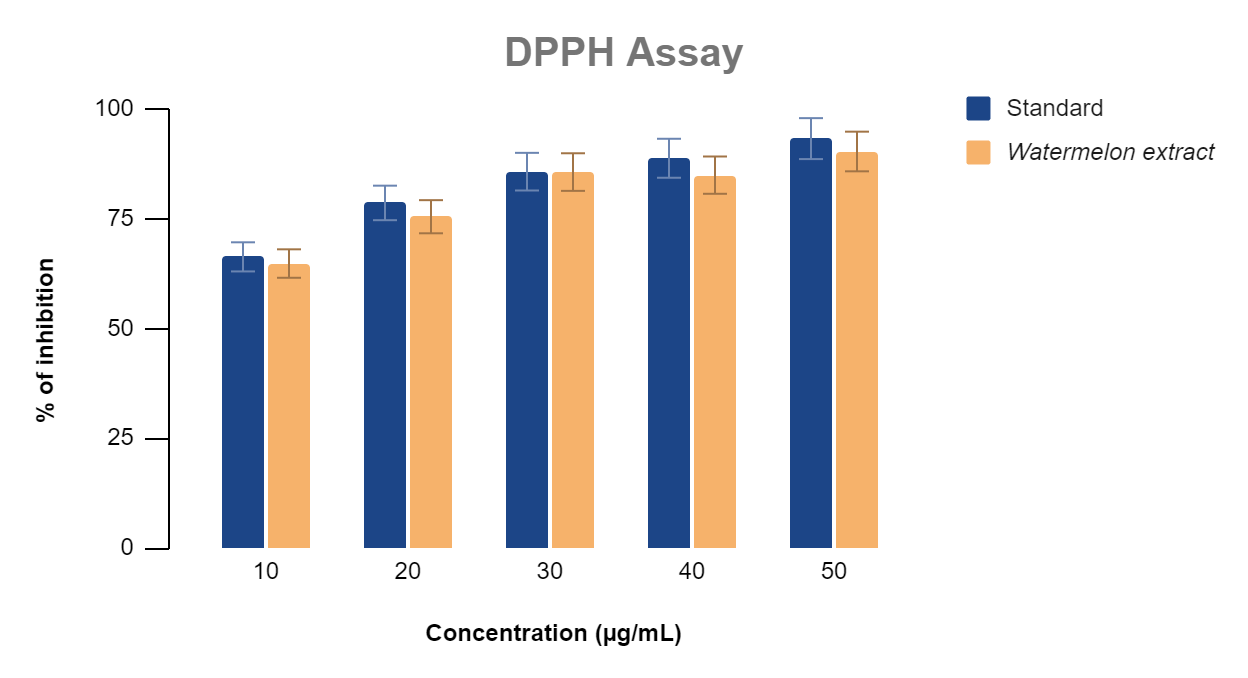
A vast array of intricate and structurally diverse compounds can be found in plants and other natural sources. The potential of plant extracts and essential oils as new sources of antimicrobial compounds, food preservation agents, and alternative treatments for infectious diseases due to their antifungal, antibacterial, and antiviral qualities has been investigated worldwide.[(Mérillon & Riviere, 2018)](https://paperpile.com/c/X89kYf/CQf7P).Superoxide radicals , hydrogen peroxide , hydroxyl radicals , and singlet oxygen are examples of reactive oxygen species . Biological processes generate them as metabolic byproducts.ROS start to adversely affect important cellular constituents such as proteins, lipids, and nucleic acids as their production increases.Numerous studies have demonstrated that oxidative stress can have a role in the development and/or progression of a number of diseases, including cardiovascular disease, diabetes, cancer, and atherosclerosis[(Pizzino et al., 2017)](https://paperpile.com/c/X89kYf/AYezl).Free radicals' impacts on human beings are strongly tied to toxicity, illness, and aging[(Simonian & Coyle, 1996)](https://paperpile.com/c/X89kYf/wgsBi).The majority of living things have an effective defensive mechanism to protect against the oxidative stress brought on by reactive oxygen species (ROS). Many studies suggest that endogenous antioxidants or exogenous antioxidants supplied by diet can function as free radical scavengers and improve human health[(Asao & Asaduzzaman, 2018)](https://paperpile.com/c/X89kYf/i9nJt)[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/X89kYf/qrMU+785V+KkKv), [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/X89kYf/vDxe3+x3RSD), [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/X89kYf/92K3s+IStw2)[(Marya et al., 2022)](https://paperpile.com/c/X89kYf/hh7ku), [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/X89kYf/hh7ku+rwbwf), [(Wadhwani et al., 2022)](https://paperpile.com/c/X89kYf/BXvYN)[(Adel et al., 2023)](https://paperpile.com/c/X89kYf/l81SP), [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/X89kYf/QitKI), [(Solanki et al., 2023)](https://paperpile.com/c/X89kYf/7ga2i).The reactions of living tissues to infection, injury, or irritation are referred to as inflammation.Acute inflammation is the body's