



**DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
PROJECT REPORT
ON
ANTIOXIDANT ACTIVITY OF SYZYGIUM AROMATICUM (CLOVE)
BUDS**

BY

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CERTIFICATION

This is to certify that this project report entitled “ANTIOXIDANT ACTIVITY OF SYZYGIUM AROMATICUM (CLOVE) BUDS” was written by OLAWUYI JULIUS OLAYINKA with Matriculation Number HND/23/SLT/FT/0515 in partial fulfillment of the requirement for the Award of Higher National Diploma (HND) in the Department of Science Laboratory Technology, Biochemistry Unit, Kwara State Polytechnic Ilorin, Kwara state.

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DEDICATION

This Project report is dedicated to Almighty God and my Parents Mr. And Mrs. OLAWUYI.

ACKNOWLEDGMENT

From the depth of my heart, I am sincerely grateful to Almighty God for His care, love and protection He bestowed upon me throughout my days in Kwara State Polytechnic Ilorin.

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ABSTRACT

13.44g of Syzygium aromaticum (clove) yielded 39% crude extract.

The antioxidant activity of crude extract was determined by the DPPH Method and the TPC measurement.

The ethanolic extract inhibited the DPPA radical by 50.94% and showed a TPC of 190mg GAE.

CHAPTER ONE INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Syzygium aromaticum (L) also known as clove, is dried flower bud belonging to the myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide.

The clove tree is composed of leaves and buds (the commercial part of the tree) and the flowering bud production begins four years after production. Afterward, they are collected either by hand or using a natural phytohormone in the pre-flowering stage (cortes-rojas et al., 2014).

Interestingly, they are commercially used for many medicinal purposes and in the perfume industry and clove is considered one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties. Several reports have documented the antibacterial, antiviral, anticarcinogenic and antifungal activities of some aromatic herbs including cinnamon, oregano, clove, thyme and mint. However, clove has gained much attention among other spices due to its potent antimicrobial and antioxidant activities.

The effective role of clove in the inhabitation of different degenerative diseases is attributed to the presence of various chemical constituents in high concentrations with antioxidant activity (Astuti et al., 2019).

Clove essential oil (CEO) is traditionally used in the treatment of burns and wounds and as a pain reliever in dental care as well as treating tooth infections and toothache. In addition to that, its use has been documented in various industrial applications and is used extensively in perfumes, soaps and as a cleansing vehicle in histological work (Sarrami N et al., 2002).

Clove are used in Indian and Chinese traditional medicine as a warming and stimulating agent.

Traditionally, cloves have been used for centuries in the treatment of vomiting, flatulence, nausea, liver, bowel and stomach disorders and as a stimulant for the nerves. In tropical Asia, cloves have been documented to relieve different microorganisms as scabies, cholera, malaria and tuberculosis. As well in America, clove has been traditionally used in inhibiting food-borne pathogens to treat viruses, worms, candida and different bacterial and protozoan infections (Bhowmik et al., 2012).

Moreover, eugenol has been widely used in dentistry because it can penetrate the dental pulp tissue and enter the bloodstream. Sesquiterpenes, isolated from clove were reported to have anti-carcinogenic activity (Miyazawa et al., 2001).

1.2 DISTRIBUTION

Syzygium aromaticum (clove) belongs to family myrtaceace, a taxon of dicotyledon plants is one of the most valuable and second most important spice in the world trade. Various synonymes used for the clove are *caryophyllus aromaticus*, *caryophyllus*, *silvestris*, *Eugenia caryophyllus*, *Jambosa caryophyllus* and *myrtus caryophyllus* (Soh and Parnell, 2015).

Clove is commonly used in cultivation and indigenous to North Maluku Islands in Indonesia. Major activator countries of clove are pemba, Zanzibar, Indonesia, Madagascar and some of wild clove varieties are found in Bacan, ternatem Motir, Tidore, Makian and Western parts of Irian Jaya. In Indian, cultivation of clove is restricted to three states Karnataka, Tamil Nadu and Kerala. Indian becomes second largest consumer of clove after the Indonesia.

