Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.5, 2015

Anti- Lipase Activity of Some Plant Extracts on the Effectiveness of Lipase Enzyme Isolated and Purified from Pseudomonas Aeruginosa

Saba T. Hashim
Department of Biology / College of Science / University of Mustansiriyah / Baghdad/ Iraq.

Abstract
Bacterial isolates obtained from food.The isolates were grown on a selective medium agar that contained tween 80 or olive oil as the only source of carbon for detection of lipase activity. The organism grows optimally in the temperature of 40C° and pH 7. The lipase present in the broth was purified with ammonium sulphate precipitation and filtration chromatography by Sephadex G-150 to obtain 0.108 folds pure enzymes.Anti lipase activity of each plant was determined by titrating the solution against 0.02M NaOH using phenolphthalein as an indicator.
Keywords: Purification, anti-lipase, plant extract, *Ps. aeruginosa*

Introduction

Many attempts have been made to isolate lipase producing microorganisms since this enzyme is used in numerous biotechnological processes including food, leather, cosmetic, detergents and pharmaceutical industries and industrial wastes management **(1).**

Lipase (Triacylglycerol acylhydrolases E.C. 3.1.1.3) is widely distributed in animals, plants and microorganism, is a soluble enzyme that catalyzes the hydrolysis of ester bonds in water insoluble, lipid substrates. Lipases thus comprise a subclass of the esterase. **(2)** Lipase hydrolyses triglycerides to fatty acid and glycerol and under certain condition, catalyses the several reaction forming glycerides from glycerols and fatty acids. (**3)**

$$\text{Triacyl glycerol} + H_2O \xrightarrow{\text{Lipase}} \text{n Cliacyl glycerol} + \text{fatty acid anion}$$

A variety of lipases are produced from both Gram-positive and Gram-negative bacteria. Greater part of bacterial lipases comes from Gram-negative bacteria and the most important Gram-negative genus is Pseudomonas which contains at least seven lipase producing species, that are *P. aeruginosa*, *P. alcaligenes*, *P. fragi*, *P. glumae*, *P. cepacia*, *P. fluorescens* and *P. putida* **(4)**.In the present study, lipase from *Pseudomonase aeruginosa* has been isolated, partially purified and characteristic with respect to its stability at different temperature and pH . As well as the study of the inhibitory effectiveness of plant extracts against lipase enzyme.

Material and Method

Collection of producer microorganism

The bacterial strain *Pseudomonas sp* used in this study was isolated from food. The isolates were identified on the basis of various morphological and biochemical characteristics. Lipolytic bacteria were typically detected and screened through the appearance of clearing zones by using a selective medium (**5**), which containing Tween 80 or olive. The diameter ratio of clear zone and colony was measured.

Optimization of lipase production

Mineral media (NH4H2PO4 0.1g, KCl 0.02g, MgSO4 0.02g, Yeast extract 0.3g, Olive oil 5ml, D/W 100mL, pH – 7.2) was used for time course optimization. 1mL of 24hr old *Pseudomonas* culture was inoculated in 100mL of mineral media and incubated on rotary shaker adjusted at 30C° and 120 rpm. After every 24hrs and lipase activity was determined.

Lipase activity

Lipase activity was determined using olive oil hydrolysis, 10% Olive oil emulsion in 2% gum acacia was used as substrate. Reaction mixture composed of 0.5mL substrate emulsion, 0.4mL 0.1M Tris-HCl buffer (pH-7.2), and 0.1mL lipase solution. In blank lipase solution was replaced with equal amount of distilled water. Reaction was carried out at 300C for 30min. Reaction was stopped by adding 20mL acetone and ethanol (1:1). Liberated fatty acids were titrated with 0.05N NaOH using phenophthalene indicator. Amount of NaOH required to achieve end point (colorless to pink) was recorded. From this enzyme units and specific activity was calculated as follow,

Determination of protein

Protein concentration was determined by the method of Lowry **(6)** Using Bovine Serum Albumin as standard (Figure 1)

