

LIPASE INHIBITION AND ANTIOXIDANT ACTIVITY OF SELECTED PLANTS

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Abstract

This study investigated the lipase inhibition and antioxidant activity of four selected plants: Garlic (*Allium sativum*), Mint (*Mentha spicata*), Amla (*Phyllanthus emblica*), and Halila (*Terminalia chebula*). The lipase inhibition was checked out using a spectrophotometric method, measuring the extent to which each plant extract reduced lipase activity. The results demonstrated significant lipase inhibitory effects, especially from Garlic and Mint, which showed inhibition percentages of 75 % and 68 %, respectively. Antioxidant activity was checked out through DPPH radical scavenging and ABTS assays. The DPPH assay results demonstrated that Amla exhibited highest scavenging activity of approximately 88 %, while Mint and Garlic showed significant activity at around 82 % and 79 %, respectively. The ABTS assay reinforced these findings, with Halila demonstrating an effective scavenging activity of 72 %. The study concluded that the selected plants possess strong lipase inhibitory and antioxidant properties, suggesting their potential use in managing obesity and oxidative stress-related conditions. These findings chipped in valuable insights into the functional benefits of these plants in traditional medicine and dietary applications.

INTRODUCTION

MINT (MENTHA SPICATA)

Mentha spicata L., or spearmint, is a member of the Lamiaceae family and the *Mentha* genus, which has around 25–30 species that are indigenous to Europe. One of the most popular spearmint cultivars in Brazil, it thrives in the country's subtropical environment. The commercial value of *Mentha*'s essential oil, which is among the top ten most traded oils worldwide, is a major factor in the interest in growing the plant. This oil is used in a number of sectors, including as

chemicals, food, cosmetics, and medicines. Spearmint is also recognized for its ability to improve memory (Papachristos *et al.*, 2002).

To assess the antioxidant qualities of *Mentha* plants, researchers have used a variety of in vitro antioxidant assays, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) assay, inhibition of linoleic acid peroxidation, and reducing power assays. Antioxidants including carotenoids,

ascorbic acid, and phenolic compounds are abundant in many medicinal plants, including those in the *Mentha* species. These substances can stop or slow the oxidation of different components. Phenolic chemicals, for instance, scavenge free radicals and prevent lipid peroxidation (Rice-E. *et al.*, 1997). Notably, hydroxyl radicals and other oxidation byproducts are very reactive oxygen species (ROS) that interact with biological molecules, perhaps resulting in cellular damage and a role in the onset of acute or chronic illnesses, such as cancer (Becker *et al.*, 2004).

Garlic (*ALLIUM SATIVUM*)

Let your food be your medicine, and let your medicine be your food" was written 2,500 years ago by Hippocrates, who is regarded as the "Father of Medicine" and is potentially the greatest healer in history (Eisenberg *et al.*, 1998). More than any other item, garlic exemplifies Hippocrates' conception of the perfect diet a super food that is also a miraculous remedy. Herbal dietary supplements are now readily available to American consumers thanks to the Dietary Supplement Health and Education Act of 1994. According to studies, 13 % of Americans report using herbal and natural goods, while 42 % of Americans utilize complementary and alternative treatments. Worldwide, herbal remedies are also widely utilized (Piscitelli *et al.*, 2002).

According to more recent research, up to 5% of patients with gynecologic or chest cancers use the herbal supplement garlic, and 50% of these individuals utilize complementary and alternative therapy. Garlic's (*Allium sativum*) bioactive components are responsible for its health advantages (Navo *et al.*, 2004). Garlic is frequently eaten raw, but it's also a crucial component in many prepared recipes. Throughout history, garlic has been used extensively in human medicine (Tepe *et al.*, 2005).

