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PHARMACOGNOSTICAL AND PHYTOPHARMACOLOGICAL INSIGHTS INTO *PIPER LONGUM* L. LEAVES WITH GC–MS AND HPTLC FINGERPRINTING

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Keywords:

Piper longum, Pharmacognosy, GC–MS, HPTLC, Antidiabetic, Anti-inflammatory, Antibacterial, Antifungal, Piperine

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ABSTRACT: Background: *Piper longum* L. (long pepper) is a well-known Ayurvedic medicinal plant used for metabolic, inflammatory, and infectious disorders, yet its leaves remain relatively underexplored despite their traditional relevance. **Objective:** This study aimed to investigate the pharmacognostic, phytochemical, and pharmacological properties of *Piper longum* leaves, focusing on their antidiabetic, anti-inflammatory, antibacterial, and antifungal activities. **Methods:** Leaves of *P. longum* were collected, authenticated, and evaluated through pharmacognostic and physicochemical analyses. Phytochemical screening identified key secondary metabolites, while GC–MS and HPTLC provided compound profiling and fingerprinting. *In-vitro* assays assessed antidiabetic activity (α-amylase and α-glucosidase inhibition), anti-inflammatory effects (albumin denaturation and HRBC membrane stabilization), and antimicrobial activity against selected bacterial and fungal pathogens. **Results:** Pharmacognostic analysis confirmed distinct diagnostic features of the leaves, while phytochemical screening identified alkaloids, flavonoids, tannins, terpenoids, saponins, phenolics, and glycosides. GC–MS revealed key constituents including asarone, β-caryophyllene, phytol derivatives, and piperine-like alkaloids and HPTLC provided reproducible chemical fingerprints. The extract showed strong, dose-dependent α-amylase and α-glucosidase inhibition, notable anti-inflammatory effects comparable to aspirin, and significant antimicrobial activity, with highest inhibition against *P. acnes* and *C. albicans*, in some cases surpassing standard drugs. **Conclusion:** These findings validate *Piper longum* leaves as a pharmacologically versatile source of bioactive compounds with broad therapeutic potential. Their combined antidiabetic, anti-inflammatory, and antimicrobial activities, supported by strong phytochemical evidence, justify traditional uses and highlight their promise for development into standardized herbal formulations and modern phytopharmaceuticals.

INTRODUCTION: Herbal medicine utilizes plant-derived and natural substances for therapeutic purposes, although many traditional claims remain insufficiently validated [1, 2].

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Their efficacy is attributed to bioactive constituents such as phenolics, flavonoids, alkaloids, terpenoids, and saponins, which confer diverse pharmacological activities [3-5].

Piper longum L. (long pepper), an aromatic climber native to South and Southeast Asia, contains piperine, lignans, and essential oils that support its extensive traditional use in managing digestive, respiratory, metabolic, and microbial disorders [6-9]. Historical records trace its use to ancient Greek and

Roman civilizations, with the term "pepper" derived from the Tamil *pippali* [10]. In modern contexts, rising global burdens of diabetes, chronic inflammation, and microbial resistance highlight the need for safer, multi-targeted alternatives to synthetic drugs. Plant-based agents such as *Gymnema sylvestre*, curcumin, and boswellic acids exemplify this potential, while increasing fungal (*C. albicans*) and bacterial resistance underscores the importance of exploring phytochemicals as novel antimicrobial candidates [11-17].

FIG. 1: *PIPER LONGUM* PLANT

MATERIALS AND METHODS: Fresh leaves of *Piper longum* L. were collected from the Western Ghats region of Tamil Nadu and taxonomically confirmed by Dr. P. Radha (CCRS). A voucher specimen was archived in the departmental herbarium for future verification. Pharmacognostic analysis began with macroscopic characterization, documenting leaf color, texture, odor, taste, size, shape, and venation, along with photographic imaging for diagnostic clarity [18]. Powdered shade-dried leaves were examined microscopically using histochemical reagents specific for lignin, cellulose, and starch [19]. Fresh leaf samples were cleared in NaOH, sectioned, and mounted in glycerin for quantitative microscopy, enabling measurement of stomatal number, stomatal index, epidermal cell density, and palisade ratio [20]. Additional morphological descriptors leaf margin, base, apex, and venation pattern were also recorded [21].

For detailed anatomical study, plant tissues were fixed in FAA, dehydrated through a tertiary butyl alcohol series, and embedded in paraffin following the classical procedure of Sass (1940) [22]. Thin transverse sections (10–12 µm) were cut, dewaxed, and stained with toluidine blue, safranin, fast green, or IKI to distinguish structural components and localize cell wall biopolymers such as lignin, cellulose, suberin, mucilage, and starch [23]. Epidermal peels and paradermal sections were prepared using 5% NaOH or Jeffrey's fluid to observe stomatal types, venation architecture, and trichome morphology [24]. Photomicrographs were captured with a Nikon LabPhoto-2 microscope under bright-field and polarized light conditions [25].

Physicochemical constants were assessed using standard protocols. Moisture content was measured as loss on drying at 105 °C [26]. Total ash was obtained after incineration at 450–600 °C [27], while acid-insoluble ash and water-soluble ash were quantified through treatment with 6 N HCl and boiling water, respectively [28, 29]. Alcohol-soluble and water-soluble extractive values were determined using 95% ethanol and distilled water extraction procedures [30, 31]. Volatile oil content was estimated by hydrodistillation using a Clevenger apparatus [32]. The swelling and foaming indices were measured to assess mucilage and saponin content [33, 34], and pH was recorded with a calibrated digital pH meter [35].

For phytochemical screening, the air-dried powdered leaves were subjected to Soxhlet extraction with ethanol. The resulting extract was concentrated under reduced pressure, and attributes such as yield, consistency, and color were documented [36, 37]. Standard qualitative assays were used to test for major secondary metabolites including phenolics, tannins, alkaloids, flavonoids, terpenoids, saponins, glycosides, steroids, carbohydrates, proteins, and quinones [38].