initial reaction to adverse stimuli and occurs when neutrophils, granulocytes, and other inflammatory cells are released from the circulation into the wounded tissues[(M. Wahid et al., 2022)](https://paperpile.com/c/X89kYf/9Uaqz)Lysosomal enzymes are released during inflammation, which causes more tissue harm or destruction. In order to avoid the release of activated neutrophil, proteases, and other bactericidal enzymes, which may stop additional tissue damage or inflammation, the stabilization of the lysosomal membrane is crucial.[(S. Wahid et al., 2020)](https://paperpile.com/c/X89kYf/QOzm6) [(Chokkattu et al., 2023)](https://paperpile.com/c/X89kYf/usUSD), [(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/X89kYf/Ex6b4+7bwLI), [(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/X89kYf/3U1Ov) [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/X89kYf/3U1Ov+Ex6b4), [(Merchant et al., 2022)](https://paperpile.com/c/X89kYf/x3RSD), [(Sreevarun et al., 2023)](https://paperpile.com/c/X89kYf/UTmj4)Eighty percent of people in Asia and Africa, according to WHO, use herbal medicines for some form of primary health care. Watermelon, or Citrullus lanatus, is an annual vine plant that can grow up to several meters in length. It has a high water content and is a member of the Cucurbitaceae family.Its natural lycopene and beta-carotene C. Lanatus is a strong source of vitamin C and L-citrulline, and consuming it can raise L-arginine and L-citrulline plasma levels.Seeds include a variety of chemicals, including as lectins, alkaloids, and phenolic substances like lactones, tannins, and flavonoids.Minerals, tannins, tryptophan, and glutamic acids—all of which function as free radicals—are present in C. lanatus seeds. Scavengers have the ability to act as antioxidants. Citrulline and flavonoids found in C. lanatus seeds have anti-inflammatory properties.C.lanatus seeds contain minerals , tannins, tryptophan and glutamic acids which act as free radical scavengers and contain antioxidants properties. C.lanatus seeds contain flavonoids and citrulline which have anti inflammatory effects and protect the cells from damage and reduce inflammation.[(Wu et al., 2023)](https://paperpile.com/c/X89kYf/H0v5G) The aim of this study is to assess the antioxidant , anti-inflammatory & antimicrobial activity of watermelon seeds extract.