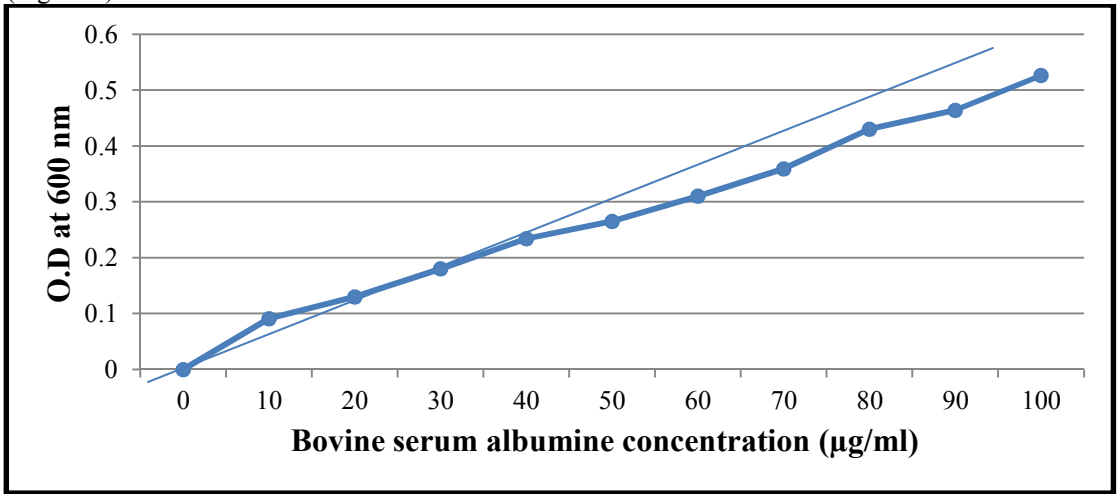


Fig (1): Standard curve of protein

The specific activity for enzyme was determined as following:-

$$\text{Specific activity (Unit /mg protein)} = \frac{\text{Enzymatic activity (Unit/ml)}}{\text{Protein concentration (mg/ml)}}$$

Determination of pH and temperature optimum

The optimum temperature and pH of purified lipase was assayed for its activity at different temperatures (20-60 C°) and different pH (3-10).

Purification of lipase

Lipase purification was carried out at 4°C. The culture medium was centrifuged at 10000 rpm for 20 min to obtain crude enzyme, then the supernatant fluid was subjected to precipitation with ammonium sulphate to 60% saturation and stirred for 2 h. The precipitate was removed by centrifugation. Lipase activity in the precipitate was determined. The precipitate were collected, dissolved in buffer pH 7.2 and dialyzed against same buffer. The enzyme mixture was loaded on Sephadex G-150 column equilibrated with 100 mM Tris buffer, pH 7.5 The enzyme was then eluted with the same buffer with a flow rate of 1 ml/min. Fractions of 3 ml were collected. The active fractions that contained lipase enzyme were pooled and assessed for protein content. The resulting enzyme was utilized for the characterization of the extracellular lipase. The protein content at each stage of enzyme purification was determined according to the Lowery method.

Analytical RP-HPLC of Purified Lipase:

Analysis of purified lipase was done by HPLC under following conditions**:** Column (250mm x 4.6mm 5 µm)**,** Mobile phase: 0.01 M Potassium di hydrogen phosphate (20%), acetonitrile(20%),methanol (40%)1% orthophosphoric acid (20%). flow rate: 1.0ml/min. Protein was detected at 280 nm.

Preparation of methanol extract

The plant was dried under shade. After drying, the plant material was powdered and extracted by soxhlet apparatus using methanol as solvent. The extract was filtered using Whatmann filter paper No.1 and concentrated at 40Cº in a rotator vacuum evaporator.

Anti-lipase activity of crude methanol extracts

The inhibitory activity of methanol extract against lipase was tested against lipase extracted from the *pseudomonas aeruginosa.* Lipase inhibitory activity of different concentrations of methanol extract for each of the *Quercus infectoria* and *Trigoella foenum graecum* was tested by mixing 100µl of each concentration of methanol extract, 8ml of oil emulsion and 1ml of lipase followed by incubation of 30 minutes. The reaction was stopped by adding 1.5ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH using phenolphthalein as an indicator **(7)** Percentage inhibition of lipase activity was calculated using the formula:

Lipase inhibition = A - B / A X 100
Where A is lipase activity, B is activity of lipase when incubated with ethanol extract.