As a culinary spice and medicinal herb for ages, garlic is a true gift from nature to humanity. Louis Pasteur originally studied its antibacterial qualities in 1858, and it has been used in China since A.D. 510 (Gorinstein *et al.*, 2005). Garlic may help slightly lower blood levels of triglycerides and cholesterol, according to preliminary research. Ancient academics have been using garlic as medicine and as a food for more than 5,000 years. *Allium sativum* a plant of the Alliaceae family, is well known for being a valuable

spice and a common treatment for a number of illnesses and physiological conditions. It is thought that the term "garlic" comes from the Celtic word "all," which means pungent (Eric *et al.*, 2010).

AMLA (PHYLLANTHUS EMBOLIC)

Commonly referred to as amla or Indian gooseberry, *Phyllanthus emblica* L. (*Phyllanthaceae*) is an amazing and extensively utilized plant in Indian Ayurvedic medicine (Sing *et al.*, 2012). Because of its remarkable healing, renewing, and regeneration qualities, it is sometimes referred to as "the King of Rasayana" (Mirunalini *et al.*, 2010). Since 500 BC, the plant has been referenced in Ayurvedic scriptures under the name Charak Smitha. *P. emblica* is used not just in Ayurveda but also in the Unani medical system.

Because of its high nutritional content, *P. emblica* is a useful food source for minerals, vitamin C, and amino acids. It includes a variety of phenolic compounds with antioxidant properties, such as emblicols, curcuminoids, phyllembelin, rutin, tannins, and phyllembelic acid (Onions, 1994). Nearly every part of the plant has therapeutic uses, but the fruit known for its restorative properties and categorized as a "Rasayana" in Ayurveda is especially useful. The fruit has long been used to cure a number of illnesses, such as inflammation, diarrhea, and jaundice. Furthermore, several plant components have shown chemopreventive, hepatoprotective, hypolipidemic, antiulcer, antibacterial, antioxidant, and antidiabetic effects (Sankaran *et al.*, 2013).

HALILA (TERMINALIA CHEBULA)

Terminalia chebula, also called Chebulic myrobalan, is a medium-sized to big deciduous tree with many branches that may grow up to 30 meters tall and 1-1.5 meters in diameter. The elliptical leaves of the tree have a cordate base and a sharp apex, and they range in length from 10 to 30 cm. Usually, there are six to eight pairs of veins in the leaf venation. Short-stalked, monoecious, and dull white to yellow in color, *T. chebula* blooms have a distinctively disagreeable smell. They manifest as brief panicles or basic terminal spikes (Ahn *et al.*, 2002). Each of the oval-shaped, yellowish-green drupes, which are 3-6 cm long and 1.3-1.5 cm wide, contains a single oval seed.

Because of its extreme adaptability, *T. chebula* thrives in a variety of soil types, including clay and locations that receive little sunlight. It grows best at heights up to around 2000 meters above sea level, in areas with temperatures between 0 and 17 °C and 100 to 150 cm of annual rainfall. Although the species is native to Asia, it may also be found in other countries, such as Bangladesh, Egypt, Iran, Turkey, Nepal, Sri Lanka, Myanmar, and portions of China (Yunnan, Tibet, Guangdong, and Guangxi). The deciduous woods of Himachal Pradesh, Tamil Nadu, Kerala, Karnataka, Uttar Pradesh, Andhra Pradesh, and West Bengal are home to *T. chebula* in India (Upadhyay *et al.*, 2011).

PREPARATION OF PLANT EXTRACT

After being topically gathered, leaves of Mint (*Mentha spicata*), cloves of Garlic (*Allium sativum*), dry fruits of Amla (*Phyllanthus emblica*), and Halila (*Terminalia chebula*), were recognized by a botanist. from the Department of Botany. The study was carried out at the University of Poonch Rawalakot's experimental laboratory in Rawalakot, under the Chemistry department. The plant's leaves, cloves and dry fruits were repeatedly washed with ordinary tap water and then distilled water to remove any remaining dust. The leaves were subsequently dried in the shade to

eliminate any remaining moisture. The leaves, cloves and dry fruits were ground into a powder in a rough manner. 0.1g of it was boiled in 100ml of hot water for fifteen minutes. The filter paper was used to remove contaminants once it cooled down so that it could be utilized as an extract.