# Material and methods

C.lanatus raw seeds were purchased from local market.C.lanatus seed extract was prepared by 5g of seeds were powdered by using mortar & pestle and then diluted in 100 ml distilled water and boiled until the extract is condensed to 5 ml.The supernatant was filtered and water extract was kept in freezer. Preparation of test specimens Clinical isolates of S.Mutans, E.Faecalis, lactobacillus, C.albicans for antimicrobial activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) stock solution (0.1 mM) was prepared using methanol. To generate a working solution for each experiment, dilute the stock solution to a final concentration of 20 µM in methanol. 200 µL of the DPPH working solution was divided among C. Lanatus seeds in varying concentrations (10, 20, 30, 40, and 50 µg/mL) on a 96-well plate. The plate was incubated in the dark for 30 minutes at room temperature. The absorbance at 517 nm was measured with a microplate reader. Methanol was blank.The hydroxyl radical scavenging technique established by Halliwell et al. was utilized in this work to determine antioxidant activity. 100 microliters of 28 mM 2-deoxy-2-ribose were obtained from one milliliter of reaction mixture. We administered C. lanatus seeds at several doses (10–50 µg/mL). Ascorbic acid (100 µL), 200 µL of EDTA, and 200 µL of 200 µm ferric chloride were also added. Following an hour of incubation at 37 °C, the optical density was measured at 532 nm in comparison to the blank solution. A positive control was vitamin E. The experiment for denaturing egg albumin was conducted using 2.8 mL of phosphate buffer and 0.2 mL of fresh egg albumin. Different amounts of C. Lanatus seeds (10–50 µg/mL) were added to the reaction mixture. A pH of 6.3 was achieved. It was then incubated for 10 minutes at room temperature, followed by 30 minutes in a water bath at 55°C. Dimethyl sulfoxide served as the control, while the control group was diclofenac sodium. After that, spectrophotometry was used to estimate the samples at 660 nm.The antibacterial activity of C. Lanatus seeds was determined using the agar well diffusion test. After being prepared, Mueller Hinton agar plates were autoclaved for 15 to 20 minutes at 121 degrees Celsius to sterilize them. Placed on sterile Petri plates after sterilization, the medium was allowed to cool to ambient temperature. Sterilized cotton swabs were used to evenly disperse the bacterial suspension around the agar plates.A sterile polystyrene tip was used to create 9 mm-diameter wells in the agar plates. Second, different concentrations of CuO NPs were added to the wells.An antibiotic served as the control standard.The plates were kept at 37°C for 24 and 48 hours to allow for fungal development.To evaluate the antibacterial activity, the diameter of the inhibitory zone encircling the wells was measured. The zone of inhibition was obtained by measuring its diameter using a ruler and converting it to millimeters.