Statistical analysis
Data obtained in the study were statistically analyzed using Analysis of Variance (ANOVA) and means were separated using Fisher's Least Significant Difference (LSD) at both 1 and 5% levels of significance **(8).**

Result
Optimization of lipase production
The strain of *Pseudomonas* aeruginosa used showed maximum lipase production after 72 hours of incubation. The activity of lipase at this point of time was found to be 0.41 u/ml. Further incubation was found to be negatively affecting the yield (Table 1). Similar results were obtained by (**9**). in their studies on *Pseudomonas alcaligenes.*

Table 1: Time course of lipase production

Lipase activity (u / ml)	Incubation Time (In Hours)
0.13	24
0.25	48
0.41	72
0.22	96

Effect of pH on lipase production
Media pH is one of the important physical parameter which can influence on bacterial growth as well as production. Many studies have been reported the importance of culture pH. **(10)** Reported that the *Pseudomonas aeruginosa* was able to grow in the pH range from 6 to 8 and produced maximum lipase (38.5 U/ml) at pH 7. **(11)** Also reported that maximum lipase activity from *Pseudomonas aeruginosa* KDP (19.32 U/ml) was obtained at pH 7.0. Similarly, in the present study maximum lipase production (0.56 U/ml) was obtained at pH 7 Fig (2).

Effect of temperature on lipase production
The enzyme activities were observed at 20C°, 30Cº, 40Cº, 50C and 50Cº. The optimum temperature was observed at 40C° for maximum production Fig(3). The optimal temperature for lipase production by *Ps. xinjiangensis* CFS14 was 40Cº (**12). (13**) Also reported the enzyme from *Psedomonas spp* worked optimally at slightly higher temperature; 50C°, at which lipase activity was 0.357 µM.min-1.mg-1.