Cloves are available throughout the year due to different harvest seasons in different countries. The different varieties of clove tree vary in canopy shape from pyramidal to cylindrical. The clove tree can live up to 100 years and above. The tree prefers to grow in well-drained soil with sufficient soil moisture. Clove tree requires heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150mm and high humidity above 10% (Danthu et al, 2014). The crop cannot withstand water logged conditions. In India clove grows well in deep black loamy soil of humid tropics and successfully grows in the red soils of mid lands of Kerala and in the hilly terrain of western that's in Karnataka and Tamil Nadu (Byng, 2016).

1.3 MORPHOLOGY AND TAXONOMY

Clove is an aromatic spice tree. The term clove is taken from French word 'clove' and 'clou' which means 'nail'. Clove is conical myrtle, medium sized tree with straight trunk which grows up to 10 to 12m in height. The branches are semi erect, grayish in color and dense leaves are large oblong to elliptic, simple obovate opposite, glabrous and possess plenty of oil glands on the lower surface.

Tree begins flowering in about 7 years and continues flowering for 80 years or more. Flowers are small, crimson in color and are hermaphrodite (bisexual) borne at the terminal ends of small branches. Each peduncle carries 3 to 4 stalked flowers and inflorescence length remains between 4 to 5cm. Initially flower buds are pale yellow in color with glossy appearance and turn green to bright red at maturity. These are 1-2cm long with cylindrical thick ovary consisting of four fleshy sepals. Buds are divided into elongated stem and a globose bulbous head which stimulates into nail. Commercially cloves used are air-dried unopened flower buds, 2.5cm in length and 1.25cm wide (Ortes-Rojas et al., 2014).

Fruit mature nine months after flowering and the red ovary gradually turns to reddish purple. The fruit nearly contains one or two seeds known as “mother of clove” The cultivated trees are rarely allowed to reach fruit stage. These are harvested when they develop dark red ellipsoid berry (Kamatou et al., 2012).

1.4 CLASSIFICATION

Syzygium aromaticum (clove) is classified both botanically and functionally due to its diverse applications in medicine, cuisine and industry. Botanically, it belongs to the myrtaceae family which includes other aromatic and medically valuable plants. Its classification is based on morphological characteristics such as leaf structure, flower arrangement and the presence of oil glands. Taxonomically classification of *Syzygium armoaticum* (L.)

Kingdom :-Plantae

Sub Kindom:- Tracheobionta

Super division:- Spermatophyta

Division: Magnoliphyta

Class:- Magnoliopsida

Sub class:- Rosidae

Order: Myrtales

Family:- Mytaceae

Genus:- *Syzygium*

Species:- *aromaticum*

Functional classification:

Clove is also functionally classified based on its uses:

- As a spice: clove is a widely used culinary spice due to its strong, pungent aroma and flavor. It is commonly included in spice blends and food preservation techniques.
- As a medicinal plant: clove is rich in eugenol, a phenolic compound with proven antioxidant, anti-inflammatory and antimicrobial activities. This makes it a valuable component in both traditional and modern medicine
- As an aromatic plant; clove oil is used in cosmetics, perfumery, aromatherapy and dental care due to its fragrance and analgesic properties.

1.5 CHEMICAL CONSTITUENTS OF CLOVE ESSENTIAL OIL

Clove essential oil, primarily extracted from the dried flower buds of *syzygium aromaticum*, is a complex mixture of bioactive compounds known for their therapeutic, aromatic and preservative properties.

From clove species three essential oils are available which are clove stem oil, clove bud oil and clove leaf oil. Each clove essential oil differs in chemical composition, flavor and color. In clove essential oil amount of secondary metabolites are affected by the nature of soil, climate, cultivation techniques and genetic factors (Veazar-Petri et al., 1985, Arslan et al., 2004).