Chemical profiling was performed using GC–MS equipped with a DB-5 MS capillary column. The instrument was operated with helium as carrier gas (1 mL/min), injector temperature 250 °C, linear oven ramp from 60–280 °C, EI ionization at 70 eV, and a mass scan range of 50–600 m/z [39]. HPTLC fingerprinting utilized silica gel 60 F_{254} plates and a mobile phase of toluene–ethyl acetate–formic acid (7:2:1). Samples were applied with a CAMAG Linomat-5 system, scanned at 254 and 366 nm, and derivatized with anisaldehyde–sulphuric acid for visualization [40].

The pharmacological activities of the extract were examined through a series of *in-vitro* assays. Antidiabetic activity was evaluated *via* α-amylase inhibition by quantifying DNS-reactive reducing sugars at 540 nm after incubation with starch substrate (250–1000 µg/mL extract) [41]. α-Glucosidase inhibition was determined by measuring glucose release during sucrose hydrolysis using a chromogenic reagent at 510 nm [42]. Anti-inflammatory potential was examined through inhibition of heat-induced albumin denaturation at 660 nm, with acetyl salicylic acid as reference [43], and by HRBC membrane stabilization based on percent hemolysis inhibition at 560 nm [44].

Antifungal effects against *Candida albicans* and *Aspergillus niger* were assessed using agar well and disc diffusion techniques on PDA plates, employing Amphotericin B as the standard drug [45]. Antibacterial activity against *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, and *Enterococcus faecalis* was tested using agar well diffusion on nutrient agar, with gentamicin as the positive control. Zones of inhibition were recorded after 24 h incubation at 37 °C and analyzed using GraphPad Prism 6.0 software [46].

RESULTS AND DISCUSSION:

Macroscopy of Leaf: The leaves are simple, alternate, and entire, borne on distinct petioles, and exhibit a lanceolate to ovate-lanceolate outline with a cordate base and acute apex. They possess a thin, membranous, glabrous surface with clearly visible basal ribs. The lamina typically measures 4–7 × 2–3 cm, while the petiole extends 1–2 cm in length.

TABLE 1: ORGANOLEPTIC CHARACTERS OF *P. LONGUM* L. (LEAF)

Sl. no.	Specification	Character
1	State	Solid
2	Nature	Fine
3	Odour	No characteristic odour
4	Touch	Smooth
5	Flow Property	Non-Free flowing
6	Appearance	Dark green in colour
7	Taste	No characteristic taste

Microscopy of Leaf: The transverse section of the petiole exhibits a reniform outline, characterized by a convex abaxial and a concave adaxial surface. The epidermis is uniseriate and covered by a thin cuticle, bearing numerous unicellular to multicellular uniseriate nonglandular trichomes. Beneath the epidermis, the hypodermis comprises 5–6 continuous layers of collenchyma along the adaxial surface, whereas the abaxial and lateral regions are interrupted by parenchymatous cells. The outer cortex consists of 3–4 layers of chlorenchyma on the upper side and small parenchyma cells below, while the inner cortex and ground tissue are composed of large, thin-walled parenchyma. The vascular system is arranged in a crescent-shaped arc containing 8–10 conjoint, collateral, and closed vascular bundles, with xylem elements positioned adaxially (mesarch configuration) and phloem oriented toward the periphery.

The midrib, in transverse section, appears biconvex with an angular adaxial surface and a semicircular abaxial surface. A uniseriate epidermis with a delicate cuticle covers both surfaces, and unicellular to 2–4-celled uniseriate nonglandular trichomes are confined to the abaxial side. The hypodermis consists of 5–6 layers of collenchyma beneath both the upper ridge and the lower epidermis. Two layers of thin-walled parenchyma occur on either side of the adaxial hypodermis and extend into the lamina as enlarged parenchyma cells. Beneath the hypodermis lie 3–4 layers of chlorenchyma, which merge into the mesophyll. A single large conjoint, collateral, closed vascular bundle is centrally positioned within the ground tissue, which is composed of broad parenchyma cells, with xylem oriented adaxially and phloem located toward the abaxial side. Epidermal peel preparations revealed a hypostomatic leaf surface with abundant anomocytic stomata, each guard cell pair surrounded by four uniformly sized subsidiary cells. Both upper and lower epidermal layers consist of large, polygonal parenchymatous cells.

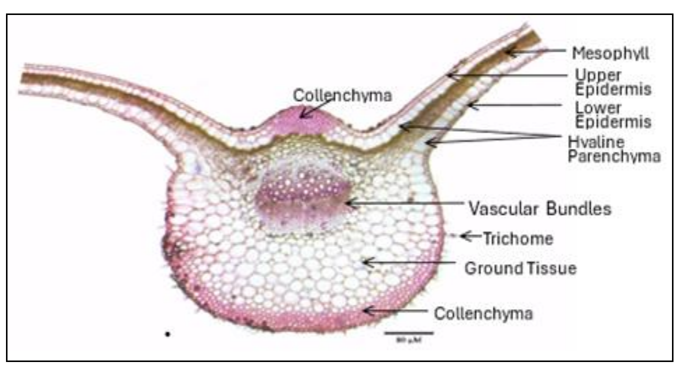

FIG. 2: TRANSVERSE SECTION OF *PIPER LONGUM* LEAF

Powder Microscopy: Microscopic analysis of *Piper longum* leaves demonstrated characteristic cellular features, including polygonal epidermal cells, anomocytic stomata, deposits of oleoresins, calcium oxalate crystals, stone cells, fibrosclereids, and fragments of xylem vessels exhibiting pits and scalariform thickenings, along with dispersed mesophyll tissue.