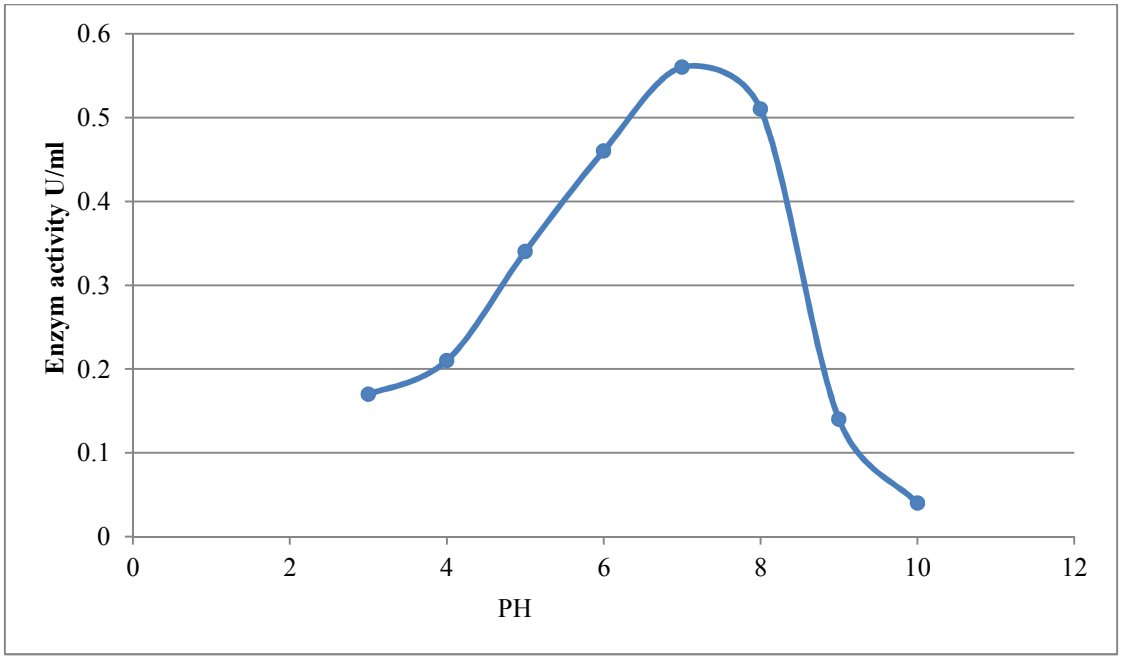


Fig (2): Effect of different pH on lipase production by *Ps. aeruginosa*

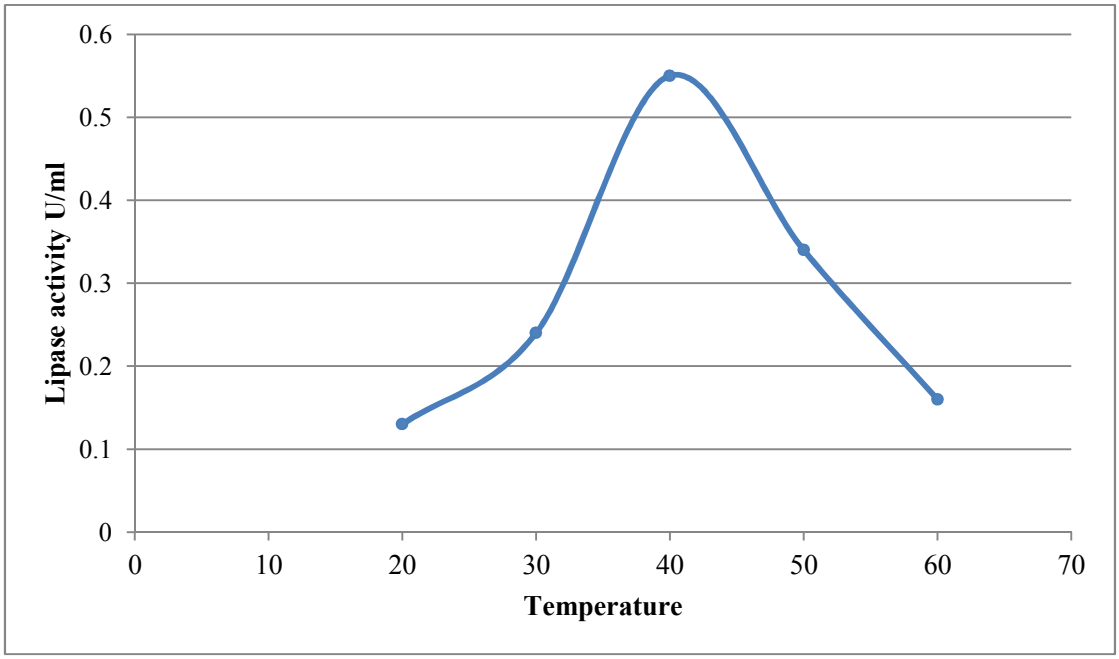


Fig (3): Effect of different temperature on lipase production by *Ps. aeruginosa*

Partial purification of lipase:

Ps. aeruginosa lipase was purified by simple steps which included precipitation with 45%, saturation of ammonium sulfate and gel filtration chromatography by Sephadex G-150, the eluted fractions of the last step contained two protein peaks Figure (4). The two peak (fractionation tubes 19-20) showed lipase activity about (0.81‹0.99) unit/ml and specific activity 3.67 unit/mg and the purification fold was 0.108 times with 1.739 % recovery was obtained after the single step purification on gel filtration chromatography As in table (2). The result was approximately compatible with other studies that deal with lipase of *Ps. aeruginosa* , such as ammonium sulphate 40% saturation(**14**). Analysis of purified lipase was done by HPLC, the individual peak was identified by comparing the retention time of peak with standard Fig (5).Fig (6) Represented the HPLC chromatogram of Gel filtration with Sephadex G -150. The purified lipase showed three peaks with retention time (4.323, 4.920 and 5.069) min the peak with retention times of 4.323min represent the presence of lipase with concentration 0.035 ppm.