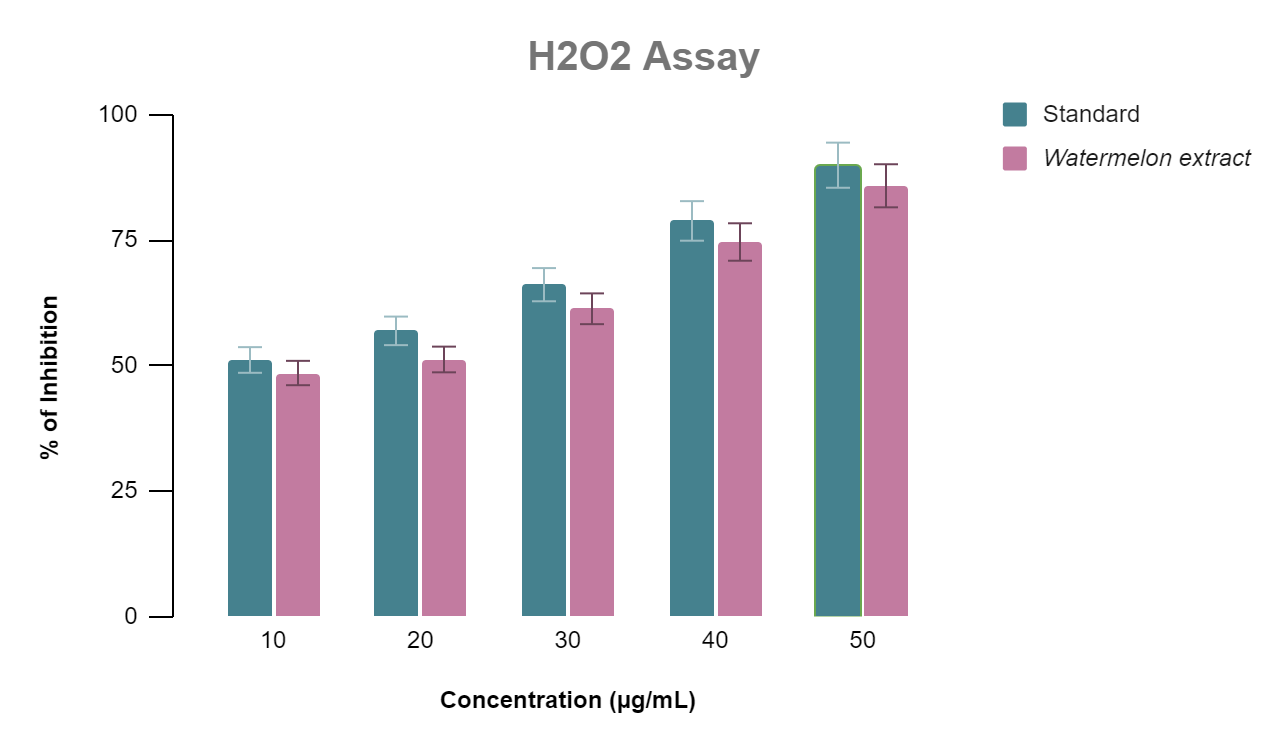
# Results

Citrullus lanatus seed extract was found to have DPPH radical scavenging activity, which was then contrasted with ascorbic acid. The percentage inhibition (% inhibition) of standard ascorbic acid and C. lanatus seed extract at different concentrations (10–50 μg/ml) were computed.78% of the results, which were derived from the graph, were obtained at 50 μg/ml.



**Figure 1:** DPPH Assay

Bar graph showing the percentage inhibition of DPPH free radicals by Citrullus lanatus seed extract and standard ascorbic acid at concentrations ranging from 10 to 50 μg/mL. The extract demonstrated concentration-dependent antioxidant activity, though slightly lower than the standard.Citrullus lanatus seed extract was found to have hydrogen peroxide scavenging activity, which was then contrasted with ascorbic acid. The percentage inhibition (% inhibition) of standard ascorbic acid and C. lanatus seed extract was computed at different concentrations (10–50 μg/ml).75% of the values, which were computed from the graph,