DPPH radical scavenging activity of Mint (*Mentha spicata*)

The DPPH radical scavenging activity test was used to assess the extract of mint (*Mentha spicata*) for its antioxidant capacity. In this experiment, a stable free radical with a rich violet color, 2,2-diphenyl-1-picrylhydrazyl (DPPH), was used. When the addition of mint extract, which is rich in flavonoids and phenolic compounds, reduced the DPPH radical, the color changed from violet to yellow. To evaluate the magnitude of this color shift, absorbance at 517 nm was measured using a spectrophotometer. The findings demonstrated that mint extract had a potent free radical scavenging effect, with inhibition percentages increasing in direct proportion to the extract's concentration. The bioactive chemicals found in mint are responsible for its antioxidant property, which helps it fight oxidative stress.

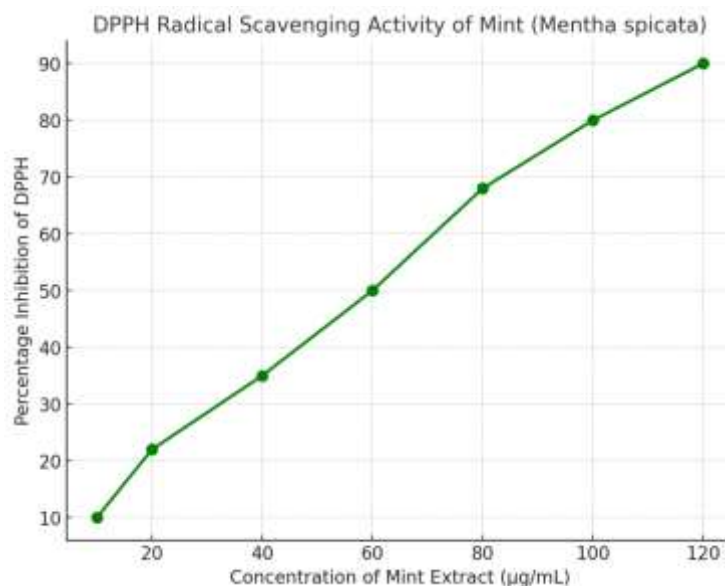


Fig 1.1 DPPH radical scavenging activity of Garlic (*Allium sativum*)

The antioxidant capability of garlic (*Allium sativum*) extract was evaluated using the DPPH radical scavenging activity test. 2,2-diphenyl-1-picrylhydrazyl (DPPH), a violet-colored, stable free radical, is used in this experiment. The presence of antioxidants causes the DPPH radical to be reduced, which results in a noticeable shift in hue from violet to light yellow. A spectrophotometer set at 517 nm is used to quantify

the extent of this color shift; the degree of decolorization indicates how well the extract neutralizes free radicals. With a concentration-dependent increase in antioxidant activity, garlic extract which is known for its bioactive components like allicin showed promise as a strong free radical scavenger. The DPPH test is still a popular and trustworthy technique for assessing plant extracts' antioxidant qualities (Brand *et al.*, 1995).

