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ISOLATION, PURIFICATION AND CHARACTERIZATION OF LLIPASE FROM *CARDIOSPERMUM HALICACABUM* GERMINATED SEEDS AND ITS HYDROLYSIS OF TRIGLYCERIDES IN NATURAL OIL

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Abstract

Cardiospermum Halicacabum is known as a medicinal herb, and its seed is used in herbal medicine. Lipases are found in a variety of taxa including bacteria, mammals and plants. However, research has shown that plant lipases are involved in numerous physiological processes other than triacylglycerol hydrolysis. The aim of this research was to get a deeper insight into the characteristics of the lipase activity in germinating *C. halicacabum* seeds the purified enzyme was quantified by HPLC as well SDS-PAGE analysis to perform a more detailed analysis at different pH, Temperatures, of their Lipase activity was done using coconut oil as substrate. *Cardiospermum halicacabum* seeds that have been germinated for 48 hours contain lipid-degrading enzyme lipase. Enzyme was extracted, purified in four steps using sephadex G-50 and CM-cellulose column chromatography, salt precipitation, dialysis, and HPLC. After 48 hours of germination, acetone fraction and 70% saturated crude extract both demonstrated lipase activity of 439.8 µg/ml and 279.1 µg/ml (dilution factor 10) respectively. At a temperature of 35^0C, crude extract at an ideal pH of 6.25 displayed a lipase concentration of 561.19 µg/ml and 91.54 x 10^3 activity units/ml, whereas acetone extract at an ideal pH of 7.25 displayed an enzyme concentration of 508.95 µg/ml and 86.23 x 10^3 activity units/ml. Also, the enzyme showed a single band with a molecular mass of 37 kDa for this lipase, which corresponded to high resolution gel filtration, as did the column-purified lipase at a concentration of 1.8 mg/ml in HPLC. This research may help to develop a natural and economical way to produce lipase enzyme from *C. halicacabum* plant seeds. which could make their economic use as industrial enzymes an appealing option. The uses of lipases in the production of biodiesel, food, cellulose pulp, detergents, oils, fat medications, fine chemistry, effluent treatment, and pharmaceuticals.

Keywords: *Cardiospermum halicacabum* lipase, high performance liquid chromatography, column chromatography, germination.

Introduction

Lipases (EC 3.1.1.3), also known as triacylglycerol ester hydrolases, catalyse the whole or partial hydrolysis of fats and oils, resulting in the release of free fatty acids, diacylglycerol, glycerol and monoglycerol.[1] Lipases act on a variety of ester compounds, with acylglycerols serving as their primary substrate. Lipases are found in high concentrations in all oilseed plants. Plant-based lipases are gaining popularity among researchers due to their low production costs and high specificity.[37,42] Because of unique qualities like as selectivity, cheap cost, availability, and simplicity of purification, these enzymes surpass animal and microbial lipases in some circumstances, making them a good choice for prospective commercial use as industrial enzymes [4]. Lipases may be produced by microorganisms such as bacteria and fungi, as well as animals and plants.[22,26,42] Plant lipases are derived from a variety of plant components, including fruits, latex, and seeds. Seeds, particularly oilseeds, are the major portions with greater lipase concentrations. Lipase concentrations are more during germination of seeds as the enzyme is very crucial for embryo's growth.[12] *Cardiospermum halicacabum* L. seed oil contains 49% of a diester generated from 1-cyano-2-hydroxymethylprop-2-ene-1-ol and 6% of another diester derived from 1-cyano-2-hydroxymethylprop-1-ene-3-ol.[23]

Plant seeds, particularly those in the germinating stage, are important sources of plant-based lipases.[19] Plant-based herbal products, such as gel, cream, shampoo, and spray, are available on the market and can help with dry, itchy skin and scalp.[33] Plant-based drugs have been used in traditional medical systems around the world to treat a variety of illnesses. Around 80% of the world's population still relies on medicinal plants for primary health care, particularly in areas where modern medicines are inaccessible.[38] *Cardiospermum halicacabum* was toxicologically evaluated and found to be safe and nontoxic up to 40 g/kg in rats.[40] Plant lipases, unlike those found in microorganisms and animals, are poorly understood.[5] The contribution of lipases within the worldwide enzyme industry market has grown significantly and it comprises a good range of applications in many sectors like food, pharmaceutical, fine chemical, oil chemical, and detergent industries in addition as in biodiesel and wastewater treatment.[6]

Plant lipases might be effective replacements for animal and microbial lipases in biocatalytic processes.[27]

Cardiosperma species found in India, America, and Africa include *C. halicacabum, C. corindum, C. ovatum, C. grandiflorum, C. hirsutum, C. barbicaule, C. vesicarium, and C. canescens*. The most studied species are *Cardiospermum halicacabum* and *Cardiospermum canescens*.[20] *C. halicacabum L.* is an annual or occasionally perennial climber plant in the *Sapindaceae* family. It flourishes in tropical and subtropical climates and can potentially be distributed across Asia and Africa. The entire plant has been used in several countries to treat rheumatism, limb stiffness, snake bite, nervous disease, and swelling.[34,39] It's a weed that grows all over India. In English, it is known as "love in a puff" or "balloon vine." In Hindi, this is known as kanphuta. The entire plant of *C. halicacabum* is used in folk remedies for its nutritional value and is known as a medicinal plant with variety of medicinal properties for treating various ailments; it has been used in various traditional systems of medicine such as Ayurveda, Unani, and Chinese medicine.[24] Cardiospermum is derived from the Latin words cardio (heart) and sperma (seed) and pertains to the seed's white heart-shaped pattern. The name Halicacabum is derived from the Latin term *halicacabus*, which describes a plant bearing inflated fruits. It's a little, delicate,

smooth climber, and the whole plant has been used to cure a range of ailments for generations.[21]

The aim of this research was to get a deeper insight into the characteristics of the lipases activity in germinating *Cardiospermum halicacabum* seeds the purified and purified enzyme was quantified by HPLC as well SDS-PAGE analysis to perform a more detailed analysis at different pH , Temperatures, of their Lipase activity was done using coconut oil as substrate.

MATERIALS AND METHODS

Seeds germination

As per botanist instructions, the matured seeds of *Cardiospermum halicacabum* were collected in communication with Siddha Gardens in Ministry Of Ayush, India. The seeds were authenticated by a botanist, Dr. M. Reema kumari, Assistant Professor, Department of Botany, mLAC College For Women, Malleshwaram, Bangalore, Karnataka, India. The seeds of *C. halicacabum* were washed with distilled water. And the seed germination was done in cocopeat at room temperature for 7 days. At the end of every 24hours (each day), a batch of seeds were harvested by removing the endosperm, washing it with distilled water and stored at 4 °C.

Extraction and isolation of lipase

The seeds of *C. halicacabum* were dehulled and the endosperm was extracted. Endosperms were rinsed in distilled water and homogenized in a cold 150 mM solution using a pestle and mortar. pH 7.5 Tris-HCl grinding buffer with 0.4M sucrose, 0.6mM EDTA, 2.0mM β-Mercaptoethanol, and 1.95% (w/v) Tween 80. The enzyme was extracted by solvent extraction using distilled water and chilled acetone as solvents. The homogenate was filtered through muslin cloth and centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant and pellet were divided into separate containers after the top layer, known as the lipid bodies (fat pad), was scraped away using a spatula. The crude lipase supernatant was kept at 4°C. The pellet was washed with tris buffer, and lipase activity in the three fractions was measured.[3]

Estimation of protein

Protein estimation was performed by measuring the absorbance at 280nm in the eluted fractions. Protein concentration was revealed by Lowry's method.[3]

Purification of lipase

The crude lipase extracted from 2-day germinated seeds was precipitated with ammonium sulphate at 30%, 50% 70% saturation. The protein precipitate was centrifuged at 5000 rpm for 20 min. The supernatant containing crude lipase was saturated with cold 50% (v/v) acetone and further subjected to dialysis. Dialysis of crude lipase was done using cellulose membrane and 0.05M Tris-Hcl buffer (pH 7.5) for 24 h. The dialysate was collected into fresh tubes and centrifuged at 5000rpm for 5 min. The supernatant (first dialysate) was collected and subjected to further analysis. For the Purification process of lipase, 70% ammonium sulphate precipitated samples were taken for dialysis and column chromatography on sephadex G-50 ion exchange column.[30]

Effect of pH

Lipase activity was determined at pH levels ranging from 5.0 to 8.0 using the following buffer system: 0.05M Tris-HCl (5.0-8.0). The pH stability was assessed by incubating the enzyme solutions in various buffers with pH ranges ranging from 5.0 to 8.0 (as mentioned above) for 1 hour at room temperature, and the protein content was then checked as previously reported.[15,43]

Effect of temperature

Lipase activity and stability were determined by assaying lipase at temperatures ranging from 30 ºC to 80 ºC in a temperature-controlled water bath using the usual technique of assay. For temperature stability, enzyme solutions

were incubated at 30-80 ºC for 30 minutes and then cooled to 4 ºC for additional 30 minutes before measuring protein concentration as discussed previously.[15,43]

Lipase activity

Lipase activity was done using coconut oil as substrate. The free fatty acids were transformed to copper soap and calorimetrically measured using p-nitrophenol as a colour reagent at 440nm, the optical density was measured against a appropriate blank. The intensity of fatty acid emitted was read off from the standard curve for 1 mint at 30 ºC.[25,32]

Y=0.2407[Conc. X]-0.2306

Activity = FFA released per mint at 35˚C,

at 6.25pH crude extract and 7.25 pH acetone extract at 35˚C X 10^3

SDS-PAGE electrophoresis

The sample was prepared by keeping 1ml enzyme samples for dialysis. The supernatant was centrifuged and taken for OD measurement. Based on the OD, 80µl of sample was mixed with the buffer and heated in boiling water bath for 5-10 minutes. SDS-PAGE was carried out in polyacrylamide gel in the presence of 0.1% SDS, as described by Laemmli on a Biorad Mini electrophoresis system. After electrophoresis, the gel was removed and placed in the staining solution, followed by the destaining solution. The gel was observed for bands. and stained with Coomassie Brillant Blue R-250. Sample protein bands and markers on the gel were determined.[17]

High performance liquid chromatography (HPLC) analysis

The concentration of lipase was evaluated using a high-performance liquid chromatography (Waters, USA) system outfitted with a UV detector and an Eclipse Plus C18 column (4.6 X 250 mm). At a flow rate of 0.5 mL/min, the mobile phase was methanol-acetonitrile (20/80, v/v). The temperature in the column was set to 25°C. A total of 20 µL of the sample (20-100 ppm) dissolved in MeOH was injected into the column, and detection was obtained at 250 nm. The peaks were combined with a standard peak.[7,36]