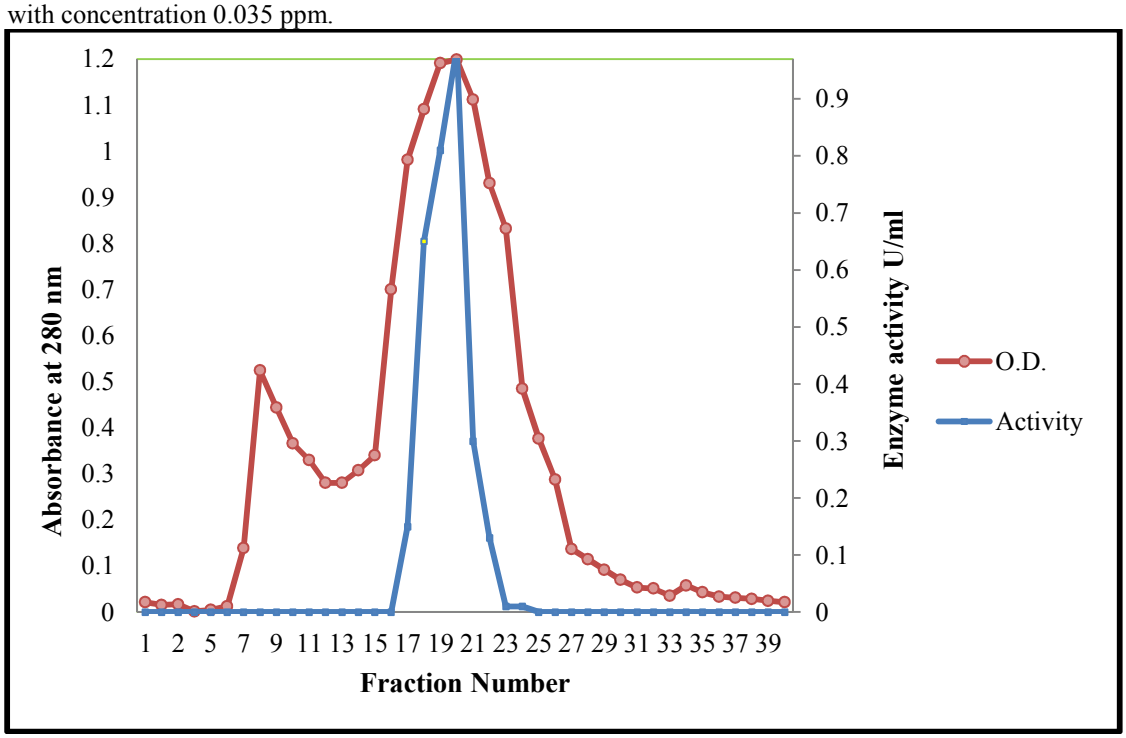
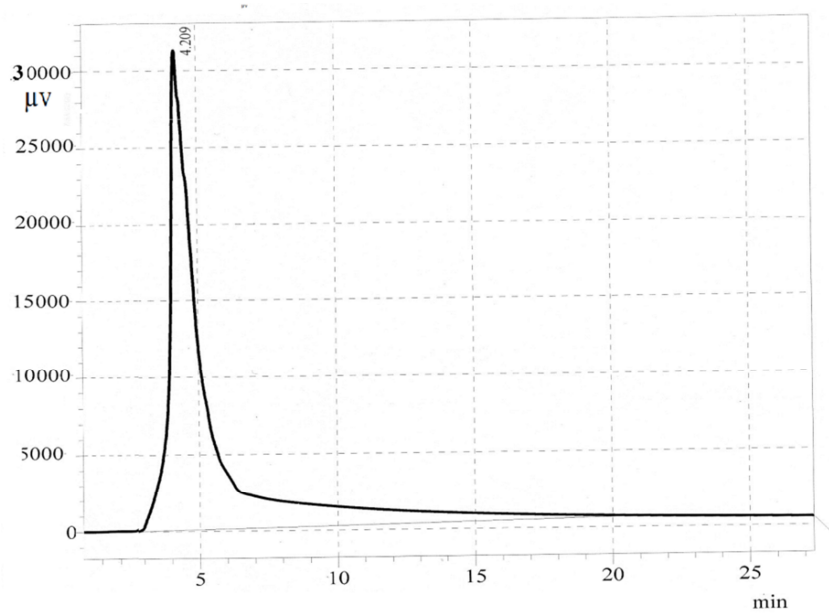


Figure (4): Gel filtration for partial purification of lipase from *Ps .aeruginosa* by Sephadex G -150 column

Table (2): Purification of *Pseudomonas aeruginosa lipase*

Purification Steps	Volume (ml)	Enzyme activity (U/ml)	protein Concentration (mg/ml)	Specific activity (U/mg)	Total activity (U/ml)	Recovery (%)	Fold purification
Crude enzyme	80	0.12	0.17	0.705	9.6	100	1
Precipitation with 45% saturation of $(NH_4)SO_4$ after dialysis	7	2.92	4	0.73	20.44	1.035	2.129
Gel filtration with Sephadex G -150 column	3	0.99	0.27	3.67	1.032	1.739	0.108