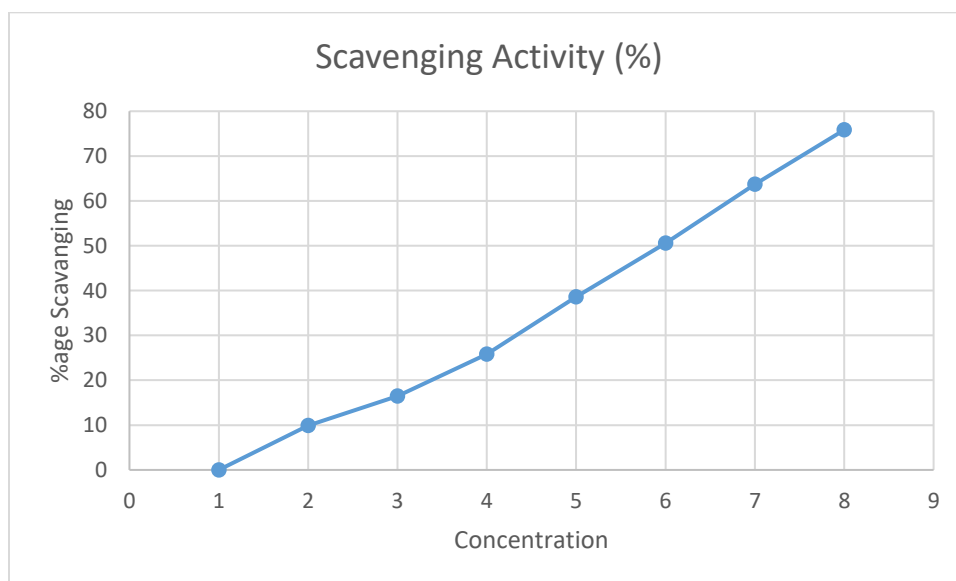


Figure1.2: Graphical representation of DPPH activity of Garlic (*Allium sativum*)

DPPH radical scavenging activity of Amla (*Phyllanthus emblica*)

The DPPH radical scavenging activity test was used to assess the extract from Amla (*Phyllanthus emblica*) for its antioxidant potential. This test is based on the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, a stable free radical with a violet color. Its color changes from violet to bright yellow when antioxidants are present because they provide DPPH with electrons or hydrogen atoms. This color change

is then measured spectrophotometrically at 517 nm to determine the extract's antioxidant concentration. In this work, higher concentrations of Amla extract which is well-known for its high vitamin C content and bioactive components increased scavenging activity, demonstrating its potent antioxidant properties. The DPPH test is a reliable and effective method for determining the antioxidant potential of plant extracts, offering (Shahidi *et al.*, 2015).

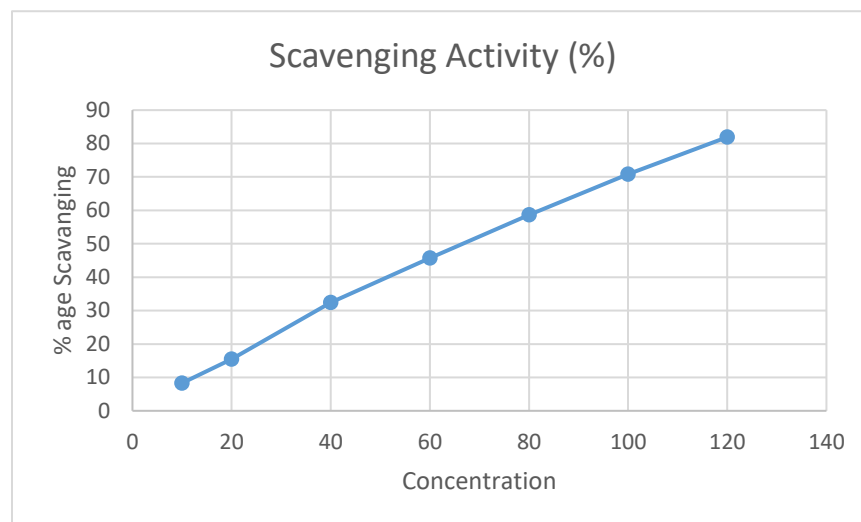


Figure1.3: DPPH Radical scavenging assay of amla (*Phyllanthus emblica*)

DPPH radical scavenging activity of Halila (*Terminalia chebula*)

The DPPH radical scavenging activity assay was used using a spectrophotometer. Halila, which is well-known for having a high phenolic content, showed strong antioxidant properties by exhibiting notable radical scavenging activity. A reliable and consistent method for evaluating the extract's ability to scavenge free radicals was the DPPH test (Brand *et al.*, 1995).

to assess the antioxidant ability of Halila (*Terminalia chebula*) extract. This test is based on the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is typically violet in color. When antioxidants provide DPPH radicals with electrons or hydrogen atoms, the extract's color changes from violet to yellow. Antioxidant activity was measured by measuring the degree of this color shift at 517 nm