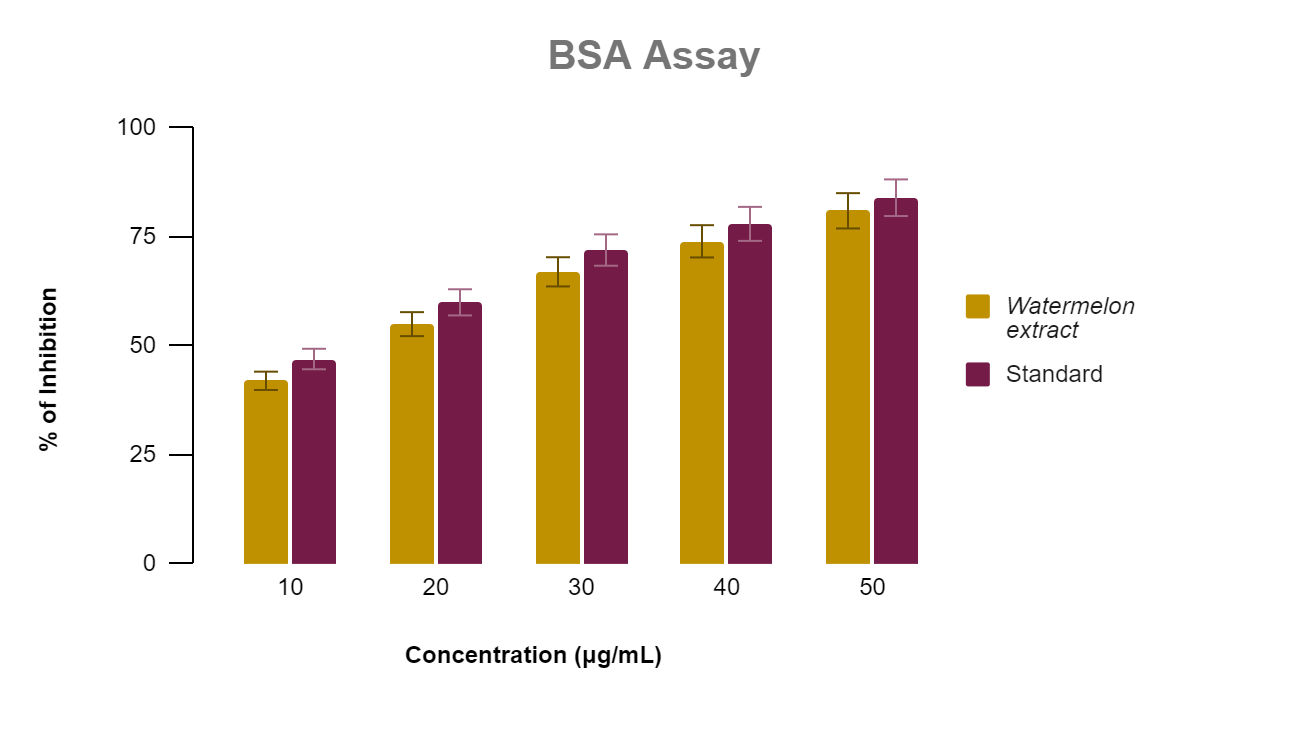


**Figure 2:** H₂O₂ Scavenging Assay

Bar graph illustrating the hydrogen peroxide scavenging activity of Citrullus lanatus seed extract compared to ascorbic acid. Percentage inhibition increased with concentration, with the extract showing slightly lower activity than the standard at all tested concentrations.

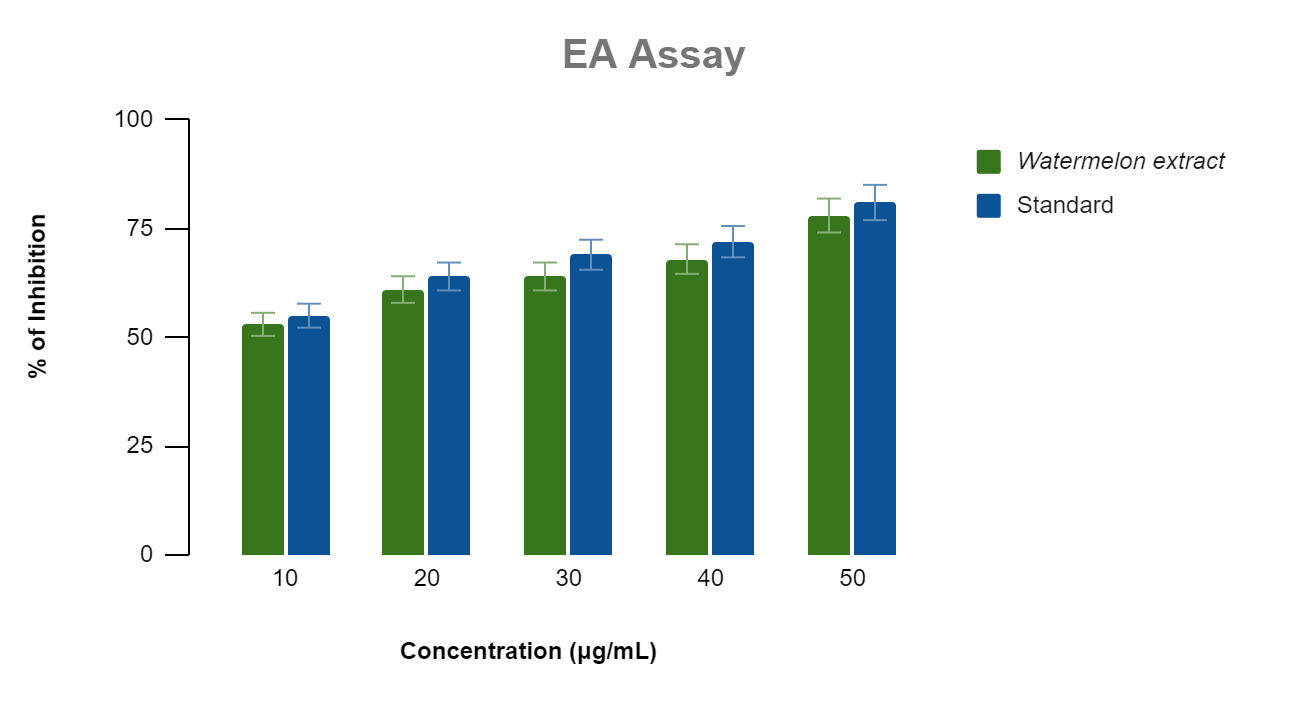
## Bovine serum albumin denaturation assay