1.5.1 THE CLOVE BUD ESSENTIAL OIL

The clove bud essential oil is yellow in color and denser than water. Alma et al, 2001 reported the presence of 18 components in clove bud essential oil. The main components characterized were eugenol (87%), chavibetal (19.7%), Bcaryophyllene (13%), eugenol acetate (8.01%), trisiloxanel, 1,1,5,5,5-hexamethyl3,- bis(trimethylsilyl) oxy) (1.7%) etc.

Further studies by (Khan et al, 2009, Matta 2010, Marya et al, 2012 and Kasai et al, 2016) reported eugenol (74.32%) followed by *trans*- β -caryophyllene (15.94%) and eugenol acetate (5.8%) as major compounds of clove bud essential oil.

(Pruthi 2001) reported that methyl-*n*-amyl Ketone (VI) was responsible for the characteristic fruity and fresh color of clove bud essential oil.

(Xu et al, 2016) also studied the chemical composition of clove bud essential oil through Gas chromatography mass spectrometry (GC-MS) and reported the presence of eugenol (I), *trans*- β -caryophyllene (iii), caryophyllene oxide (vii), eugenol acetate (iv), d-selinene (Viii), cadinene (ix), 2-pinene (x) e.t.c.

(Lee et al, 2009) detected total 9 components in clove bud essential oil among the eugenol (1,49.0%), 3-phenylprop-2-enal (xi, 14.32%) and *trans*- β -caryophyllene (iii, 7.5%) were major compounds.

(Fankem et al, 2017) showed the presence of oxygenated monoterpenes (89.06%), monoterpenes (0.04%), sesquiterpenes (10.6%) and linear components (0.03%) in clove bud essential oil and eugenol (1,81.62%) as major compound.

Recent study by (Muhammed et al, 2018) reported that monoterpenes were dominant component of clove bud essential oil and major compound was found to eugenol (1,76%).

1.5.2 THE CLOVE LEAF ESSENTIAL OIL

Clove leaf essential oil has characteristic pleasant odor and faint yellow color. (Jirovetz et al, 2006) reported the presence of 23 compounds with eugenol (I, 76.8%) *trans*- β -caryophyllene (iii, 17.4%) eugenol acetate (iv, 1.2%), d-humulene (xiii, 2.1%) as major compounds (Kapahi and Thappa, 1989) also determined the presence of eugenol (I, 87.8%), *trans*- β -caryophyllene (iii, 13.0%) and d-humulene (xiii,

1.5%) in clove leaf essential oil.

(Srivastava et al, 2005) analyzed 22 compounds represent 99.9% of oil with eugenol (i), iso eugenol (xiii), B-caryophyllene (iii), α -humulene (xii) and γ -cadinene (xiv) as major compounds.

1.5.3 THE CLOVE STEM OIL

The clove stem oil is not commercially used as the clove bud oil as the constituents responsible for fruity odor of clove oil are present in lesser amount and results in the flatter odor of clove stem oil but free eugenol (i) was present in much higher quantity in stem oil than the bud oil. (Patil and Dhale, 2013).

1.5.4 THE CLOVE ROOT OIL

The clove root oil was obtained by steam distillation with yield of about 6%. Freshly distilled root oil was bright yellow in color and having 85-95% of eugenol (i) (Pruthi, 2001).

1.6 AIMS AND OBJECTIVES

AIM

The primary aim of this study is to evaluate the antioxidant activity of *Syzygium aromaticum* (clove) buds using standard biochemical assays.

OBJECTIVES

To achieve the aim of this study, the following objectives have been outlined:-

- To collect and properly identify *Syzygium aromaticum* (clove) buds from reliable source for experimental use.
- To prepare clove bud extracts using suitable solvent (methanol).

- To evaluate the antioxidant activity of the clove extracts using the DPPH radical scavenging assay.
- To compare the antioxidant potential of the different solvent extracts of clove buds.
- To assess the potential of *Syzygium aromaticum* as a natural source of antioxidant for health applications.