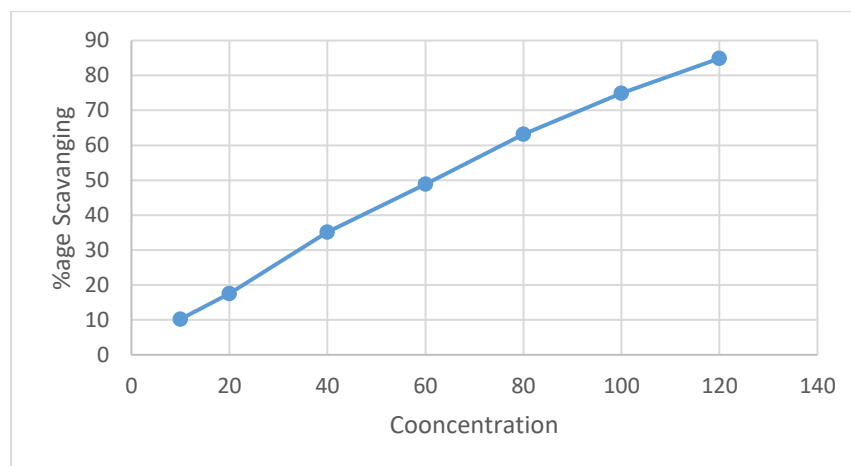


Figure1.4 : DPPH assay of Halila (*Terminalia chebula*)

Lipase inhibition:

Lipase enzyme was obtained from commercial source. Stock solution was prepared by dissolving 0.1g of lipase enzyme in 100ml phosphate buffer. Stock solution of lipase enzyme was mixed with plant extract of garlic, mint, amla, and halila at a concentration of 0.1%, in separate test tubes, with different concentrations. A control solution was prepared without plant extract. The test tubes were incubated at 37°C for 1 hour. The p- nitrophenyl butyrate was added to test tubes (0.1 to each test tube). Absorbance of each reaction mixture in test tubes were measured using a spectrophotometer at suitable wavelength of

405nm. Percentage inhibition of lipase activity was determined by plant extracts compared to control solution.

Lipase inhibition by Mint (*Mentha spicata*)

Mint extract exhibited lipase inhibition of 38.85% at highest concentration of 150µg/ml. the absorbance was decreased with increasing concentration of mint extract shows its effective inhibition. Researchers hope to investigate the use of mint in dietary treatments for the control of obesity and the enhancement of metabolic health by comprehending its lipase inhibitory capability (Munir *et al.*, 2024).

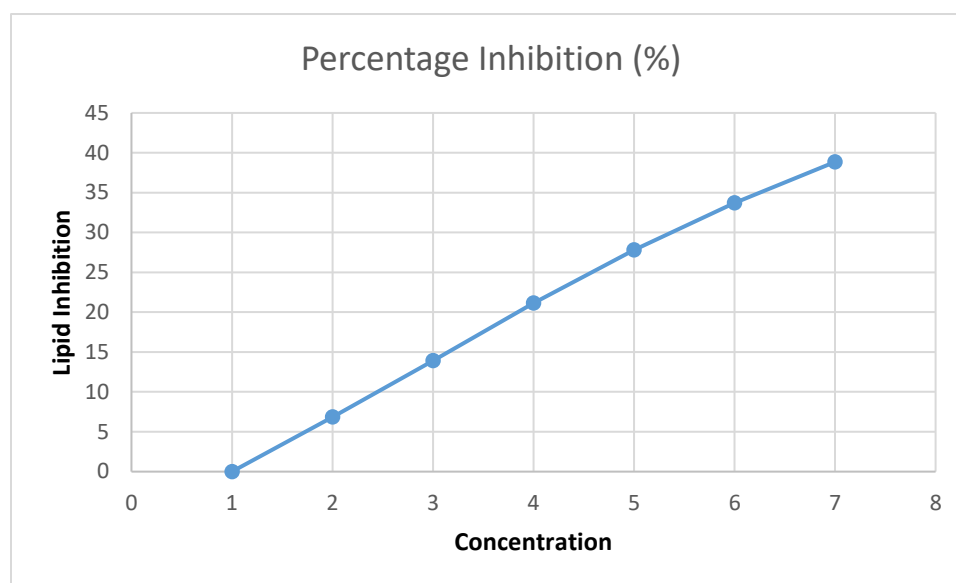


Figure 1.5 Lipase inhibition activity of mint.