Fig(5): HPLC chromatogram of standard lipase

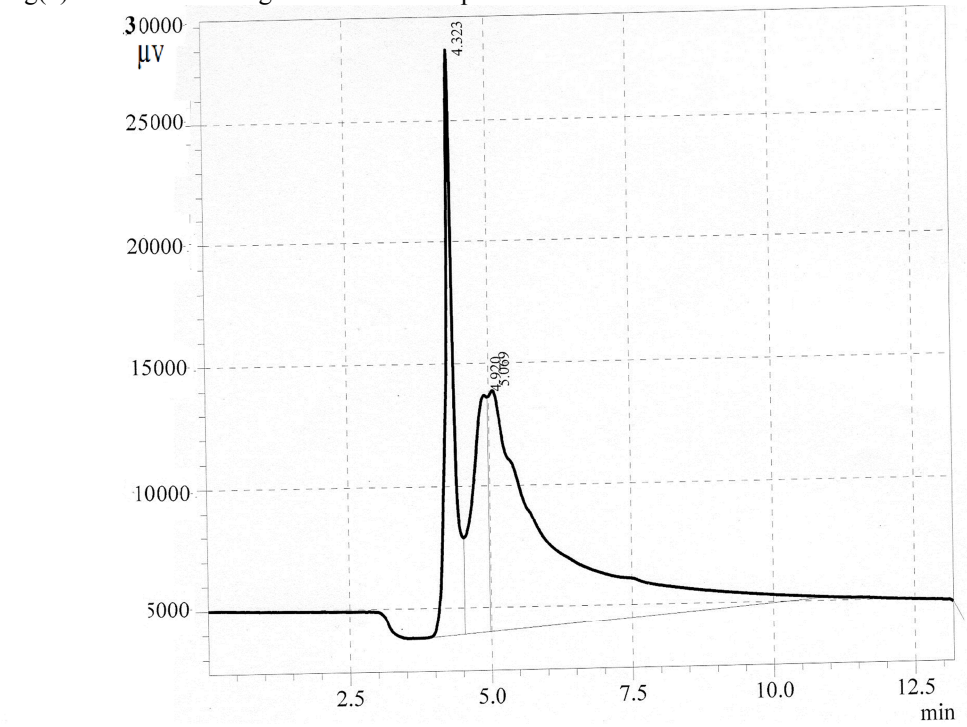


Fig (6): HPLC chromatogram of gel filtration chromatography by Sephadex G-150 of purified lipase

Lipase inhibitory activity of methanolic extract for each of the *Quercus infectoria* and *Trigoella foenum graecum*

*Ps.*aeruginosa lipase was treated with different concentration of plant extract to determine their effect on the production and activity of this enzyme. The methanolic extract varied in their effect *Quercus infectoria* reduced the effectiveness of the enzyme activity more than *Trigoella foenum graecum* Where the specific gravity about (0.36, 1.58) U/mg respectively in the concentration of 20 mg/ml Table (3). In this study we demonstrated that galls of *Quercus infectoria*, has strong anti lipase activity (82.8) % while *Trigoella foenum* graecum has a weak inhibitor activity (49.5) % at concentration 20 mg/ml Table (4).The galls of *Quercus infectoria* is a rich source of tannins which are polyphenolic compounds. Polyphenols from plants have an affinity for proteins, primarily through hydrophobic, as well as hydrogen bonding and therefore exhibit inhibitory activity for enzymes, because of aggregation of enzyme proteins (**15**). As well as contain *Quercus infectoria* on quercetin **(16)** which could play an important role against lipase producing microorganisms **(17).** In addition to containing *Quercus infectoria* on alkaloids which are inhibitors of the enzyme lipase **(18)**.

Table (3): Specific gravity of the lipase enzyme in different concentrations of plant extract.

Rang of Specific activity (U/mg)± Standard error		
Concentration mg/ml	*Quercus infectoria*	*Trigoella foenum graecum*
0	b3.67±0.02	a3.67±0.02
1.25	b3.07±0.02	a3.41±0.01
2.5	b2.63±0.01	a2.93±0.02
5	b2.15±0.03	a2.48±0.04
10	b0.41±0.02	a2.15±0.02
20	b0.63±0.02	a1.58±0.02

- The different numbers refer to significant differences.
- Probability (P≤0.01).
- The result means to five replicate.

Table (3): Anti-lipase activity for eachof *Quercus infectoria* and *Trigoella foenum graecum* against lipase.