CHAPTER TWO MATERIALS AND METHODOLOGY

2.1 PLANT MATERIAL

The plant material used in this study was the dried flower buds of *Syzygium aromaticum* L. (clove)

The *Syzygium aromaticum* (clove) were freshly purchased at Oja Oba Market Ilorin South Kwara State. *Syzygium aromaticum* L. buds purchased at Oja Oba Market are of good quality and are widely acknowledge as authentic and reliable (Okello et al, 2017).

The clove buds were also subjected to morphological identification and voucher authentication. This step was essential to ensure the botanical identity and quality of the plant material, as varieties in plant species can significantly influence phytochemical content and bioactivity (Smith & Jones, 2020). After confirmation, the clove buds were carefully sorted to remove any adulterants or foreign matter, such as broken stems, dirt or debris the cleaned buds were then air-dried at ambient temperature (25-30°C) in a shaded and well-ventilated area. The drying process was conducted away from direct sunlight to prevent the degradation of sensitive volatile

and phenolic compounds such as eugenol, the primary bioactive compound in cloves (Nara Simhan & Shamasundar, 2018).

2.2 APPARATUS AND GLASSWARES

The apparatus and glassware were utilized during the experimental procedure for assessing the antioxidant activity of *Syzygium aromaticum* (clove) buds:

Beakers, weighing balance burette, measuring cylinder, round bottom flask, water bath, conical flask spatula, soxhlet extractor heating mantle, magnetic stirrer, foils, multifunctional kitchen blender, separator funnel, test tube racks, test tube, test tube holder cellulose thimble, 721 visible spectrophotometer, glass rod and distillation apparatus.

All glassware was thoroughly washed and rinsed with distilled water before and after use to prevent contamination and ensure accuracy in experimental results (Oyeleke & Manga, 2008).

2.3 REAGENTS

The following reagents were used in the evaluation of the antioxidant activity of *Syzygium aromaticum* (clove) buds: The reagents used were of high analytical grade and were used without further purification:

Methanol (CH₃OH), Distilled water, concentrated sulphuric acid (CH₂SO₄), Acetic Anhydride, Acetic acid, chloroform Butylated hydroxianisole, sodium nitroprusside (SUP), Ferric Oxide, sodium hydroxide, pyridine, Wagner's, Hager's, Dragendroff, DPPH solution.

All reagents were freshly prepared and properly stored to maintain stability and reliability of results (Sofowora, 1993; Harborne, 1998).

2.4 EXTRACTION OF PLANT MATERIAL

The dried clove bud were pulverized using a high-powered multifunction kitchen blender SAMSUNG (model no: 20221) with 500w and 32000RP, made in japan. The powered clove sample was kept in a plastic container and used for the solvent extraction.

14.44g of dried pulverized clove sample was packed into cellulose thimble and place in a 1L beaker, 300ml of methanol solvent was measured and transferred into the beaker to cover the sample in the thimble. A magnetic bar was place at the bottom of the beaker, the beaker and its content was placed on a magnetic-stirrer temperature regulated hot plate the extraction was done for about 2 hours.

The colored extract solution was removed and another 200ml of fresh methanol was added and the extraction process repeated until the sample was exhaustively extracted. All the extraction were poured together and transferred into a 1L round bottom flask. The extract solution was distilled to remove the methanol solvent. The concentrated extract was subsequently transferred into a beaker and placed in a water bath, heating was done until all solvent almost completely evaporated. The beaker and its content were left to cool at ambient temperature and until it dried,

The weight of the crude extract obtained was determined form which the extract yield was calculated.

The crude methanol extract of clove obtained was labelled CME and was kept in the laboratory and ambient temperature for further analysis.

The yield was calculated as follows:

$$\% \text{ Extract yield} = \frac{\text{weight of crude extract}}{\text{Weight of clove sample}} \times 100$$

2.5 DPPH RADICAL SCAVENGING ASSAY

By utilizing the stable radical, 2, 2-diphenyl –picrylhydrazyl (DPPH), as per the procedure described by Blois (1958), the antioxidant activity of *Syzygium aromaticum* (clove) buds extract was assayed.