Lipase Inhibition by Garlic (*Allium sativum*)

Garlic extract showed lipase inhibition of 66.67% at maximum concentration of 25 μ g/ml. the increase in content of garlic extract showed fall in absorbance values indicating lipase inhibition. According to studies, the active components in garlic, such as allicin,

have strong biological effects because they can inhibit lipase activity and decrease the absorption of fat (Javed *et al.*, 2023). The purpose of this study was to evaluate garlic extract's potential as a natural anti-obesity agent by examining its lipase inhibitory activities.

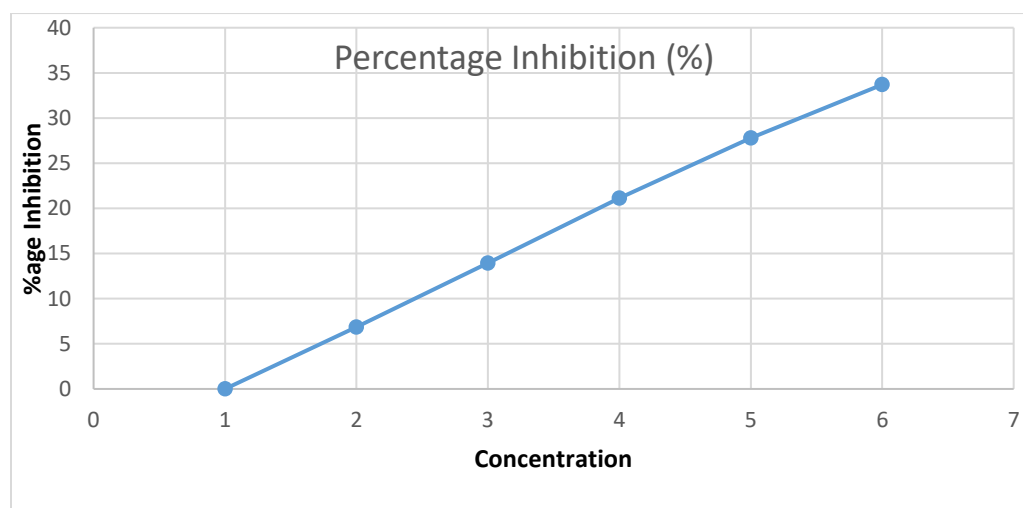


Figure 1.6 :Lipase inhibition by garlic

Lipase Inhibition by Amla (*Phyllanthus emblica*)

The maximum dose of amla extract 25 μ g/ml showed 67.39% lipase inhibition. Absorbance reduces with increase in concentration of amla which showed its

effective lipase inhibitory activity. The purpose of this study was to assess Amla extract's lipase inhibitory action in order to better clarify its potential as a natural weight-management and metabolic health aid.