	Inhibition %	
Concentration (mg/ml)	*Quercus infectoria*	*Trigoella foenum graecum*
1.25	16.1	7
2.5	28.3	20.2
5	41.4	32.3
10	61.6	41.4
20	82.8	49.5

References

1. Sharma, R.; Chisti, Y.and Banerjee, U.C.(2001). Production, purification, characterization, and applications of lipases. Biotechnol. Adv, 19: 627-662.
2. Jaeger, K. E. and Eggert, T. (2003) .Lipases for biotechnology, Curr Opin Biotechnol, 13: 390-397.
3. Brockerhoff , H. and Jensen, R. (1974) Lipolytic enzymes, Academic Press, NewYork, 29-85.
4. Kojima, Y., M. Kobayashi, and S. Shimizu. 2003. A Novel Lipase from Pseudomonas fluorescens HU380: Gene Cloning, Overproduction, Reneturation-Activation, Two-Step Purification, and Characterization. Journal of bioscience and bioengineering 96(3): 242-249.
5. Loo, J.L.; Lai, O.M..; Long, K. and Ghazali, H.M. (2006). Identification and characterization of a locally isolated lipolytic microfungus *Geotrichum candidum*. Malays. J. Microbiol., 2:22-29.
6. Lowry, O. H., Rosebrough,N. G.; Farr, A.L.and Randall, R. J.(1951).Protein measurement with folin phenol reagent. J. Biol. Chem., 193:265-275.
7. Zheng, C.; Duan, Y.; Gao, J.and Ruan, Z.(2010) Screening for Anti-lipase Properties of 37 Traditional Chinese Medicinal Herbs. J Chin Med Assoc, 73: 319-324.
8. Sanders, D.H., 1990. Statistics; a Fresh Approach. 4th Edn., McGraw Hill Inc., Singapore
9. Gerritse G, Hommes RWJ and Quax WJ, 1998. Development of a lipase fermentation process that uses a recombinant *Pseudomonas alcaligenes* strain. *Appl. Env. Microbiol,* 64(7): 2644-2451.
10. Benattouche, Z., and Abbouni, B. (2012). Production, optimization and characterization of the lipase from Pseudomonas aeruginosa. Romanian Biotechnological Letters, 17(2): 7187- 7193.
11. Sujatha, K. and Dhandayuthapani, K.(2013). Optimization of process parameters for the extracellular lipase production by newly isolated Pseudomonas aeruginosaKDP. Int.J.Curr.Microbiol.App.Sci, 2(7): 116-122.
12. Khemika, L.; Angkhameen, B. and Hataichanoke, N. (2012). Investigation of isolated lipase producing

bacteria from oil contaminated soil with proteomic analysis of its proteins responsive to lipase inducer. J. Biol. Sci., 12 (3): 161-167.

13. Tembhurkar,V.R.; Kulkarni, M.B. and Peshwe, S.A.(2012). Optimization of Lipase Production by *Pseudomonas* spp. in submerged batch process in shake flask culture. Science Research Reporter, 2(1):46-50.
14. Zouaoui, B.; Bouziane, A. and Ghalem, B.R. (2012) Production, optimization and purification of lipase from *Pseudomonas aeruginosa.* African Journal of Microbiology Research, Vol. 6(20):4417-4423.
15. Birari, R.B. and Bhutani, K.K. Pancreatic Lipase Potential. (2007) Drug Discovery Inhibitors from Natural Sources: Unexplored Today, 12: 19
16. Hashim,S.T.(2013). Bacteriological and Biochemical study for effect of phenolic extract of Quercus infectoria against some food-born pathogenic bacteria. Indian of applied research,3:52-55.
17. Gatto,M. T.; Falcocchio, F.; Grippa,E. ; Mazzanti,G.Battinelli,L.; Nicolosi,G. Lambusta, D. and Sasoa, L.(2002). Antimicrobial and Anti-Lipase Activity of Quercetin and Its C2-C16 3-O-Acyl-Esters. Bioorganic & Medicinal Chemistry,10: 269–272.
18. Kim, K.J; Lee M.S.; Jo, K.and Hwang, J.K. (2011) Piperidine alkaloids from Piper retrofractum Vahl. protect against high-fat diet-induced obesity by regulating lipid metabolism and activating AMP-activated protein kinase. Biochem Biophys Res Commun, 22: 219-225.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar