Journal of Biological Sciences and Public Health ISSN 3007-9535
December, 2024, 2: 11-18

Extraction of lipase from waste fruit seeds and its biomedical applications

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ABSTARCT

Background: Lipases have widespread applications in the field of leather and detergent industry. **Aim:** The aim of this study was the production of lipase from various fruit seeds and its usage in detergent industry and in laundry product for removing oil and fat stains. **Methods:** In the present study three waste fruit seeds (water melon, honeydew melon, and cantaloupe melon) were used for the extraction and partial purification of lipase. Initially extraction of crude enzyme was carried out and partial purification of lipase done by ammonium sulphate precipitation method. Both crude and purified lipase product were screened for lipolytic activity using tween 80. Purified extract of all the samples showed the maximum tween 80 hydrolysis compared to the crude extract. The antibacterial activity of crude and purified extracts were performed against six clinical pathogens (*Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Klebsiella pneumonia, Staphylococcus aureus*, and *Staphylococcus epidermidis*). **Results:** The purified extract of water melon showed maximum zone of inhibition against all the pathogens except *Staphylococcus aureus* and *Klebsiella pneumonia*. Purified extract of honeydew melon seeds had great antibacterial effect compared to crude extract. Purified extract showed maximum zone of inhibition against all the pathogens. Purified extract of cantaloupe melon showed maximum inhibition of all tested bacterial pathogens compared to crude extract. Similarly, lipolytic enzyme extracted from waste fruit seed were also efficiently involved in the removal of oil and fat stains. **Conclusion:** it was concluded that extract of waste fruit seeds may serve as an antibacterial and destaining agents.

Key words: Aantibacterial activity, Lipase production, waste fruit seeds, destaining effect, bacterial pathogens

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Article Information:
Received: 13-02-2024
Revised: 20-11-2024
Accepted: 02-12-2024
Published: 22-12-2024

Author Contribution:
SA conceived the idea, designed the experiments, analyzed and interpreted the results, and was a major contributor in manuscript writing.
MM and HJ performed all the experiments and wrote the first draft of the manuscript.
KA contributed to formal analysis, interpreted results and manuscript review.

INTRODUCTION

Lipase is a biocatalysts that catalyze triacylglycerol hydrolysis into mono-and diacylglycerols and free fatty acids (Farzad Mardani Kataki, 2016). Lipases have widespread applications, could be found in various industries, including but not limited to the: pharmaceutical, food, leather, textile, biofuel and paper production, detergent, cosmetic and fat-processing (Singh et al., 2010; Saisubramanian et al., 2006; Alonso et al., 2005; Barros et al., 2010; Sharma et al., 2001). Most of the commercially produced lipases are used as flavor improvement components as well as they are used in processing of dairy products such as: meat, vegetables, fruit, milk product, baked foods, and beer, as well as used in household care, biodiesel, cosmetics, and pharmaceuticals (Kumar et al., 2016; Ansorge-Schumacher and Thum, 2013; Tecelão et al., 2012; Patel, 2011; Tan et al., 2010; Farahat et al., 1990). In food production lipid with high level of unsaturated fatty acid (Reshma et al., 2008), fragrance production and ripening of cheeses are also lipase applications (Salah et al., 2007). Lipase also have played valuable role in detergent industry as a cleaning agent. Enzyme based detergents are cheaper and does not induce any harm to the environment. Now biological cleaning system becoming more beneficial than caustic or acidic cleaning system (D'Souza and Mawson, 2005). In medical applications lipases serve as therapeutic agents in combination with other components against various diseases such as: dyspepsia, gastrointestinal disturbances, cutaneous manifestations of digestive allergies, cancer treatment, lipases also serve as diagnostic tool in medicine, thereby justifying the growing demands

(Tardelli et al., 2020; Sarmah et al., 2018; Arpigny et al., 1999). Lipases are gaining increasing prominence in biotechnology due to their versatility in both synthesis and hydrolysis processes (Ali et al., 2023).