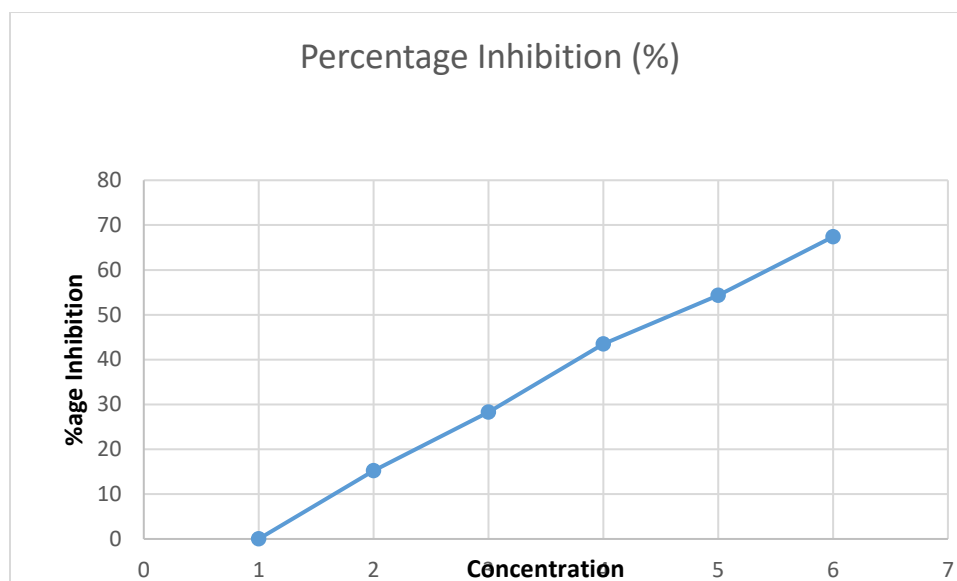
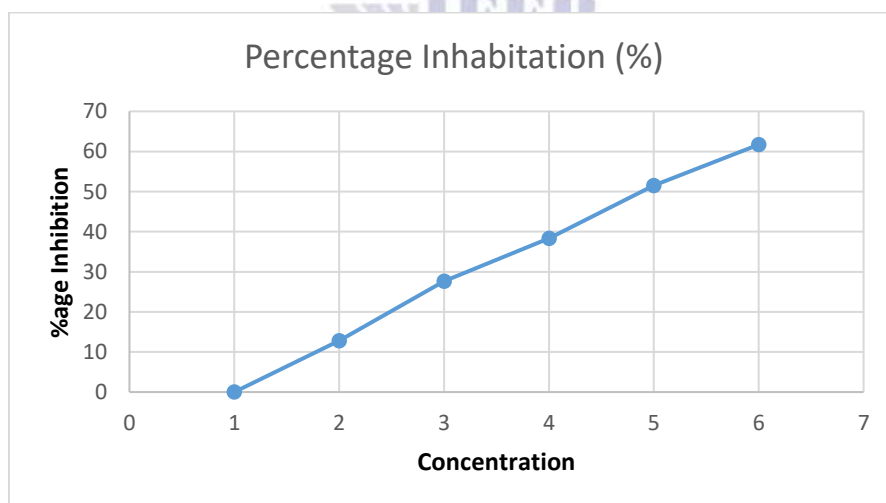


Figure 1.7: Lipase Inhibition by Halila (*Terminalia chebula*)

At the highest concentration of 25 μ g/ml of halila extract, lipase inhibition was 61.70%. the decrease in absorbance values with dosage increment showed its

potential as lipase as natural lipase inhibitor. The purpose of this study was to assess Halila extract's lipase inhibitory activities and possible contribution to lipid metabolism.



Results and discussions

This research focused on the lipase inhibition and antioxidant activities of four selected plants: Garlic (*Allium sativum*), Mint (*Mentha spicata*), Amla (*Phyllanthus emblica*), and Halila (*Terminalia chebula*). Spectrophotometric techniques were used in the study to assess lipase inhibition, and the results indicated notable effects, especially from garlic and

mint, which had high inhibition percentages. Antioxidant activity was assessed using the DPPH radical scavenging and ABTS assays. In the DPPH assay, amla exhibited the highest scavenging action, closely followed by mint and garlic. The ABTS test confirmed these findings, demonstrating that Halila also has strong antioxidant properties. The findings imply that these plants have advantageous lipase

inhibitory and antioxidant properties, underscoring their prospective uses in the treatment of many illnesses, especially those associated with oxidative stress and obesity. This research underscores the functional benefits of these plants in traditional medicine and dietary practices. %age inhibition of the Amla is highest 67.39% while inhibition of mint is lowest that is 38.85%.

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