The DPPH assay is based on the reduction of the DPPH radical which exhibits a deep violet color to a yellow-colored diphenylpicrylhydrazine upon reaction with hydrogen-donating antioxidants.

Varying concentrations of the clove buds extract were prepared for each test tube, 1 ml of the extract was added to 1 ml of freshly prepared 1 mM DPPH solution in methanol. The mixture was vigorously shaken and incubated in the dark at room temperature for 1 hour to ensure complete reaction.

After incubation the absorbance of the resulting solution was measured at 517 nm using UV-visible spectrophotometer. A blank measurement was also done at the same wavelength.

The DPPH scavenging ability was calculated as follows:

$$\% \text{ DPPH antiradical Activity} = \frac{100 - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \times 100$$

A solution of ascorbic acid was used as standard for the DPPH measurement. 0.1 g of ascorbic acid was dissolved and made up to 10 ml solution with distilled water.

25ml of the standard was measured into a clean test tube, 0.0394g of DPPH solution was added and was kept for 1 hour before absorbance measurement at 517nm was read.

$$\% \text{ DPPH antiradical Activity} = \frac{100 - (\text{Ab simple} - \text{Ab blank})}{\text{Ab control}} \times 100$$

A control sample was determined by adding 1ml of DPPH solution to 2.5ml water and was kept in the dark for 1 hour and absorbance measurement was read at 517nm (Ab control).

2.6. DETERMINATION OF TOTAL PHENOLIC CONTENT

1ml of extract was dissolved in methanol, 5ml of Folin – ciocalteu reagent (FCR) in distilled water was added and 4ml of 70% sodium carbonate (Na_2CO_3) solution was added.

The mixture was incubated at 40°C for 40min and the absorbance at 760nm was measured. A blank measurement was also carried out by adding methanol 1ml of FCR and incubated at 40°C following the same procedure as for sample

$$\text{TPC (Mg GAE quipoly)} = (v/m$$

C = Concentration of Gallic acid equivalent obtained from calibration curve

V = Volume of extract solution

M = Mass of Extract in g

2.6.1 Measurement of TPC of standard for calibration curve solution six (6)

standard gallic acid (0Ng/ml (blank), 504g/ml, 100Ng/ml, 150Ng/ml, 200Ng/ml and 250Ng/ml) these were treated using the same procedure as the extract.

1ml Gallic acid of each solutions was measured into a clean test-tube, then 5ml of 10% Folin-ciocalteu reagent (FCR) was added and mixed gently and 4ml of 7% of Na_2CO_3 was added subsequently. A blue coloration was developed immediately in each test tube. The mixture was incubated for 30mins at 40°C . A blank test solution was carried out by adding methanol Absorbance was measured at 760nm.

CHAPTER THREE

3.1 PERCENTAGE CALCULATION OF EXTRACT YIELD

The percentage crude extract yield of *Syzygium aromaticum* L. (clove) is calculated as follows:

$$\% \text{ Extract yield} = \frac{\text{Weight of crude extract} \times 100}{\text{Weight of clove sample}}$$

Given value:

$$\text{Weight of crude extract} = 5.25\text{g}$$

$$\text{Weight of clove sample} = 13.44\text{g}$$

$$\% \text{ Extract yield} = \frac{\text{Weight of crude extract} \times 100}{\text{Weight of clove sample}}$$

$$\% \text{ Extract yield} = \frac{5.25 \times 100}{13.44}$$

$$\% \text{ Extract yield} = 39\%$$

Therefore the percentage extract yield = 39%

Table 3.2: (TPC) Calibration curve table

Concentration of standard Galic Acid (GA) mg/ml	Absorbance of 760nm		AV
	A1	A2	AV
25	0.701	0.700	0.7005
50	1.105	1.103	1.1040
75	0.985	0.985	0.9850
Blank	0.000	0.000	0.000
100	1.391	1.391	1.3910
125	1.650	1.650	1.6500
150	1.951	1.951	1.9510

A1 and A2 are duplicates of absorbance measured while AV is the average absorbance calculated from A1 and A2

Table 3.3: Result of DPPH Antioxidant Capacity and TPC of sample

Sample	DPPH Scavenging Ability	Total Phenolic Content
<i>Syzygium aromaticum</i> L. (Clove)	50.94%	190

3.4 IC₅₀ value of clove extract

Sample	IC ₅₀ (mg/ml)
Clove extract	58.6

3.4.1 Determination of IC₅₀ values

The IC₅₀ values is the concentration of sample required to inhibit 50% of DPPH radicals, it is determined by plotting % inhibition against concentration and extrapolating the regression line.

3.4.2 Absorbance values of clove extract at different concentrations.

Concentration	Absorbance	% inhibition
20	0.221	27.85
40	0.329	46.43
60	0.453	60.71

CONCLUSION

The project showed that *Syzygium aromaticum* (clove) buds have antioxidant activity. The DPPH test gave result of 50.94% inhibition which means the extract can help reduce free radicals that cause damage to cells. The total phenolic content (TPC) also showed that the extract contains a good amount of phenolic compounds, which are known for their antioxidant properties.

This results support the traditional use of clove in treating illness related to oxidative stress. The presence of antioxidants in the extract suggests that clove buds could be useful in preventing or managing diseases such as cancer, diabetes and heart conditions.

Syzygium aromaticum has the potential to be used as a natural antioxidants in herbal medicine, food preservation or cosmetics.

DISCUSSION

The present study investigated the antioxidant potential of *Syzygium aromaticum* (clove) buds using two in vitro assays:

2,2-diphenyl-2 –picrylhydrazyl (DPPH) radical scavenging activity and total phenolic content (TPC) determination.

The DPPH assay is a widely accepted method for evaluating the free radical scavenging ability to plant extracts. The result obtained showed that the clove bud extract exhibited considerable DPPH radical scavenging activity, indicating its ability to donate hydrogen or electrons to neutralize free radicals. A % inhibition of 50.94% was recorded at the tested concentration, which suggests moderate antioxidant activity. Although not as high as synthetic antioxidants like ascorbic acid, this result supports the traditional use of clove in managing oxidative stress related conditions. (Narasimhan R. & Shamasundar B.A., 2018).

Furthermore, the total phenolic content (TPC), of the extract was determined using the folin-ciocalteu reagent. Phenolic compounds are known to be effective antioxidants due to their redox properties, which allow them to act as reducing agents, hydrogen and singlet oxygen quenchers. The TPC value obtained for clove buds reflects a significant presence of these bioactive compounds, which likely contributed to the radical scavenging effect observed in the DPPH assay.

Overall, the findings from both the DPPH and TPC assays confirm that *Syzygium aromaticum* buds possess notable antioxidant potential, supporting their use in traditional medicine and their potential as natural antioxidants in food and

pharmaceutical applications (Bhowmilk D. et al., 2012)

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APPENDIX

DPPH

$$\%DPPH = 100 - \frac{[Ab\ standard - Ab\ blank]}{Ab\ Control} \times 100$$

$$Absorbance\ standard = 0.7638$$

$$Absorbance\ blank = 0.000$$

$$Absorbance\ control = 1.557$$

$$\begin{aligned}\% DPPH &= 100 - \frac{[0.7638 - 0.000]}{1.557} \times 100 \\ &= 100 - \frac{[0.7638 - 100]}{1.557} \\ &= 100 - 49.055 \\ &= 50.94\%\end{aligned}$$

For Total phenolic content [TPC]

$$TPC\ [mg\ GAEquipolg] = C \times V/M$$

$$Concentration = 1.9mg/ml$$

$$Volume\ [v] = 10ml$$

$$Mass\ [m] = 0.1g$$

$$\text{TPC} = \frac{1.9 \times 10}{10}$$

$$\text{TPC} = 190\text{mg GAE/g}$$

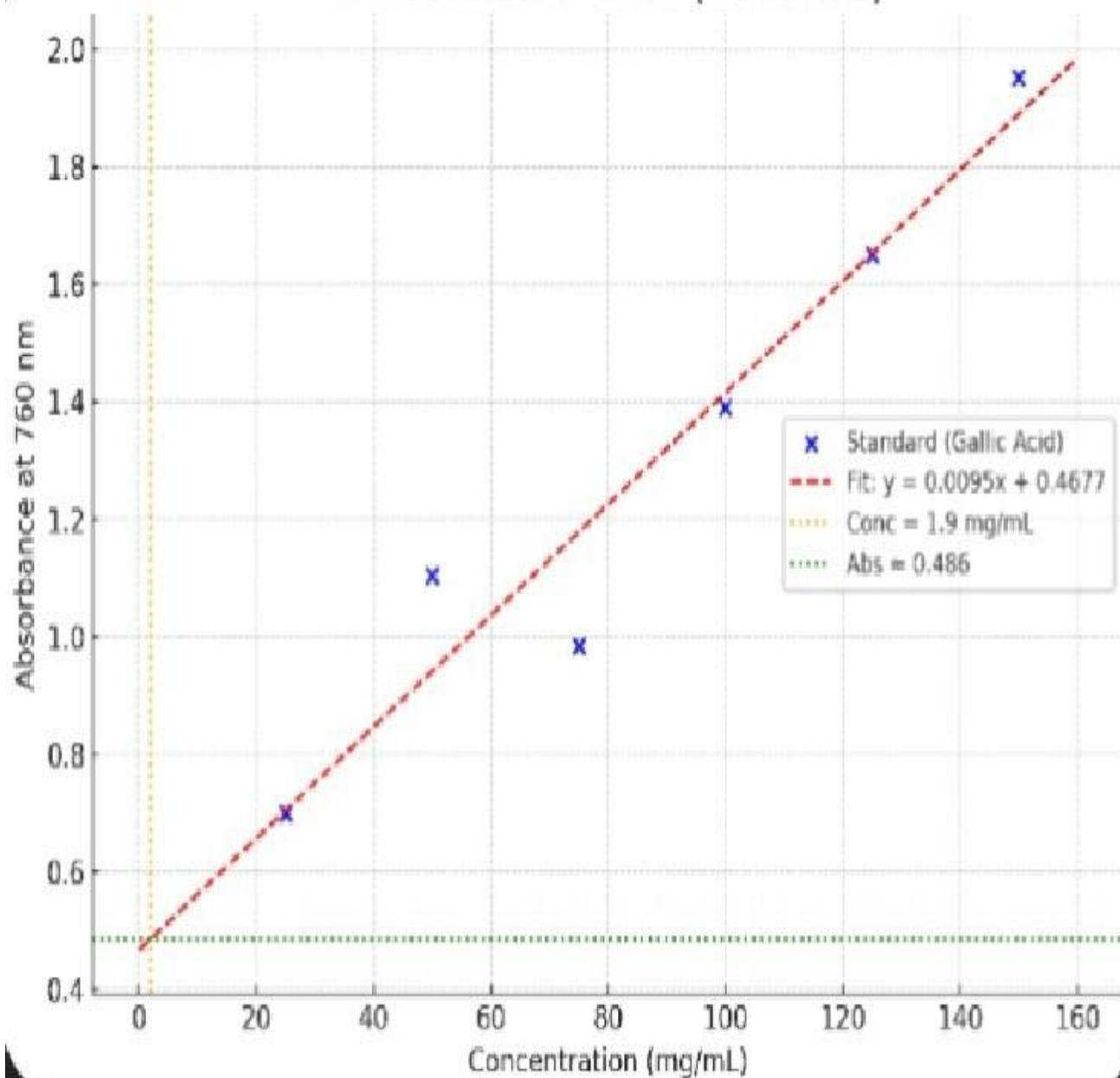
FOR IC₅₀

$$y = 0.569x + 16.65$$

$$1C_{50} = \frac{50 - 16.65}{0.569}$$

$$= 58.6 \mu\text{g/ml}$$

TPC Calibration Curve (Gallic Acid)



GRAPH OF %INHIBITION AGAINST CONCENTRATION