RESULTS

Extraction and isolation

Several industrial processes have been developed with lipase, a very versatile enzyme. Lipase may come from a variety of sources, including animal products, vegetables, and microbes.[10,18] Lipase enzyme was extracted and isolated from the germinated seeds of *Cardiospermum halicacabum.* Seeds were germinated at room temperature for 24, 48, 72 and 96 hrs using distilled water and acetone as solvents. Endosperms of seeds were collected and homogenate was prepared using Tris HCl as grinding buffer. After centrifugation, the pellet and supernatant were taken in to separate tube and employed to measure enzyme activities. When lipase concentration was determined for crude extract and acetone fraction table 1, showed that 48hrs of both the fraction showing the highest concentration with crude extract showing concentration of 224.88 µg/ml [Dilution factor 10] and acetone fraction giving 128.85 µg/ml [Dilution factor 10]. Based on the concentration of lipase obtained at different germination time, 48hrs of crude extract and acetone fraction extract were used for further analysis. Figure 1, shows Seed germination, Extraction, isolation, and Purification of Lipase. Protein concentration was determined by Lowry's method in the eluted fractions by using BSA as standard and measuring the absorbance at 280nm is shown in figure 2.

Purification of lipase

Different purification processes were used for *Cardiospermum halicacabum L.* seeds. Both crude and acetone fractions were precipitated with ammonium sulphate. Dialysis was then performed, and the dialyzed protein was purified using ion exchange

chromatography on a sephadex G-50 column. The eluted proteins were then submitted to SDS-PAGE, followed by HPLC. The purification process for *Cardiospermum halicacabum* seed, lipase was used by precipitating it with ammonium sulphate at 30%, 50% 70% saturation. As a result, for both 48hrs crude and acetone fraction, 70% saturated protein sample showed highest activity with concentration of 439.8 μg/ml [Dilution factor 10] and 279.1 μg/ml [Dilution factor 10] as shown in table 2. To further purify the enzyme, both 70% saturated crude and acetone fractions were subjected to dialysis. After dialysing samples with Tris-HCl buffer for 24h, the diasylate were collected in separate tubes and further purified using sephadex G-50 ion exchange column. figure 3, shows the chromatogram for both crude extract and acetone fraction for sephadex G-50 ion exchange column. As a result, the peaks were showing highest absorbance from tube no 16-20 for crude extract samples were pooled together and 17-20 tube no's were pooled together of acetone extract for further analysis.

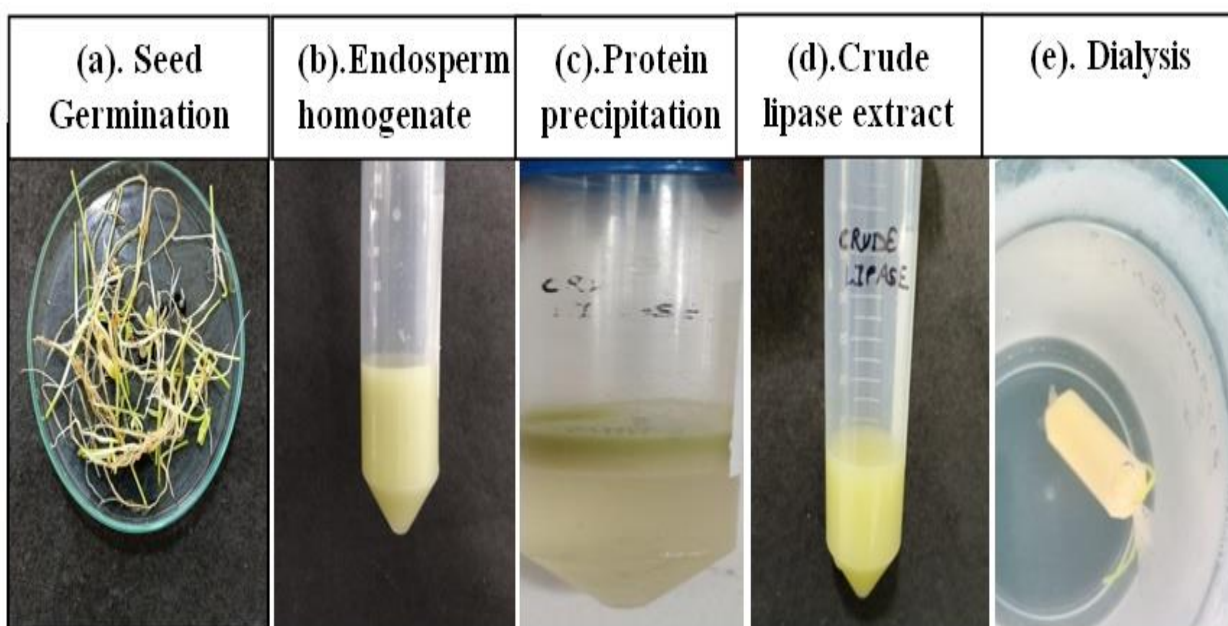

Fig. 1: Seed germination, Extraction, isolation, and Purification of Lipase.

Table 1. Lipase protein concentrations of crude extract and acetone fraction with different germination time.

Sl. No.	Germination Time	Protien Sample	OD at 650nm	Conc μg/mL
1	24 Hrs	Crude extract	0.228	113.4 [Dilution factor 10]
2	24 Hrs	Acetone fraction	0.097	48.25 [Dilution factor 10]
3	48 Hrs	Crude extract	0.452	224.88 [Dilution factor 10]
4	48 Hrs	Acetone fraction	0.259	128.85 [Dilution factor 10]
5	72 Hrs	Crude extract	0.154	38.31 [Dilution factor 5]
6	72 Hrs	Acetone fraction	0.086	21.39 [Dilution factor 5]
7	96 Hrs	Crude extract	0.321	79.85 [Dilution factor 5]
8	96 Hrs	Acetone fraction	0.102	25.37 [Dilution factor 5]

*48h germinated seeds showing highest activity of lipase.

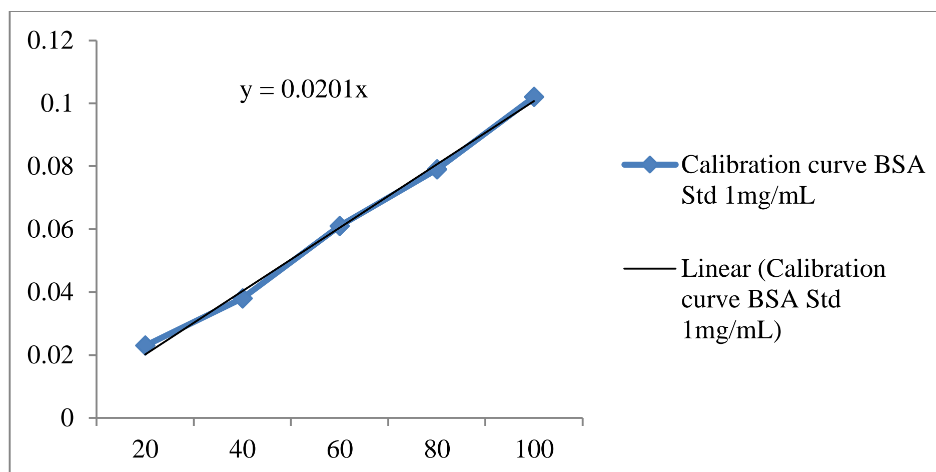

Fig. 2: Standard BSA 1mg/ml calibration curve.

Table 2. Lipase activity of 48hrs crude and acetone fraction on precipitating enzyme with ammonium sulphate.

Sl. No	Protien Sample	% saturation	OD at 650nm	Conc µg/ml [Dilution factor 10]
1	48 Hrs Crude extract	30%	0.151	75.12
		50%	0.165	82.08
		70%	0.884	439.8
2	48 Hrs Acetone fraction	30%	0.083	61.19
		50%	0.119	59.20
		70%	0.561	279.1

*Both crude extract and acetone fraction showing highest activity in 70% Saturation.

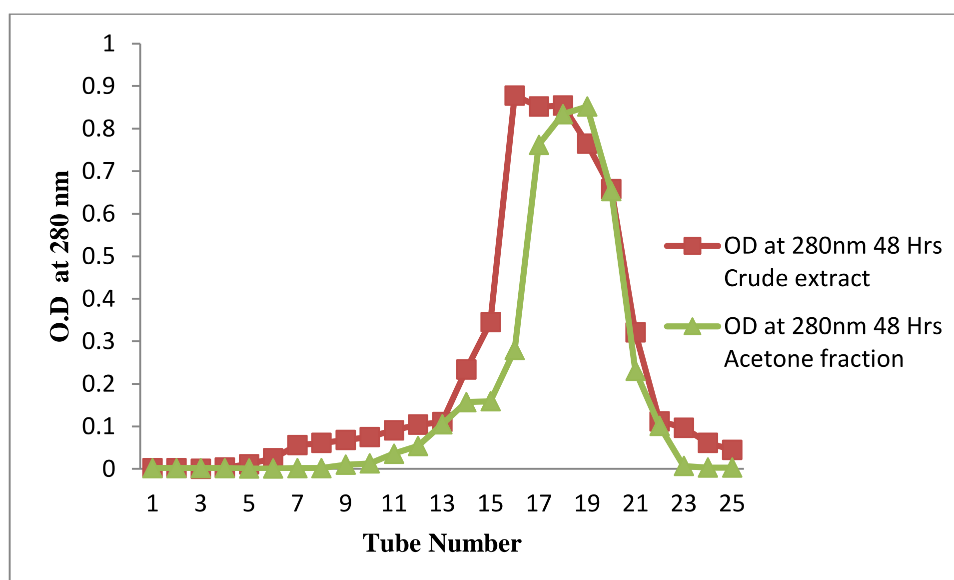

Fig. 3: Lipase activity of 48hrs crude and acetone fraction on precipitating enzyme with ammonium sulphate

Effect of pH on lipase activity

The effect of pH on the *C. halicacabum* seed lipase activity were examined in the range of 5.0-8.0 using the following buffer system; 0.05M Tris-HCl (5.0-8.0). The pH activities of the Crude extract at 6.25 shown 242.11 µg/mL [Dilution factor 10] and acetone extract at pH 7.25 were shown 203.49 µg/mL concentration µg/mL [Dilution factor 10]. Very little activities were found below pH-6 and at or above 7 pH. The optimum lipase pH range for two crude and acetone fraction is 6 to 7 ranges for 1 h at 37 °C. The residual lipase activity was measured using standard assay procedures. The optimum of pH on lipase crude extract and acetone fraction extract were showed in table 3 and figure 4.

Table 3. Effect of pH on lipase activity

	Crude extract		Acetone fraction	
pH 100µl Sample	OD at 650nm	Conc µg/mL [Dilution factor 10]	OD at 650nm	Conc µg/mL [Dilution factor 10]
5	0.342	84.236	0.145	29.77
5.25	0.351	86.45	0.167	34.29
5.5	0.335	82.51	0.168	34.49
5.75	0.403	99.26	0.174	35.72
6	0.453	111.57	0.177	36.34
6.25	0.983	242.11	0.178	36.55
6.5	0.548	134.97	0.213	43.73
6.75	0.349	85.96	0.346	71.04
7	0.355	87.43	0.387	79.46
7.25	0.328	80.78	0.991	203.49
7.5	0.312	76.84	0.924	189.73
7.75	0.201	49.5	0.345	70.84
8	0.190	46.79	0.329	67.55

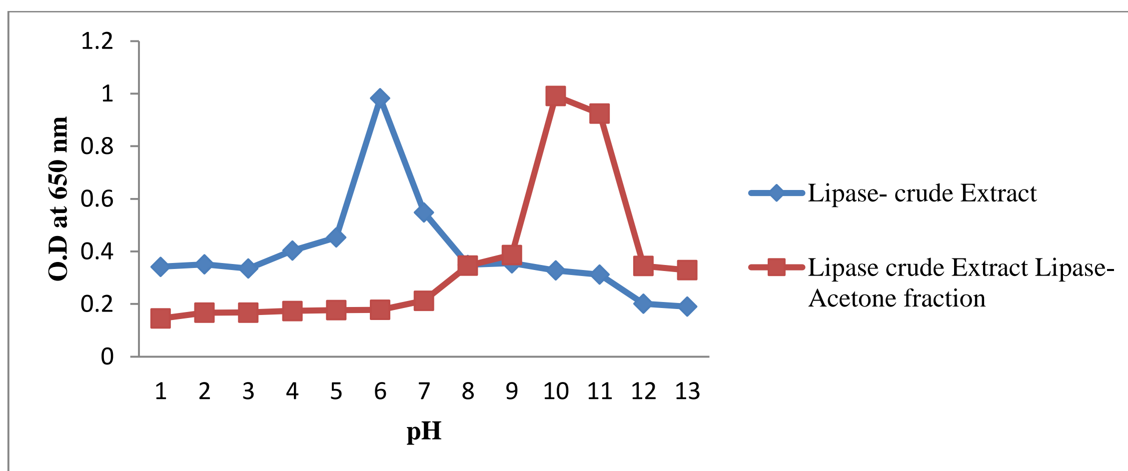

Fig. 4: Optimum of pH on lipase crude extract and acetone fraction extract.

Effect of temperature on lipase activity

Lipase activity was measured at various temperatures ranging from 30 °C to 80 °C for 30 minutes to identify the ideal temperature. The temperature activities of the Crude extract at 35 °C shown 233.56 µg/mL [Dilution factor 10] and Acetone extract at 35 ^{0}C were shown the 186.44 µg/mL [Dilution factor 10]. Very little activities were found below 30 ^{0}C and above 35 ^{0}C. The optimum of temperature of lipase crude extract and acetone fraction extract were showed in table 4 and figure 5.

Table 4. Effect of temperature on lipase activity

	Crude extract		Acetone fraction	
Temp ˚C 100µl Sample	OD at 650nm	Conc µg/mL [Dilution factor 10]	OD at 650nm	Conc µg/mL [Dilution factor 10]
30	0.987	225.34	1.086	179.5
35	1.023	233.56	1.128	186.44
40	0.676	154.33	0.898	148.42
45	0.661	150.91	0.672	111.07
50	0.632	144.29	0.655	108.26
55	0.249	56.84	0.621	102.64
60	0.231	52.73	0.384	63.47
65	0.230	52.51	0.353	58.34
70	0.215	49.08	0.320	52.89
75	0.210	47.94	0.319	52.72
80	0.210	47.94	0.299	49.42

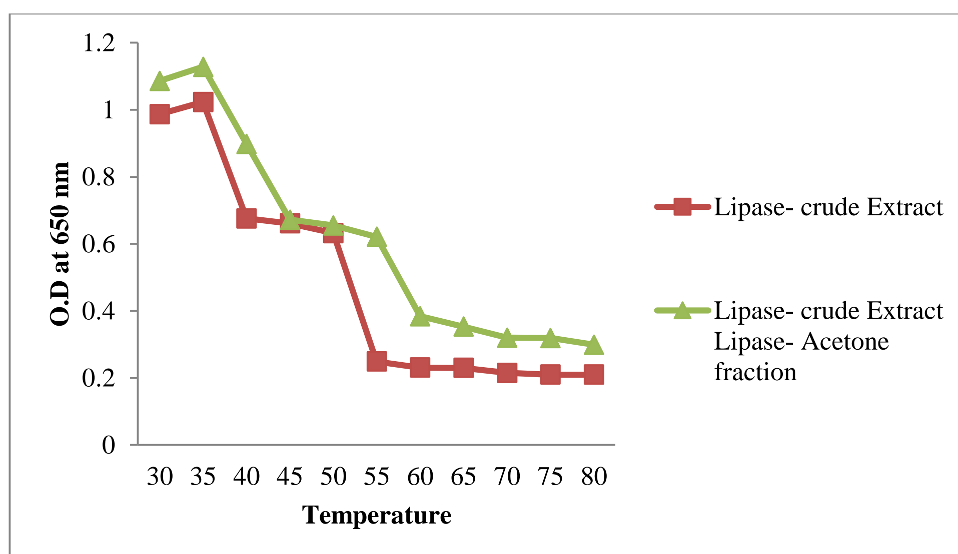

Fig. 5: Optimum temperature of lipase crude extract and acetone fraction extract

Lipase activity

Oilseeds are primarily composed of triacylglycerols, which account for 20% to 50% of their dry weight. Numerous lipases hydrolyze triacylglycerol from seeds to produce free fatty acids as well as glycerol. Phosphorylation converts dihydroxyacetone phosphate (DHAP) to glycerol. Acetate-COA is activated into acetyl-COA in the peroxisome, where it begins the process of β-oxidation. During germination, acetyl-COA, produced by β-oxidation, enters the glyoxylate cycle and subsequently participates in glycogen to provide the embryo with energy (28,29). Coconut oil was used as a substrate for lipase activity. Released free fatty acids were transformed to copper soap and calorimetrically measured using p-nitrophenol as a colour reagent. Figure 6-Standard curve showed that there was an increase in enzyme activity with increase in enzyme concentration. The lipase activity of both crude extract 6.25pH at 35^0C shown the activity of 91.54 X 10^3 Units/mL and acetone extract 7.25pH at 35 ^{0}C is shown 86.23 X 10^3 Units/mL is shown in table 5.

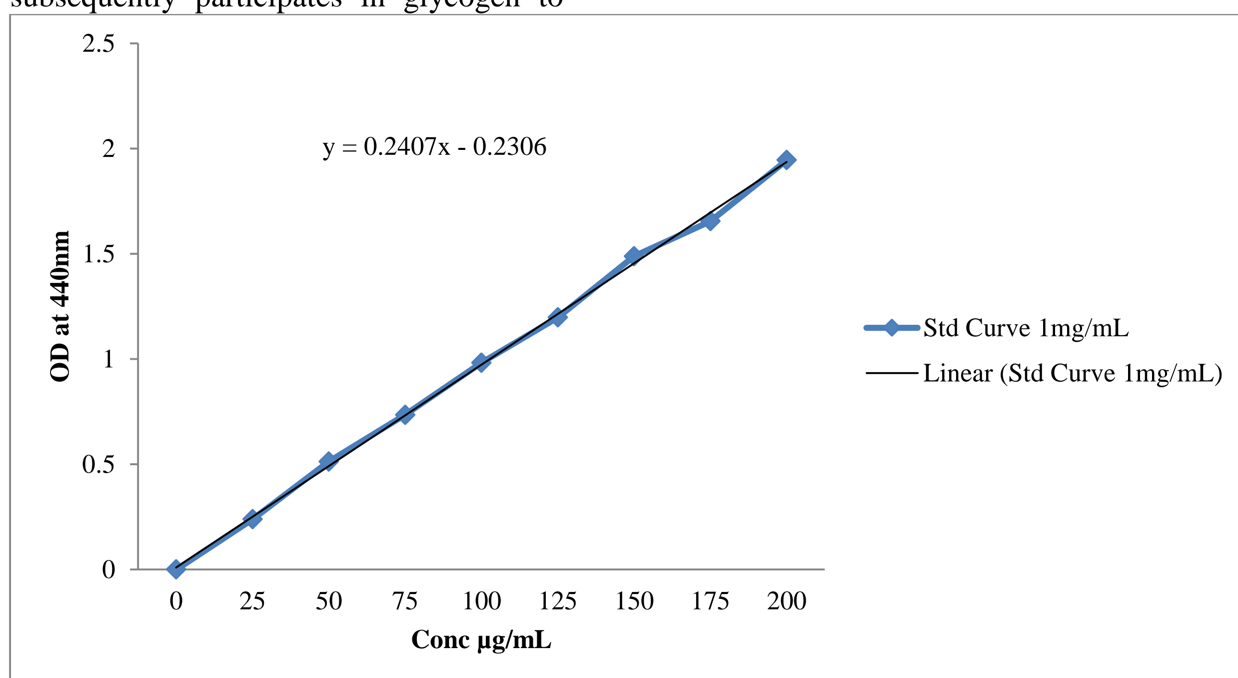

Fig. 6: Standard curve of effect of enzyme concentration on lipase activity

Table 5. Lipase activity of crude extract and Acetone extract

Enzyme Activity				
Sample 100µl	**OD at 650nm**	**Conc µg/mL [Dilution factor 10]**	**OD at 440nm**	**Activity Units/mL**
Crude extract 6.25pH Temp 35 ˚C	1.128	561.19	1.973	91.54 X 10^3
Acetone extract 7.25pH Temp 35 ˚C	1.023	508.95	1.845	86.23 X 10^3

SDS-PAGE electrophoresis

SDS-PAGE gel, electrophoretic examination revealed that the purified lipase of crude extract, first dilysate, and column purified lipase has a single band with a relative molecular weight of 37kDa, indicating that the resulting lipase has a single component.

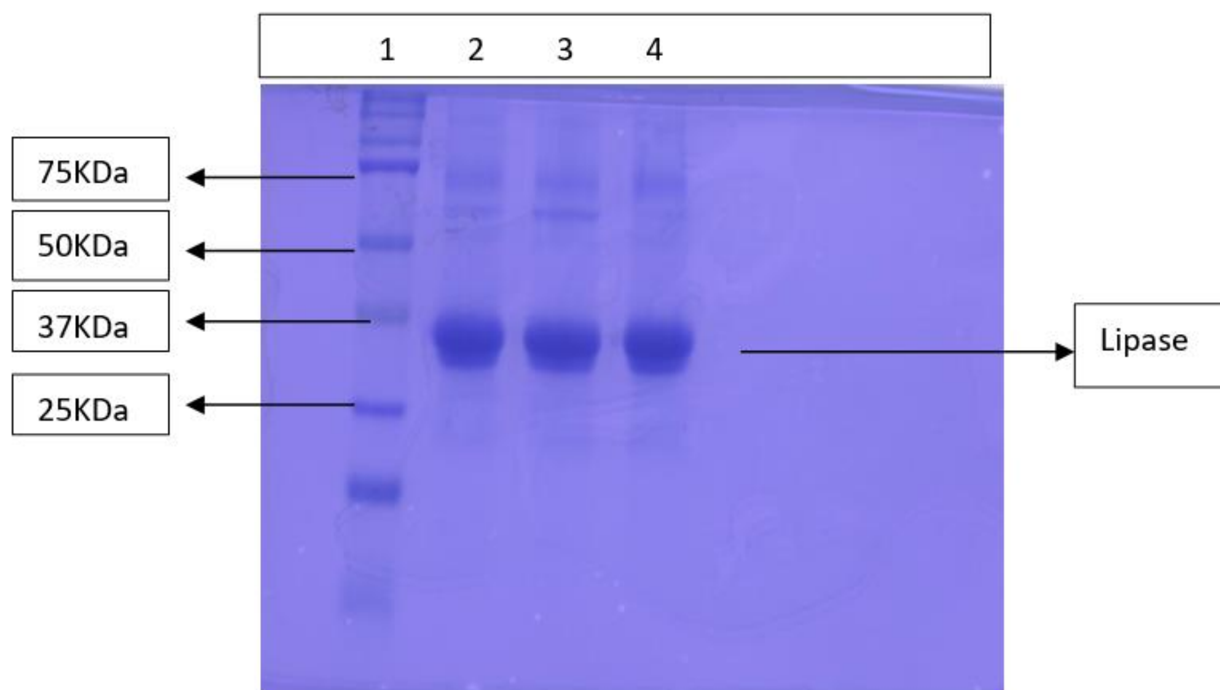

Fig. 7: SDS- PAGE slab gel electrophoresis of lipase 1. Ladder, 2. Crude extract, 3. First Dialysate, 4. Column purified.

HPLC analysis of purified lipase enzyme

Standard: Lipase from porcine pancreas Type II, 100-650 units/mg protein (using olive oil and 30 min incubation)) CAS Number: 9001-62-1. 100 % standard Concentration: 100mg/mL were showed a peak area % 5442278 and the sample lipase purified concentration 100 µL with peak area % is 19733 with 1.8 mg/mL. Chromatograms of Sample lipase with standard Lipase from porcine pancreas were showed in figure 8.

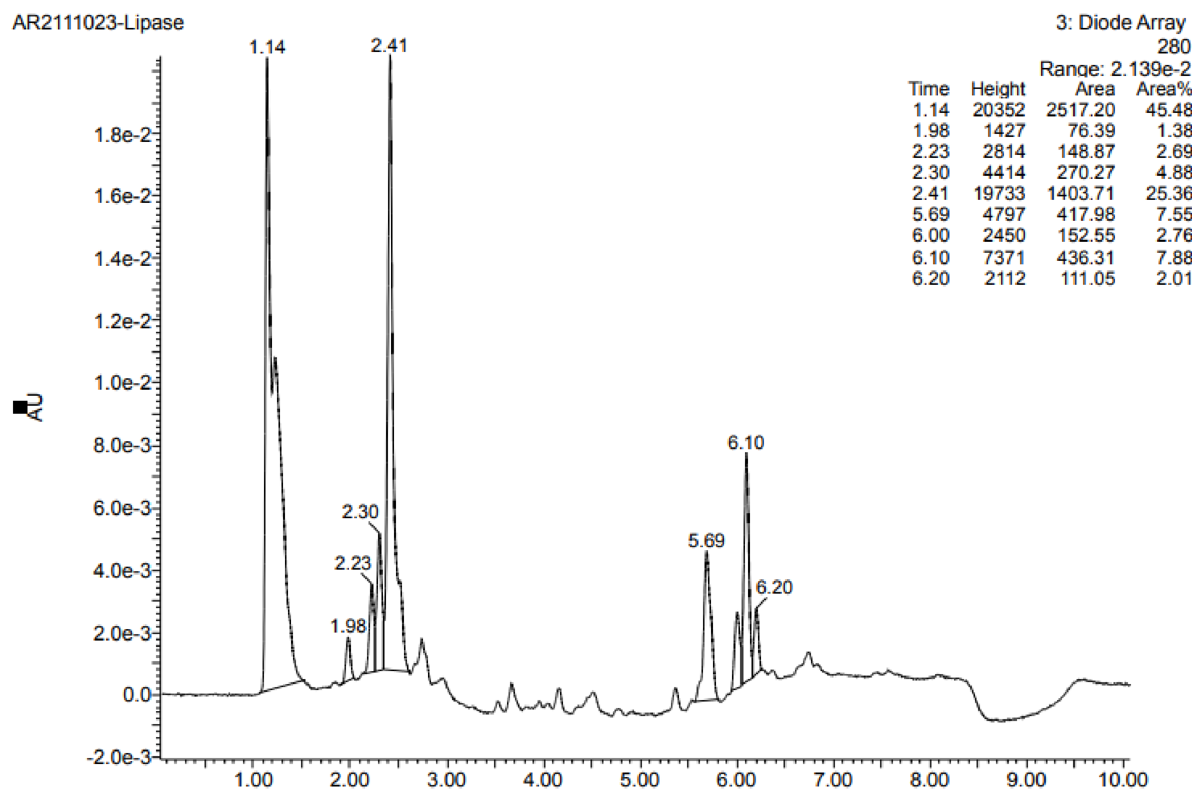

Fig. 8: Chromatograms of Sample lipase with standard Lipase from porcine pancreas.

DISCUSSION

Plant lipases have mostly been researched as an esterase for the hydrolysis of triacylglycerol. These are Lipases are utilized in numerous sectors, including food, pharmaceuticals, biodiesel, fine chemicals, oil chemicals and industrial detergent. Seedlings are indeed an essential stage in plant development which can be employed to forecast plant production and development.[4] In germinating seeds, lipid catabolism provides energy essential precursors for embryonic development.[9] *C. halicacabum* is widely employed in conventional medicine. Previous research has concentrated on the aerial portions, but the seeds have not received as much attention.[14] Syed Atif Raza et al., was reported the presence of various phytochemicals like aglycones, flavones, glycosides, triterpenoids, variety of fatty acids and volatile esters.[35] *Cardiospermum halicacabum* was used as a phytotherapy approach used to treat in atopic dermatitis.[13,13] Store oil breakdown is vital in many plants' life cycles because it provides carbon skeletons that assist seedling development shortly after germination. Lipases catalase triacylglycerol hydrolysis, releasing free fatty acids and glycerol. A variety of lipases have been isolated to near homogeneity from seed tissues and their in vitro activities have been investigated.[2,11]

A simple procedure for the extraction of the Lipase enzyme and isolated from the germinated seeds of *Cardiospermum halicacabum*. Seeds were germinated at room temperature for 24, 48, 72 and 96hrs using distilled water and acetone as solvents. Different purification processes were used for *C. halicacabum* L. seeds. Both crude and acetone fractions were precipitated with ammonium sulphate. This was followed by dialysis and dialyzed protein was further purified

by ion exchange chromatography using sephadex G-50 column.[28]

The eluted proteins were further subjected to SDS-PAGE, and at last HPLC. The purification process for Cardiospermum halicacabum seed, lipase was used by precipitating it with ammonium sulphate at 30%, 50% 70% saturation. As a result, for both 48hrs crude and acetone fraction, 70% saturated protein sample showed highest activity with concentration of 439.8 µg/ml. Electrophoretic analysis showed that the purified of Crude extract, First Dialysate, Column purified lipase has a single band with a relative molecular weight of 37kDa on SDS-PAGE gel. Jung *et al.,* 2013 was Purified and characterization activity of lipase from oat seeds similarly the lipase were shown the 40 kDa protein.[16]

It is widely accepted that abiotic factors such as pH and temperature can have a strong influence on enzymatic processes. Lipases can metabolise well in a wide range of pH and temperature conditions, and lipases are most commonly effective in an alkaline medium. The stability of lipase pH activities of the Crude extract at 6.25 shown 242.11 µg/mL and acetone extract at pH 7.25 were shown 203.49 µg/mL. Very little activities were found below pH-6 and at or above 7 pH and temperature activities of the Crude extract at 35°C shown 233.56 µg/mL and Acetone extract at 35 ^{o}C were shown the 186.44 µg/mL. Very little activities were found below 30^{0}C and above 35 ^{o}C.[29] Coconut oil was used as the substrate for the Lipase activity. The free fatty acids were transformed to copper soap and calorimetrically measured using p-nitrophenol as a colour reagent. The lipase activity of both crude extract 6.25pH at 35 ^{o}C shown the activity of 91.54 X 103 Units/mL and acetone extract 7.25pH at 35°C is shown 86.23 X 103 Units/mL.

Standard Lipase from porcine pancreas Type II, 100-650 units/mg protein. 100 % standard Concentration 100mg/mL were showed a peak area % 5442278 and the sample lipase purified concentration 100 µL with peak area % is 19733 with 1.8 mg/mL. Chromatograms of Sample lipase was observed. As a consequence of their high activity at low temperatures, inorganic solvents have a high affinity for lipases from oilseeds, which makes them effective biocatalysts for the degradation of oilseeds. Most cereal seed lipases can survive high temperatures and alkali conditions, including those from rice, wheat, barley, oat, and maize.[8] The research findings, yield, and properties promise towards the vast industrial application of lipases. In addition, the results showed that this unique lipase was a new example of a lipase, which may have a huge industrial potential in the sectors of pharmaceutical and other fields like high salt wastewater treatment, bioremediation in fat polluted environment.

CONCLUSION

In this present research work, *C. halicacabum* seeds are the valuable source of crude lipase with concentration of 1.8 mg/mL and having molecular weight of 37 kDa. The results show that 24 hrs germinating *C. halicacabum* seeds can act as good source of lipase enzyme. Therefore, germinating *C. halicacabum* be exploited in industrial processes. Seed lipase resources may provide more cost-effective and quicker production techniques for essential biocatalysts. Lipases are enzymes that are employed in food processing to release fatty acids into food items by selectively hydrolyzing fats and oils contained in a range of foods. Recognizing the significance of seed lipase applications may lead to their increased utilization in business and medicine. Lipases are also employed in the pharmaceutical and fine chemical industries throughout the manufacturing process. Lipase chemoselectivity allow it to be used for racemic mixture resolution

and compound removal Examples include detergents, biodiesel, wastewater pretreatment due to its ability to hydrolyze oil and other vegetable oils and many more. This study makes way for further analysis on applications of the enzyme.

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