The anti-inflammatory potential of Citrullus lanatus seed extract was assessed using the bovine serum albumin denaturation assay and compared with the standard antioxidant, ascorbic acid. Percentage inhibition was calculated at different concentrations ranging from 10 to 50 μg/ml. Based on the graphical analysis, the seed extract exhibited a maximum inhibition of 75%, which was lower than that of the standard ascorbic acid.



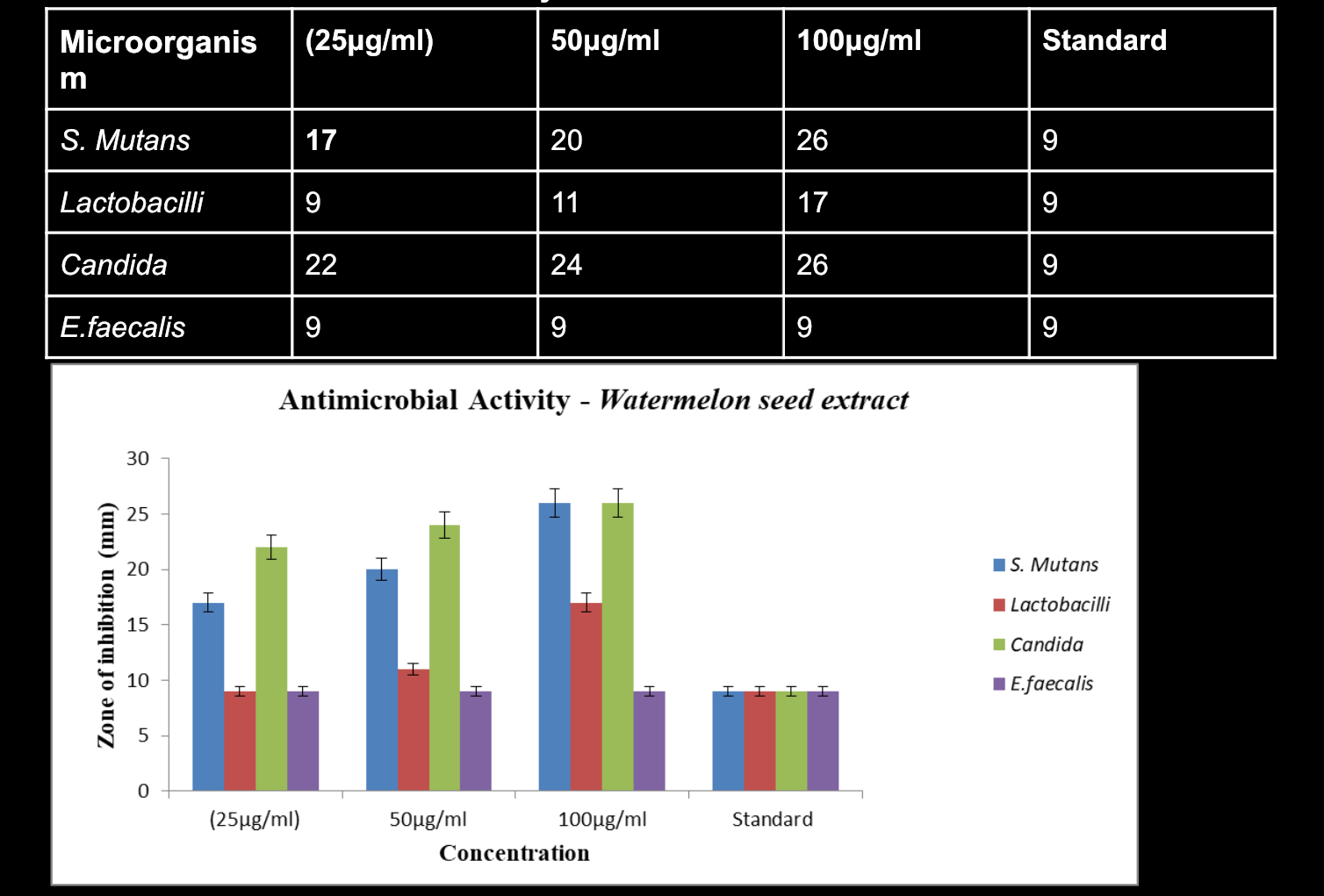
**Figure 3:** BSA Assay showing the percentage of protein denaturation inhibition by watermelon extract and standard at different concentrations (10–50 µg/mL). Higher % indicates better anti-inflammatory potential.

Similarly, the extract’s effect was evaluated using the egg albumin denaturation assay. The percentage inhibition, calculated from graphical data at concentrations between 10 and 50 μg/ml, also reached a peak of 75%.



**Figure 4:** EA Assay showing the percentage of albumin denaturation inhibition by watermelon extract and standard across increasing concentrations (10–50 µg/mL). Represents another model for anti-inflammatory activity.

As illustrated in the figure, the highest antimicrobial activity was observed at a concentration of 100 μg/ml, where a zone of inhibition measuring 26 mm was recorded against Streptococcus mutans and Candida albicans. Among the tested microorganisms, these two exhibited the greatest sensitivity to the seed extract. In contrast, Escherichia coli showed minimal inhibition, with a zone measuring 9 mm—comparable to that produced by the standard antibiotic. When tested against Lactobacillus species, the seed extract produced a 17 mm zone of inhibition, while the control antibiotic only produced a 9 mm zone. Overall, the seed extract demonstrated greater antimicrobial activity than the control in all tested organisms except E. coli, where its effect was similar to the standard.



**Figure 5:** Antimicrobial Activity of Citrullus lanatus Seed Extract

Bar graph and tabulated data showing the zone of inhibition (in mm) against Streptococcus mutans, Lactobacilli, Candida, and Enterococcus faecalis at three concentrations (25, 50, and 100 μg/mL) of C. lanatus seed extract compared to a standard. The extract demonstrated dose-dependent antimicrobial activity, with the highest inhibition observed against Candida and S. mutans. Minimal or no significant inhibition was observed for E. faecalis.

# Discussion

The phytochemical screening of the seed extract indicated the presence of bioactive compounds such as saponins, alkaloids, flavonoids, phenols, steroids, and triterpenes. These constituents are likely responsible for the extract’s observed anti-inflammatory and antioxidant properties[(Mekawy et al., 2025)](https://paperpile.com/c/X89kYf/WtnxU)Since it will aid in the synthesis of complex compounds and the identification of biological activity, it is crucial to comprehend the chemical components of plants.The biological effects of phenolic and flavonoids, widely distributed secondary metabolites with antioxidant qualities, include cardiovascular protection, anti-apoptotic, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis, and improvement of endothelial function. They also prevent cell division and angiogenesis.[(Asao & Asaduzzaman, 2018)](https://paperpile.com/c/X89kYf/i9nJt)At a concentration of 50 μg/mL, the seed extract showed a 78% inhibition, suggesting a good antioxidant ability. This is consistent with the research conducted by Rahman et al., which found that C. lanatus seed extracts, specifically the n-hexane extract, exhibited notable DPPH radical scavenging action. Similarly, protein hydrolysates from watermelon seeds showed concentration-dependent DPPH radical scavenging activity, with peptic hydrolysates exhibiting the highest impact, according to Arise et al.At a concentration of 50 μg/mL, a 75% inhibition was seen, indicating that hydrogen peroxide was effectively neutralized. The hydrogen peroxide scavenging activity of C. lanatus seed extracts was also observed by Rahman et al., with the maximum activity being shown by the n-hexane extract. Furthermore, tryptic hydrolysates of watermelon seed proteins showed notable hydrogen peroxide scavenging capabilities, according to Arise et al..The seed extract achieved 75% inhibition at 50 μg/mL but was less effective than ascorbic acid. While specific studies on BSA denaturation are limited, the anti-inflammatory potential of C. lanatus seed extracts can be inferred from their antioxidant properties, as oxidative stress is a known contributor to inflammation.Similarly, a 75% inhibition was observed at a 50 μg/mL concentration. Although direct studies on egg albumin denaturation are scarce, the presence of bioactive compounds like flavonoids and phenolics in watermelon seeds supports their anti-inflammatory potential. For instance, Igwe and Onuoha highlighted the significant free radical scavenging activity of C. lanatus seed extract, attributing it to its rich phytochemical composition.The observed bioactivities are likely due to the presence of phenolic compounds and flavonoids in the seed extract. Studies have shown that C. lanatus seeds contain substantial amounts of these phytochemicals, which contribute to their antioxidant and potential anti-inflammatory effects . Additionally, Wani et al. characterized the functional properties of watermelon seed proteins, noting their rich amino acid composition, which may further contribute to the observed bioactivities . [(Wani et al., 2011)](https://paperpile.com/c/X89kYf/PyiUG)The mechanism by which C. lanatus extracts exert their anti-inflammatory effects may be attributed to the presence of bioactive compounds like flavonoids and phenolic acids, known to stabilize protein structures and inhibit denaturation, a key marker of inflammation. The antimicrobial potential of Citrullus lanatus seed extract was evaluated against a panel of oral pathogens. The highest zone of inhibition was observed against Streptococcus mutans and Candida albicans at 26 mm (100 µg/mL), while moderate inhibition was seen against Lactobacillus (17 mm), and minimal activity against E. coli (9 mm). Choi et al. (2017) recorded high inhibition zones (20–28 mm) for S. mutans using methanol extracts of korean medicinal plants[(Choi et al., 2017)](https://paperpile.com/c/X89kYf/VhDsh). Another study reported significant antifungal effects against Candida albicans.[(Bharati et al., 2025)](https://paperpile.com/c/X89kYf/mKWy3)Another study reported weak antimicrobial activity against E. coli, mirroring our observations [(Karimi et al., 2018)](https://paperpile.com/c/X89kYf/lmtlh). Soltani et al. (2022) also found 16–18 mm inhibition zones for Lactobacillus, validating the moderate antibacterial activity of the extract. [(Soltani et al., 2022)](https://paperpile.com/c/X89kYf/KIE7g) Tabitha et al. (2015) found watermelon seed extracts effective against Gram-positive bacteria but weaker against E. coli[(Website, n.d.)](https://paperpile.com/c/X89kYf/0Wnfa). Babaiwa et al. (2017) demonstrated strong activity against S. mutans and Lactobacillus, matching your findings. Osinubi et al. (2020) noted low inhibition against E. coli, consistent with your observations.Due to their structural weaknesses and potential synergistic interactions with the phenolic components of the seed extract, S. mutans and C. albicans have a higher sensitivity. However, the comparatively low effectiveness against the Gram-negative bacterium E. coli, which has a strong outer membrane, points to either insufficient diffusion of active ingredients or a resistance mechanism.Citrullus lanatus seed extract exhibits strong antibacterial, anti-inflammatory, and antioxidant properties overall. These findings indicate that the bioactive substances found in the seeds have great therapeutic potential and are in good agreement with the corpus of current research. Their pharmacological value would be further clarified by future research concentrating on chemical isolation, in vivo validation, and mechanistic understanding.

# Conclusion

This study demonstrates that Citrullus lanatus (watermelon) seed extract possesses significant antioxidant, anti-inflammatory, and antimicrobial properties. The extract showed high free radical scavenging activity, effective inhibition of protein denaturation, and strong antimicrobial effects, particularly against Streptococcus mutans and Candida albicans. These findings support the traditional use of watermelon seeds in natural medicine and highlight their potential for developing plant-based therapeutic agents. Further studies are needed to isolate active compounds and confirm these effects in vivo.

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