In animals, plants and micro-organisms lipases are found (Chandra et al., 2020; Bharathi and Rajalakshmi, 2019; Javed et al., 2018; Seth et al., 2014; Ejedegba et al., 2007). In biotransformation reactions plant lipases may represent remarkable substitute for microbial and animal lipases (Palocci et al., 2003). Lipases isolated from bean, sunflower Seed (*Heliantus annuus L.)*, canola (*Brassica napus L.*), rice (*Oryza sativa*), wheat (*Triticum aestivum L.*), corn (*Zea mays L.*), barley (*Hordeum vulgare*), and sesame (*Sesamum indicum L.*) (Jensen, 1983). During germination of seeds such as white melon (*Cucumeropsis manni*) represent the good source of lipase, which are hydrolyzed by the action of endogenous lipases (Jachmanian at el., 1995). The activity of the lipase is high during germination and it is rate controlling (Ejedegba et al., 2007). In some cases, plant lipases are more beneficial than animal or microbial lipases due to some features like they are very specific in their activity, they have low cost, they are easily available and can be purified easily (Barros et al., 2010).

Recently, seed lipases have gained great attention in the field of biocatalysts. Due to some very interesting features such as specificity, low cost, availability, and easy purification these enzyme present advantages over lipases which are obtain from animals or microbes and is represent a great substitute for possible commercial use as industrial enzymes (Polizeli et al., 2008; Paques and Macedo, 2006; Enujiugha et al., 2004; Hellyer, et al. 1999). The aim of current research was to extract and purify lipase from the waste fruit seeds of water melon (*Citrullus lanatus*), honeydew melon (*Cucumis melon*) and cantaloupe (*Cucumismelo cantalupensis*) and to evaluate their biomedical applications.

MATERIALS AND METHODS

Chemicals, glassware and equipments

All chemicals and solutions including; Nutrient Agar, Nutrient Broth, NaCl solution, Na-Phosphate (Na_2HPO_4-NaH_2PO_4) buffers pH (8.0), 3 mM DDT (dithiothreitol), 1 mM EDTA, 10 Mm sodium metabisulphate, and 50 mM Tris buffer (pH 8.0), Muller Hington Agar, peptone, tween 80, $CaCl_2$, 0.01 M phosphate buffer, 2% formaldehyde 1.5 ml blue tips, Laminar flow, beakers, Petri plates, mortar and pestle, centrifuge machine, autoclave, incubator 37^0C, shaking incubator 37^0C, test tubes and test tubes rack were from sigma Aldrich and Oxide company.

Seed collection and germination

Waste fruit seeds of Water Melon (*Citrullus lanatus*), Honeydew Melon (*Cucumis melon*) and Cantaloupe (*Cucumismelo cantalupensis*) were collected from household waste and rinsed with the running tap water to clean the seeds. All seeds were dried with tissue papers and allowed to germinate in Petri plates on filter paper. The seeds were moistened with distilled water to keep the moisture balance for seed germination. Furthermore, NaCl solution and Na-Phosphate (Na_2HPO_4-NaH_2PO_4) buffers (pH 8) were added and kept in a dark room for 120 h or up to seed germination. The germinating seeds were washed with distilled water for further analysis.

Extraction and purification

The germinated seeds were collected and lipase extraction was done using homogenization in a food processor with the solution containing 1 mM EDTA, 10 mM sodium metabisulphite, 50 mM Tris buffer (pH 8.0) and 3 mM DDT (dithiothreitol). After homogenization samples clarification was done by using centrifugation at 3000 rpm for 30 min (Polizelli et al., 2008; Tan et al., 2015). The filtrate again was dissolved in 100 mL distilled water and again centrifuge at 7500 rpm for 1 min. The supernatant was collected can be used as crude lipase extract. Crude lipase extract was saturated with ammonium sulphate (80%), and again centrifuged at 10,000 rpm for 20 min. Collected pellet was dissolved in 20 mL Tris-Cl buffer (10 mM, pH 8.5) and dialyzed for overnight (Eze et al., 2005). The dialyzed enzyme was used as partially purified lipase extract.

Lipolytic assay

Lipase activity of both crude and purified lipase extract was done by agar well diffusion method (Balouiri et al., 2016). The activity was done on Tween 80 medium composed of peptone, NaCl, $CaCl_2.2H_2O$, Tween 80, and agar. The lipase activity was checked by making small wells in the medium and filled with both crude and purified lipase extracts, and left for 24 h at 37°C. Zone of clearance around the wells showed Tween 80 hydrolysis (Kumar et al., 2012).

Antibacterial activity

Antibacterial activity of both crude and purified lipases extracts of Water Melon (*Citrullus lanatus*), Honeydew Melon (*Cucumis melon*) and Cantaloupe (*Cucumismelo cantalupensis*) was checked by agar well diffusion method (Balouiri et al., 2016). For this purpose, six microbial strains: *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* strains were grown in nutrient broth medium at 37°C for overnight. Bacterial cultures were mixed with MH agar medium, poured Petri dishes and placed at room temperature under laminar flow to solidify. After solidification six

wells were made in each plate with the help of sterilized yellow tips (to make 5 mm well). In three wells 100 µl crude extract of proteases were added whereas in remaining three wells 100 µl purified proteases extract were added. All these plates were placed at 37°C and zone of inhibition was recorded after 24 h of incubation. The growth sensitivity was recorded as (0) for no sensitivity, 1-3 for low sensitivity, >3-7 for moderate sensitivity, and >7 to 15 for highest sensitivity.

Enzymatic distaining

Lipases also used for the removal of different kind of stains like grease, butter, vegetable oil and olive oil. For this activity three white cotton cloth pieces were taken and permeated with grease, butter and olive oil stains. In one plate only water was added, in second Petri plate, detergent (soup) was added, and in third petri plate crude lipase extract and in fourth purified lipase extract were added. All plates were placed at room temperature for 30 min, and the destaining activity was recorded.

Statistical analysis

Each test was repeated thrice and mean from absolute data was calculated (http ://easycalculation. com/statistics/mean .php)

RESULTS

Extraction and lipolytic effect

In the present study 3 types of waste fruit seeds such as water melon (*C. lanatus*), honeydew melon (*C. melon*) and cantaloupe melon (*C. cantalupensis*) were used for the extraction of lipase. Seeds were germinated for 120 h and crude lipase extract was extracted. Purified lipase extract were extracted using ammonium sulphate. Results revealed that tween 80 hydrolysis was shown by purified lipase extracts of *C. lanatus*, *C. melon*, and *C. cantalupensis* compared to crude lipase extract (Figure 1). Maximum tween 80 hydrolysis was shown by purified extract of cantaloupe melon (CM) but crude extract did not show tween 80 hydrolysis. Purified extract of watermelon (WM) also showed tween 80 hydrolysis but crude show little tween 80 hydrolysis. Similarly purified extract of honeydew melon (M) also show maximum tween 80 hydrolysis but crude showed little tween 80 hydrolysis.

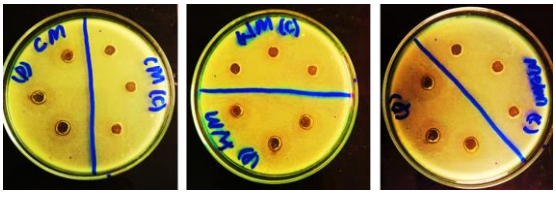

Figure 1: Tween 80 hydrolysis by crude and purified lipase extracts of seeds of watermelon, cantaloupe melon and honeydew melon. WM (Water melon); **CM** (cantaloupe melon); **melon** (honeydew melon); **P** (Purified extract); **C** (Crude extract).

Antibacterial activity of lipase extracts of water melon seeds

Antibacterial assay of crude and purified lipase extracts of seeds of water melon was analyzed against clinical pathogens through agar well diffusion method and it was observed that purified lipase extract had significant bactericidal effect against all tested pathogens except *S. aureus* and *K. pneumonia* (Figure 2). The maximum zone of inhibition was recorded as *E. coli* (11.3±0.0 mm), *P. aeruginosa* (7.3±0.0 mm), *S. pyogenes* (10.0±0.0 mm), and *S. epidermidis* (7.0±0.0 mm). on the other hand, the maximum zone of inhibition of *E. coli* (7.3±0.0 mm) while moderate inhibition of *P. aeruginosa* (5.0±0.0 mm), S. *pyogenes* (6.6±0.0 mm), and *S. epidermidis* (5.0±0.0 mm) was recorded in case of crude lipase extract (Figure 2).

Antibacterial activity of lipase extracts of honeydew melon

Results revealed that purified lipase extract of honeydew melon seeds had great antibacterial effect against all tested bacteria compared to crude extract (Figure 3). Purified lipase extract showed maximum inhibition of *E. coli*, *P. aeruginosa S. pyogenes K. pneumonia S. aureus* and *S. epidermidis* (9.3±0.0 mm, 10.6±0.0 mm, 8.0±0.0 mm, 8.3±0.0 mm, 13±0.0 mm, and 7.3±0.0 mm). Similarly, crude lipase extract showed the maximum zone of inhibition as well: 10±0.0 mm for *P. aeruginosa*, 8.0±0.0 mm for *S. pyogenes*, and 12±0.0 mm for *S. aureus*, 7.0±0.0 mm for *E. coli*, and 7.0±0.0 mm for *S. epidermidis*, while moderate inhibition of *K. pneumonia* (5.0±0.0 mm) was recorded (Figure 2).

Antibacterial activity of extract of cantaloupe melon seeds

It was observed that purified lipase extract of cantaloupe melon seeds showed maximum inhibition of *S. pyogenes* (13.0±0.0 mm), *E. coli* (11.0±0.0mm), *S. epidermidis* (11.0±0.0 mm), *K. pneumonia* (7.3±0.0 mm), while moderate inhibition of *S. aureus* (6.3±0.0 mm), and *P. aeruginosa* (6.0±0.0 mm) were recorded, respectively (Figure 2). On the other hand, crude extract showed maximum inhibition of *S. pyogenes* (11±0.0 mm), and *S. epidermidis* (8.0±0.0 mm), while moderate inhibition of *E. coli* (6.0±0.0 mm) were recorded. The antibacterial effect is shown in figure 3.

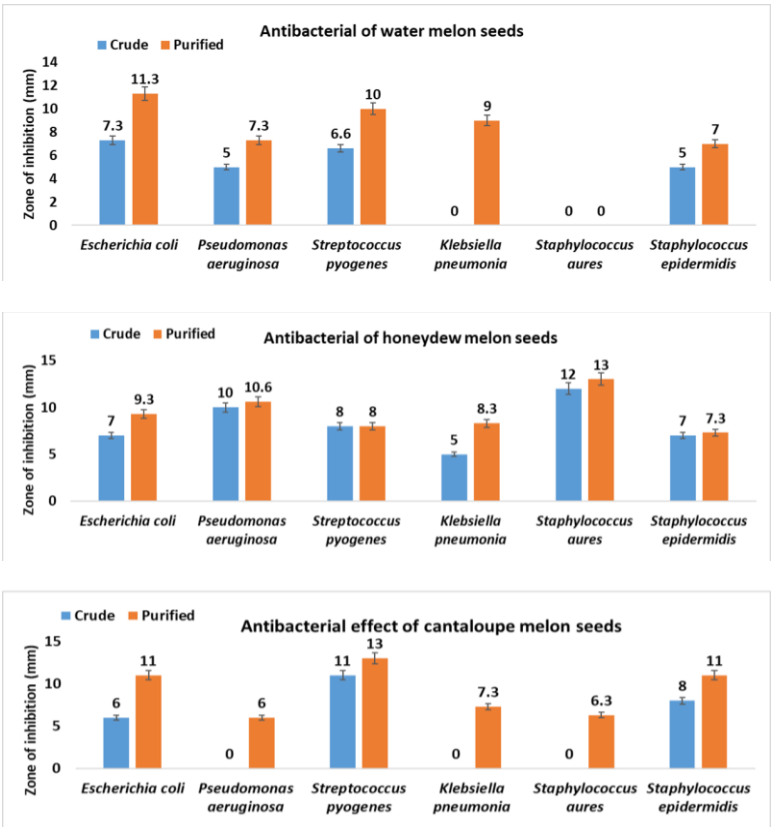

Figure 2: Antibacterial efficacy of crude and purified lipase extracts obtained from various fruit seeds.

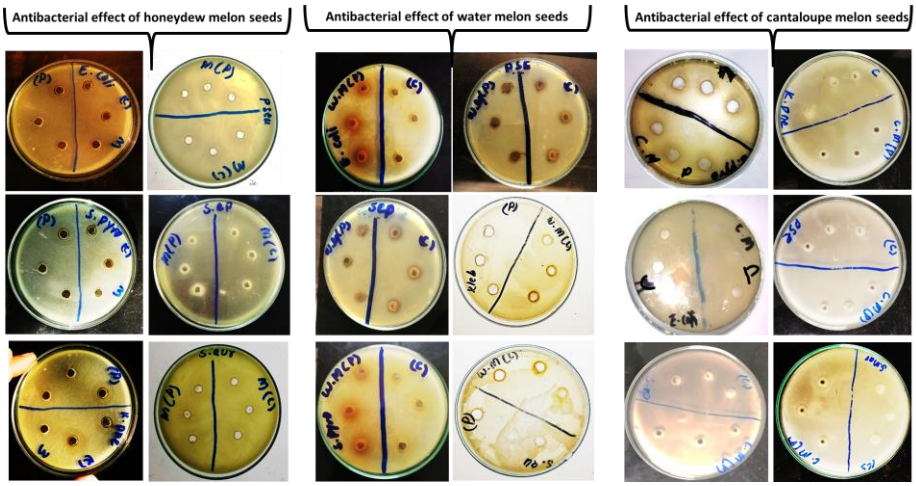

Figure 3: Antibacterial efficacy of crude and purified lipase extracts obtained from various fruit seeds against bacterial pathogens through agar well diffusion method.

Figure 4: Destaining effect of purified lipases obtained from various fruit seeds.

De-staining effect

Results revealed that the piece of cloth washed only with water have visible stains. It was observed that there was incomplete removal of stains when washing was done only with the water and water mixed with soup, whereas complete removal of stains occurred with addition of purified lipase extracts obtained from honeydew melon (M), water melon (WM) and cantaloupe melon (CM) (Figure 4).

DISCUSSION

Lipases can be obtained from animals, microorganisms and plants (Mohd Zin et al., 2017; Ray, 2012). In the current study, lipases produced from seeds of water melon (*Citrullus lanatus*), honeydew melon (*Cucumis melon*) and cantaloupe (*Cucumismelo cantalupensis*), and our findings agree with previous literature (Salaberría et al., 2017; Lampi et al., 2015; Mohd Zin et al., 2017). They demonstrated that latex, leaves, bran, seeds, beans, and fruits are examples of plant materials that include lipases. Researchers isolated lipases from various soucres of seeds such as castor beans, African beans, elms, sunflowers, physic nuts, lupins, linseeds, coconuts, almonds, black cumin, wheat grain, rice, corn, oats, barley, sesame, sorghum, and other seeds because these are especially rich in plant lipases and have wide range of application in detergents, leather industry, paper industry and fine chemistry (Salaberría et al., 2017; Mohd Zin et al., 2017; Qiu et al., 2017; Huang et al., 2017; Hamden et al., 2017; Schneider et al., 2016; Moreau et al., 2016; Lampi et al., 2015; Ahmad et al., 2015).

Lee et al. (2015) performed antibacterial effect of mustard plant against *E. coli* through agar well diffusion method. Zainab and Batool (2018) also reported that microbial lipase showed inhibition *E. coli* and *S. aureus*. Yassein et al. (2021) demonstrated the antibacterial effect of lipase against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis,* and Methicillin-resistant *Staphylococcus aureus* (MRSA). Antibacterial effect of crude and purified extract of seeds of watermelon, honey dew, melon and cantaloupe melon was analyzed against clinical pathogens (*E. coli, P. aeruginosa, S. pyogenes, K. pneumonia, S. aureus* and *S. epidermidis*) through agar well diffusion method. Results revealed that purified extracts of all seeds exhibited bactericidal effect against both Gram-negative and Gram-positive bacteria compared to crude extracts. Purified extract of water melon showed highest inhibition of *E. coli*, melon showed maximum inhibition of *S. aureus* and cantaloupe melon showed highest inhibition of *S. pyogenes*. Our results are consistent with the findings of Prabhawathi et al. (2014), who demonstrated that the majority of Gram-positive bacteria include lipoteichoic acids, and the lipid tail that is present in these bacteria is crucial to their ability to adhere to surfaces. This lipid tail may be subject to lipase action, which would stop it from adhering to a surface. In the current study purified lipase inhibited the growth of Gram-positive bacteria. Similarly, lipase's action on the lipopolysaccharide of Gram-negative cell walls and the esters of exopolysaccharide were also found. Additionally, lipase cause the lipid bilayer to hydrolyze by acting on the lipoprotein, lipopolysaccharide, and phospholipids that envelop the peptidoglycan layer. Yassein et al. (2021) confirmed the antibacterial and antibiofilm properties of crude lipase against the tested human pathogens. Our results are agreed with the findings of Ngai and Ng, (2004).

Fruit seed-based lipases (honeydew melon, cantaloupe melon and water melon) shown the ability to remove stains from fabrics containing grease, butter, and olive oil in the current study and outcomes are consistent with the findings of Sajna et al. (2013) and Bacha et al. (2018). Lipases are beneficial in detergent industry because they are ecofriendly and also enhance the ability of detergents to remove the stains of grease and oils (Chauhan et al., 2013). Lipases are stable at wide range of pH and temperature (Cherif et al., 2011). In the current study, lipases production was carried out at pH 8 and 8.5 and room temperature (28±2°C). however, the lipolytic activity was observed at pH 7 and 37°C. It is reported that microbial based lipases have great effect in stains removal (Saraswat et al., 2017). Therefore, the current study illustrated that seed based lipases could be used in detergent and laundry industry for the removal of various stains like olive oil stains, vegetables oil stains, butter and grease stains. It was observed that purified lipases actively remove the stains and keep the clothes shiner and brighter compared to the crude lipases and detergent used as a control. So, lipase could be widely used in detergent industry. Our results agree with the Sharma et al. (2017), who demonstrated that detergent which contain enzyme also improve the fabric quality and keep the color shiner and brighter (Sharma et al., 2017).

CONCLUSION

In the present study, lipase produced from various fruit seeds such as honeydew melon, cantaloupe melon and water melon and evaluated for the antibacterial and detergent efficacy. It was concluded that seeds based lipase had potential role in the field of laundry and pharmaceutical industries and could be used as a therapeutic agent as well as a detergent. For the optimal application, lipases should be produced and characterized in the future. To further satisfy the needs of the industrial sector, further creative methods for boosting lipase output could be used.

Acknowledgements: Authors are grateful to The University of Azad Jammu and Kashmir, Muzaffarabad for providing research facilities.

Statement of conflict of interest
No conflict of interest

Funding statement
No funding source

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