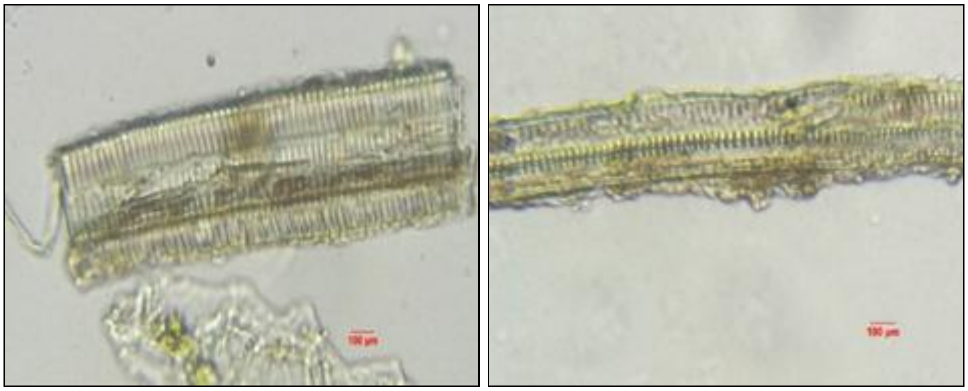

FIG. 3: XYLEM VESSEL WITH SCALARIFORM THICKENING AND XYLEM VESSEL WITH PITTED AND SCALARIFORM THICKENING

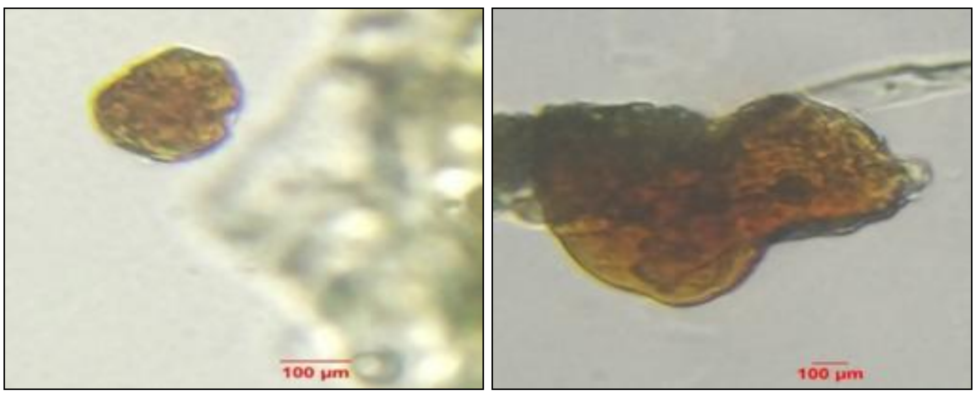

FIG. 4: BROWN CONTENT/OLEORESIN CONTENT

FIG. 5: SPIRAL THICKENING OF XYLEM VESSEL

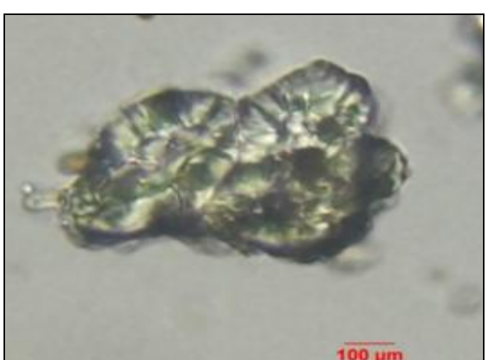

FIG. 6: STONE CELLS

FIG. 7: FIBROSCLEREID

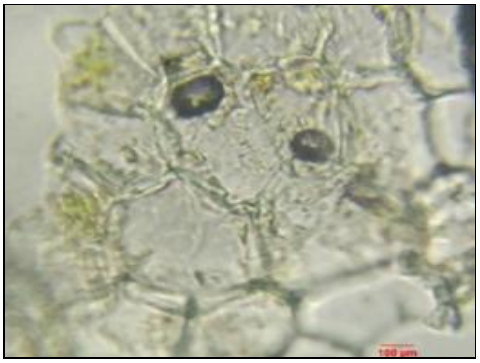

FIG. 8: EPIDERMAL CELLS

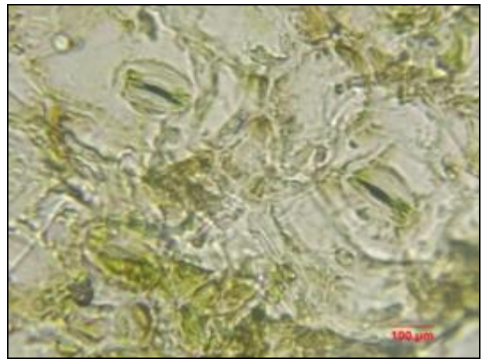

FIG. 9: TETRACYTIC STOMATA

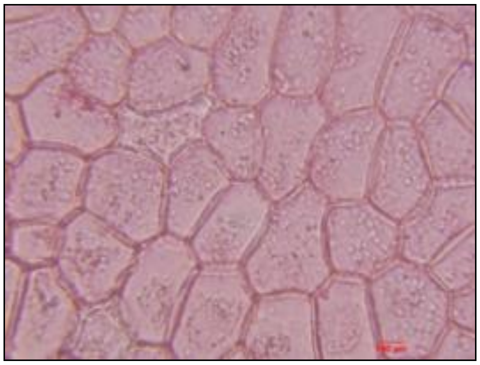

FIG. 10: UPPER EPIDERMIS

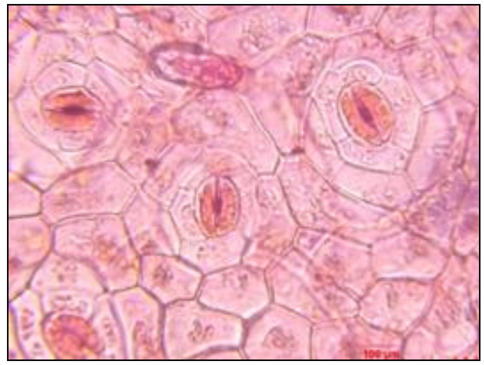

FIG. 11: LOWER EPIDERMIS

FIG. 12: VEIN TERMINATIONS

FIG. 13: VEIN ISLET

TABLE 2: QUANTITATIVE MICROSCOPY

Parameter	*Piper longum*	
	Upper	Lower
Stomatal number	-	210-235
Epidermal number	550-570	680-610
Stomatal Index	-	23.59- 27.89
Vein islet	2	
Vein termination	8	
Palisade ratio	5-6	

TABLE 3: PHYSICO- CHEMICAL ANALYSIS

Sl. no.	Parameters	Results
1.	Loss on drying (%)	18.45
2.	Total ash (%)	11.48
3.	Acid insoluble ash (%)	0.75
4.	Water soluble ash (%)	2.50
5.	Water soluble extractive (%)	17.42
6.	Alcohol soluble extractive (%)	11.66
7.	Swelling index	1.5 ml/g
8.	Foaming index	100
9.	Volatile oil (%)	2
10.	pH	5.14

TABLE 4: ETHANOLIC EXTRACTION RESULTS OF *PIPER LONGUM* L. LEAVES

Parameter	Observation / Result
Plant Material Used	100 g of shade-dried *Piper longum* leaf powder
Solvent Used	Ethanol (99%)
Extraction Method	Soxhlet Extraction
Duration of Extraction	8–10 hours
Solvent Volume Used	1000 mL
Weight of Crude Extract Obtained	10.85 g
Extractive Yield (%)	10.85% w/w
Physical Appearance	Dark green, sticky semi-solid extract
Odour	Aromatic, characteristic of *Piperaceae*
Solubility	Soluble in ethanol

TABLE 5: PRELIMINARY PHYTOCHEMICAL ANALYSIS (+ PRESENT; - ABSENCE)

Tests	Result
Saponins	-
Tannins	+
Phenols	+
Terpenoids	+
Alkaloids	+
Flavanoids	+
Steroids	+
Glycosides	+
Carbohydrates	-
Quinones	-
Proteins	-

HPTLC Analysis of *Piper longum* Extract: High-performance thin-layer chromatography (HPTLC) of the ethanolic leaf extract revealed a chemically diverse profile under both UV (254 and 366 nm) and post-derivatization conditions.

Distinct chromatographic bands were observed within Rf ranges of 0.08–0.72 (254 nm), 0.08–0.71 (366 nm) and exhibited purple and green coloration after derivatization. Prominent, reproducible bands at Rf 0.62–0.72 corresponded to marker alkaloids such as piperine, validating both chemical diversity and method reliability.

Densitometric analysis at 254 nm (Track 1, 5 µL) displayed ten peaks, with the major peak at Rf ≈ 0.58 (33.68% area), while moderate peaks at Rf 0.68–0.81 and minor peaks below Rf 0.35 indicated additional secondary metabolites. Track 2 (7 µL) exhibited ten peaks, with the dominant peak at Rf 0.58 (33.68%) identified as piperine, and secondary peaks at Rf 0.74 (14.97%) and Rf 0.81 (12.40%) likely representing related amide alkaloids such as piperlongumine or pipernonaline; minor peaks (Rf 0.05–0.27) suggested additional constituents.

At 366 nm, Track 1 (5 µL) showed nine peaks across Rf 0.02–0.86, with the major peak at Rf 0.68 (37.05%) and high-intensity peaks at Rf 0.08 and 0.58 (16.29% each), together accounting for ~70% of total area. Track 2 (7 µL) revealed nine peaks, dominated by Rf 0.68 (41.57%), followed by Rf 0.57 (18.94%) and Rf 0.07 (12.78%), with additional minor peaks confirming a reproducible chemical profile.

At 575 nm, Track 1 (5 µL) displayed 11 peaks, with major compounds at Rf 0.61 (28.14%) and Rf 0.86 (24.85%), and moderate peaks at Rf 0.44 (13.76%) and Rf 0.71 (8.37%). Track 2 (7 µL) showed 12 resolved peaks, with prominent peaks at Rf 0.59 (28.13%) and Rf 0.86 (25.31%), moderate peaks at Rf 0.42 (13.44%), 0.76 (6.58%), and 0.51 (6.10%), and minor peaks (<5%), reflecting a highly diverse and well-resolved phytochemical profile.

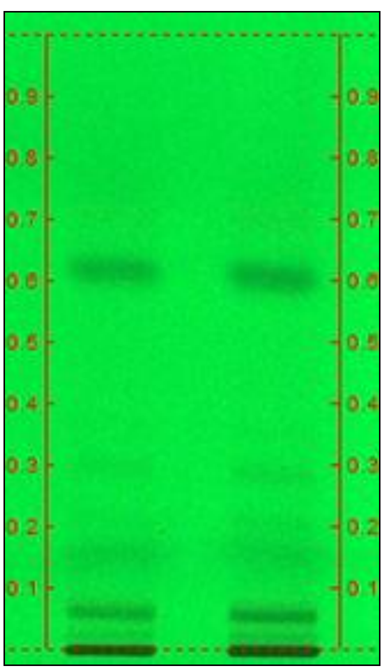

FIG. 14: HPTLC UNDER UV SHORT

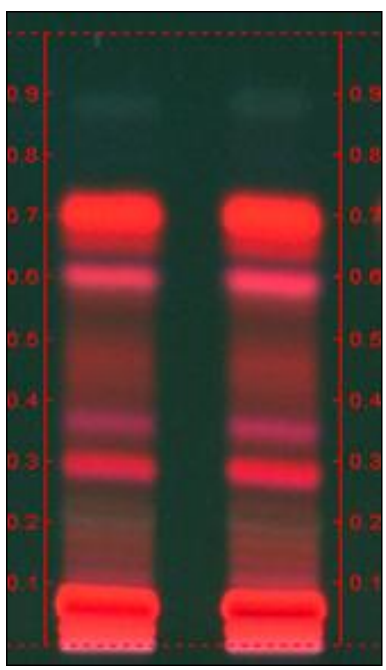

FIG. 15: HPTLC UNDER UV LONG

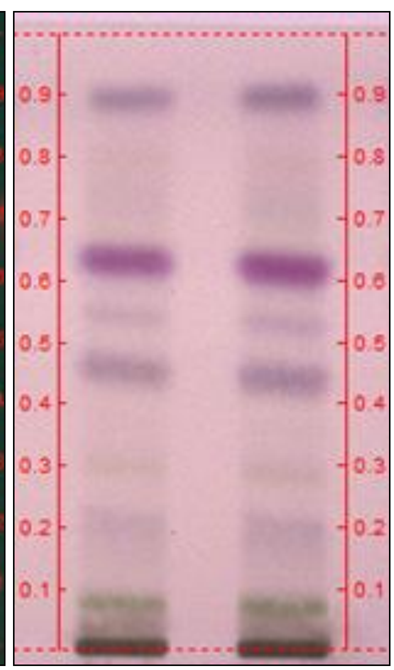

FIG. 16: HPTLC UNDER WHITE LIGHT AFTER DERIVATISATION

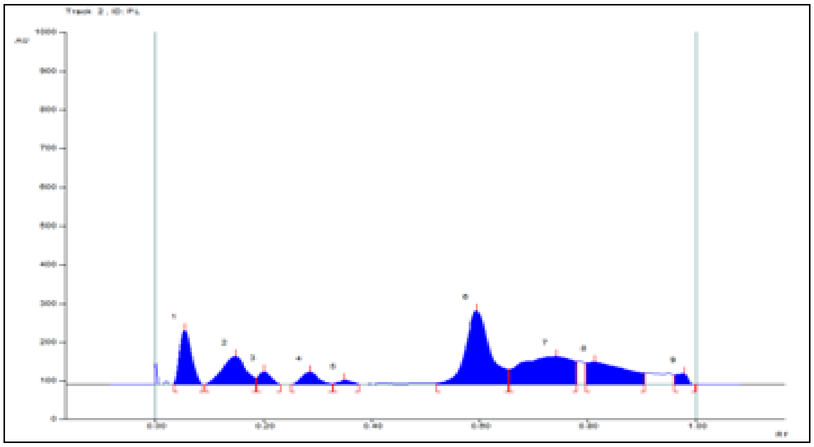

FIG. 17: HPTLC TRACK 1- 5 µL, 254 NM

TABLE 6: HPTLC DENSITOGRAM PROFILE (TRACK 1 – 5, µL254 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	1.7 AU	0.05 Rf	140.7 AU	22.05%	0.09 Rf	0.0 AU	2080.6 AU	10.55%
2	0.09 Rf	0.0 AU	0.15 Rf	72.7 AU	11.39%	0.19 Rf	17.4 AU	2181.8 AU	11.06%
3	0.19 Rf	17.5 AU	0.20 Rf	32.9 AU	5.15%	0.23 Rf	0.0 AU	513.5 AU	2.60%
4	0.25 Rf	0.1 AU	0.29 Rf	31.9 AU	4.99%	0.33 Rf	1.6 AU	685.8 AU	3.48%
5	0.33 Rf	2.1 AU	0.35 Rf	11.3AU	1.77%	0.38 Rf	0.9 AU	184.8 AU	0.94%
6	0.52 Rf	3.4 AU	0.60 Rf	190.6 AU	29.87%	0.65 Rf	39.3 AU	5987.0 AU	30.35%
7	0.66 Rf	39.4 AU	0.74 Rf	72.8 AU	11.40%	0.78 Rf	60.3 AU	4687.2 AU	23.76%
8	0.80 Rf	56.7 AU	0.81 Rf	57.7 AU	9.05%	0.91 Rf	30.1 AU	2981.6 AU	15.11%
9	0.96 Rf	24.8 AU	0.98 Rf	27.6 AU	4.32%	1.00 Rf	0.1 AU	426.8 AU	2.16%

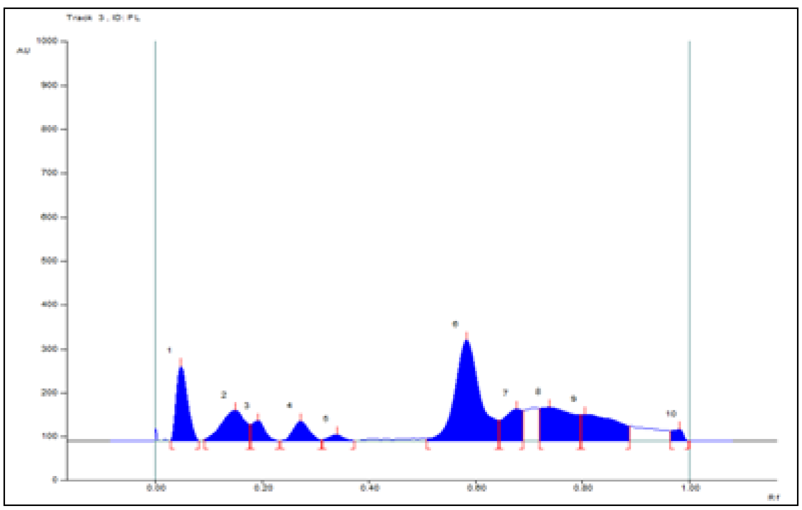

FIG. 18: HPTLC TRACK 2 – 7 µL, 254 NM

TABLE 7: : HPTLC DENSITOGRAM PROFILE (TRACK 2 – 7 µL, 254 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.6 AU	0.05 R1	170.3AU	21.00%	0.08 Rf	0.8 AU	2422.3 AU	10.93%
2	0.09 R1	3.5 AU	0.15 R1	69.5 AU	8.57%	0.18 Rf	37.2AU	2147.0 AU	9.69%
3	0.18 R1	37.4 AU	0.19 Rf	45.5 AU	5.61%	0.23 Rf	0.1 AU	763.2 AU	3.44%
4	0.24 Rf	0.4 AU	0.27 Rf	44.7 AU	5.51%	0.31 Rf	2.2 AU	909.0 AU	4.10%
5	0.31 Rf	2.4 AU	0.34 Rf	14.2 AU	1.75%	0.37 Rf	0.2 AU	257.1 AU	1.16%
6	0.51 Rf	4.6 AU	0.58 R1	230.6 AU	28.44%	0.64 Rf	46.4 AU	7463.5 AU	33.68%
7	0.64 Rf	46.4 AU	0.68 R1	72.5 AU	8.95%	0.69 Rf	69.7 AU	1747.4 AU	7.88%
8	0.72 Rf	74.0 AU	0.74 R1	76.4 AU	9.43%	0.80 Rf	59.1 AU	3317.0 AU	14.97%
9	0.80 Rf	59.3 AU	0.81 Rf	60.5 AU	7.46%	0.89 Rf	33.9 AU	2748.7 AU	12.40%
10	0.97 Rf	23.1 AU	0.98 Rf	26.7 AU	3.29%	1.00 Rf	0.0 AU	387.1 AU	1.75%

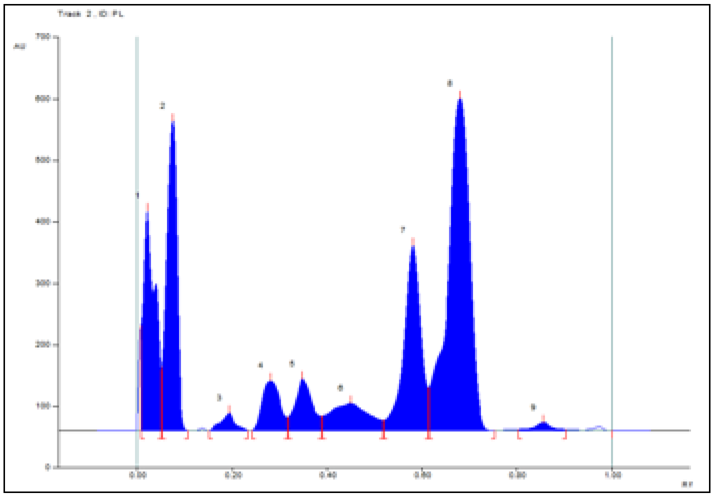

FIG. 19: HPTLC TRACK 1 – 5 µL, 366 NM

TABLE 8: HPTLC DENSITOGRAM PROFILE (TRACK 1 – 5 µL, 366 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	170.6 AU	0.02 Rf	357.8 AU	18.32%	0.05 Rf	98.7 AU	6405.9 AU	13.89%
2	0.05 Rf	103.9 AU	0.08 Rf	503.3 AU	25.77%	0.11 Rf	0.0 AU	7514.6 AU	16.29%
3	0.15 Rf	0.2 AU	0.19 Rf	28.6 AU	1.47%	0.23 Rf	0.7 AU	571.8 AU	1.24%
4	0.24 Rf	0.3 AU	0.28 Rf	80.9 AU	4.14%	0.32 Rf	21.6 AU	2111.4 AU	4.58%
5	0.32 Rf	21.9 AU	0.35 Rf	83.7 AU	4.28%	0.39. Rf	24.0 AU	2072.0 AU	4.49%
6	0.39 Rf	24.1 AU	0.45 Rf	44.8 AU	2.29%	0.52 Rf	16.9.AU	2501.0 AU	5.42%
7	0.52 R1	16.9.AU	0.58 R1	300.2 AU	15.37.9%	0.62 Rf	69.1 AU	7510.2 AU	16.29%
8	0.62 Rf	69.6 AU	0.68 Rf	540.3 AU	27.66%	0.75 R1	0.0 AU	17084.1 AU	37.05%
9	0.81 Rf	1.6 AU	0.86 Rf	13.6 AU	0.70%	0.90 Rf	1.7 AU	345.4 AU	0.75%

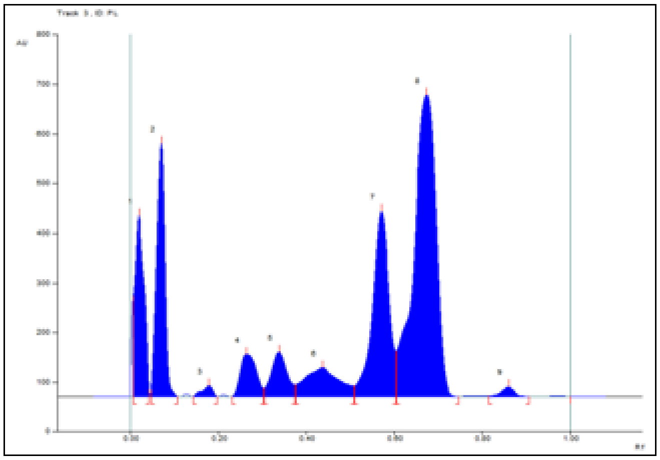

FIG. 20: HPTLC TRACK 2 – 7 µL, 366 NM

TABLE 9: HPTLC DENSITOGRAM PROFILE (TRACK 2 – 7 µL, 366 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	202.5 AU	0.02 Rf	364.5 AU	17.08%	0.04 Rf	15.5 AU	5474.5 AU	10.62%
2	0.05 Rf	13.3 AU	0.07 Rf	510.5 AU	23.91%	0.11 Rf	0.1 AU	6590.2 AU	12.78%
3	0.14 Rf	0.4.AU	0.18 Rf	22.4 AU	1.05%	0.20 Rf	0.3 AU	387.4 AU	0.75%
4	0.23 Rf	0.1 AU	0.26 Rf	85.9 AU	4.02%	0.31 Rf	17.0 AU	2146.9 AU	4.16%
5	0.31 Rf	17.5 AU	0.34 Rf	90.5 AU	4.24%	0.38 Rf	24.3 AU	2228.2 AU	4.32%
6	0.38 Rf	24.4 AU	0.44 Rf	59.0 AU	2.77%	0.51 Rf	21.7 AU	3111.6 AU	6.04%
7	0.51 Rf	21.8 AU	0.57 Rf	373.3 AU	17.49%	0.61 Rf	91.6 AU	9765.7 AU	18.94%
8	0.61 Rf	92.2 AU	0.68 Rf	608.2 AU	28.49%	0.75 Rf	0.0 AU	21433.1 AU	41.57%
9	0.82 Rf	1.6.AU	0.86 Rf	20.3 AU	0.95%	0.91 Rf	0.2 AU	420.0 AU	0.81%

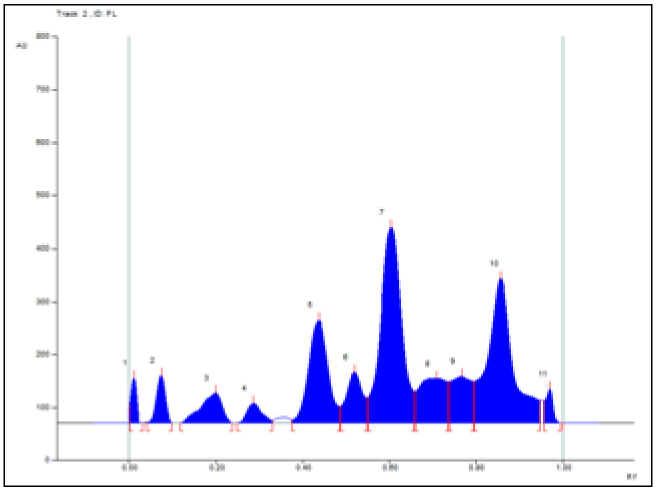

FIG. 21: HPTLC TRACK 1- 5 µL, 575 NM

TABLE 10: HPTLC DENSITOGRAM PROFILE (TRACK 1 – 5 µL, 575 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	34.0 AU	0.01 Rf	85.7 AU	5.95%	0.03 Rf	0.1 AU	769.2 AU	1.74%
2	0.04 Rf	1.6 AU	0.08 R1	90.3 AU	6.27%	0.10 Rf	0.4 AU	1273.4 AU	2.88%
3	0.12 Rf	0.1 AU	0.20 Rf	57.1 AU	3.97%	0.24 Rf	0.0 AU	1926.2 AU	4.36%
4	0.25 Rf	0.1 AU	0.29 R1	37.1 AU	2.58%	0.33 Rf	4.1 AU	882.1 AU	2.00%
5	0.38 Rf	5.8 AU	0.44 Rf	194.6 AU	13.51%	0.49 Rf	30.8 AU	6075.4 AU	13.76%
6	0.49 Rf	31.2AU	0.52 Rf	96.3 AU	6.69%	0.55 Rf	47.3 AU	2494.3 AU	5.65%
7	0.55 Rf	48.0 AU	0.61 R1	368.8 AU	25.61%	0.66 Rf	59.9 AU	12430.2 AU	28.14%
8	0.66 Rf	60.0 AU	0.71 R1	84.6 AU	5.87%	0.74 Rf	77.3 AU	3696.3 AU	8.37%
9	0,74 Rf	77.7 AU	0.77 Rf	88.0 AU	6.11%	0.80 Rf	78.1 AU	2897.1 AU	6.56%
10	0.80 Rf	78.3 AU	0.88 R1	273.7 AU	19.00%	0.95 Rf	42.7 AU	10974.5 AU	24.85%
11	0.96 Rf	41.5 AU	0.97 Rf	63.9 AU	4.44%	1.00 Rf	0.1 AU	748.7 AU	1.70%

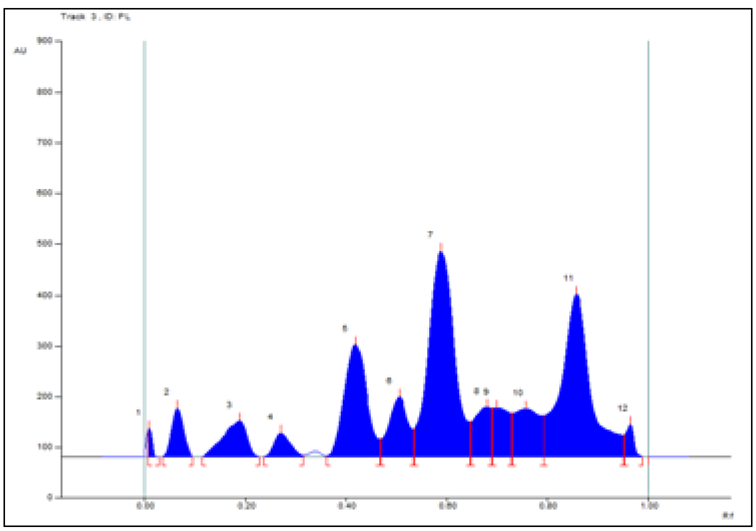

FIG. 22: HPTLC TRACK 2 – 7 µL, 575 NM

TABLE 11: HPTLC DENSITOGRAM PROFILE (TRACK 2 – 7 µL, 575 NM)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	52.2 AU	0.01 Rf	56.1 AU	3.32%	0.03 Rf	0.0 AU	323.3 AU	0.63%
2	0.04 Rf	0.5 AU	0.06 Rf	95.5 AU	5.65%	0.10 Rf	0.3 AU	1550.5 AU	3.03%
3	0.12 R1	0.2 AU	0.19 Rf	71.0 AU	4.20%	0.23 Rf	0.1 AU	2337.0 AU	4.57%
4	0.24 R1	0.7 AU	0.27 Rf	46.7 AU	2.76%	0.32 Rf	3.4 AU	1041.6 AU	2.04%
5	0.36 Rf	2.0 AU	0.42 Rf	221.5AU	13.11%	0.47 Rf	34.7 AU	6877.6 AU	13.44%
6	0,47 Rf	34.9 AU	0.51 R1	118.6 AU	7.02%	0.54 Rf	54.5AU	3121.0 AU	6.10%

7	0.54 Rf	55.3 AU	0.59 Rf	404.6 AU	23.94%	0.65 Rf	68.8.AU	14393.9 AU	28.13%
8	0.65 R1	69.2 AU	0.68 Rf	98.8 AU	5.84%	0.69 R1	96.3 AU	2279.1 AU	4.45%
9	0.69 R1	96.3 AU	0.70 Rf	97.0 AU	5.74%	0.73 Rf	85.9 AU	2230.7 AU	4.36%
10	0.73 Rf	86.0 AU	0.76 Rf	94.7 AU	5.60%	0.79 Rf	80.6 AU	3364.9 AU	6.58%
11	0.80 Rf	80.7 AU	0.86 Rf	321.0 AU	18.99%	0.95 R1	42.5 AU	12950.1 AU	25.31%
12	0.95 Rf	43.1 AU	0.97 Rf	64.6 AU	3.82%	0.99 Rf	0.8 AU	703.4 AU	1.37%

GC–MS (GAS Chromatography–Mass Spectrometry) Analysis: GC–MS analysis of *Piper longum* leaf extract revealed a chemically diverse profile, with major constituents including alkaloids (notably piperine), terpenoids (β-caryophyllene, humulene, α-pinene, limonene), phenolics, esters, fatty acids, and hydrocarbons. The dominant compound was 1,3-bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one (31.93%), followed by 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.39%), asarone (2.62%), benzene,1,2,3-trimethoxy-5-(2-propenyl)- (3.32%), and 9-phenanthrenemethyl 2,6-dimethylbenzoate (3.37%). Minor peaks, including cyclotrisiloxane derivatives and esters, reflect a complex array of phenylpropanoids, terpenoids, heterocycles, esters, amides, and siloxanes. The major compounds likely underlie the observed antidiabetic, anti-inflammatory, antimicrobial, antioxidant, and neuroprotective activities, while minor constituents may act synergistically; alkaloids contribute primarily to antidiabetic and antimicrobial effects, terpenoids to anti-inflammatory and antimicrobial actions, and phenolics to antioxidant and anti-inflammatory potential.

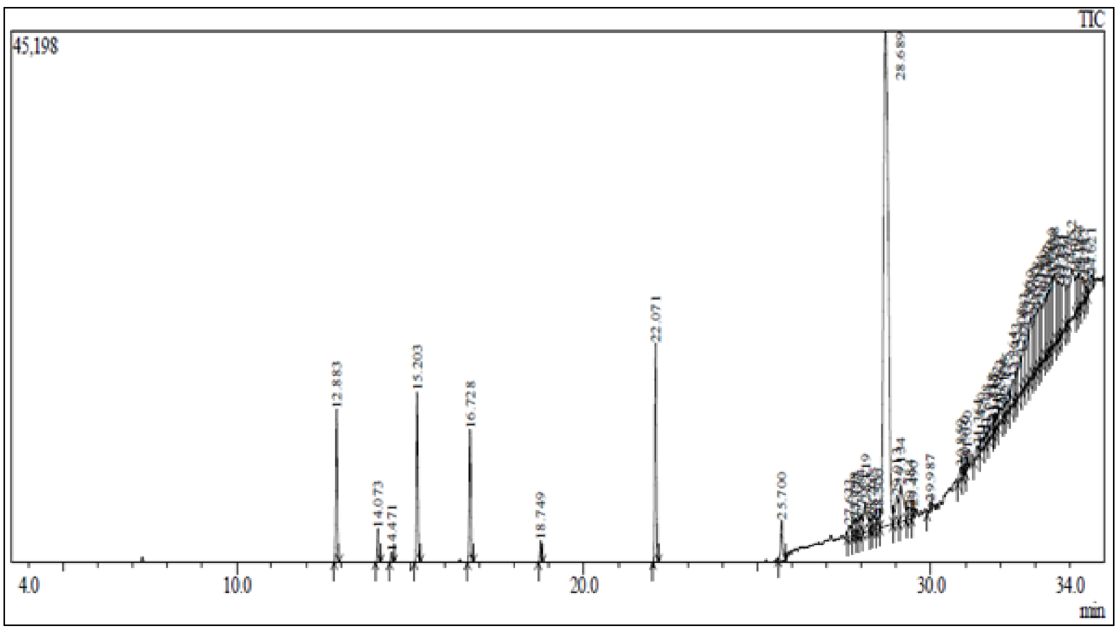

FIG. 23: CHROMATOGRAM - GCMS

TABLE 12: RESULT – GCMS

Peak	Retention Time	Start Time	End Time	m/Z	Area	Area %	Height	Height %	A/H	Name
1	12.883	12.808	12.958	TIC	38187	2.89	13054	5.51	2.93	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-
2	14.073	14	14.142	TIC	8320	0.63	2877	1.21	2.89	2-(Benzylamino)-1-phenylethanol, N,O-bis(trimethylsilyl)-
3	14.471	14.408	14.533	TIC	2447	0.19	885	0.37	2.76	Peroxide, 1,1-dimethylethyl 1-methyl-1-phenylethyl
4	15.203	15.125	15.292	TIC	43878	3.32	14505	6.13	3.03	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-
5	16.728	16.65	16.817	TIC	34686	2.62	11320	4.78	3.06	Asarone
6	18.749	18.683	18.817	TIC	5234	0.4	1879	0.79	2.79	2-Imino-4-methylpentanenitrile
7	22.071	21.992	22.15	TIC	57997	4.39	18679	7.89	3.1	3,7,11,15-Tetramethyl-2-

										hexadecen-1-ol
8	25.7	25.6	25.825	TIC	14423	1.09	3391	1.43	4.25	Benzonitrile, m-phenethyl-
9	27.633	27.592	27.75	TIC	7778	0.59	925	0.39	8.41	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole
10	27.798	27.75	27.833	TIC	5606	0.42	1321	0.56	4.24	4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one
11	27.877	27.833	27.917	TIC	5398	0.41	1221	0.52	4.42	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole
12	27.984	27.917	28.042	TIC	9099	0.69	1490	0.63	6.11	1-Heptene, 1,3-diphenyl-1-(trimethylsilyloxy)-
13	28.119	28.042	28.225	TIC	16942	1.28	2571	1.09	6.59	Vinyl crotonate
14	28.267	28.225	28.3	TIC	3827	0.29	916	0.39	4.18	2-1-Phenyl ethylidene-hydrazono-3-methyl-2,3-dihydrobenzothiazole
15	28.345	28.3	28.4	TIC	5878	0.44	1190	0.5	4.94	1-Heptene, 1,3-diphenyl-1-(trimethylsilyloxy)-
16	28.5	28.4	28.533	TIC	7256	0.55	1046	0.44	6.94	N-Ethylformamide
17	28.689	28.533	28.908	TIC	422152	31.93	42135	17.79	10.02	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one
18	29.013	28.908	29.075	TIC	16532	1.25	2355	0.99	7.02	Amylene hydrate
19	29.134	29.075	29.3	TIC	26312	1.99	3108	1.31	8.47	Vinyl crotonate
20	29.384	29.3	29.442	TIC	7477	0.57	1041	0.44	7.18	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
21	29.49	29.442	29.533	TIC	3660	0.28	865	0.37	4.23	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative
22	29.987	29.892	30.017	TIC	2970	0.22	860	0.36	3.45	1,3-Dioxolane, 2-(3-bromo-3-buten-1-yl)-
23	30.859	30.775	30.908	TIC	4927	0.37	1012	0.43	4.87	4-Cyclopentene-1,3-dione
24	30.97	30.908	31	TIC	3035	0.23	841	0.36	3.61	4-Cyclopentene-1,3-dione
25	31.05	31	31.092	TIC	3615	0.27	989	0.42	3.66	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
26	31.361	31.233	31.4	TIC	4612	0.35	763	0.32	6.04	Glycine, 2-cyclohexyl-N-(but-3-yn-1-yl)oxycarbonyl-, dodecyl ester
27	31.45	31.4	31.567	TIC	6692	0.51	924	0.39	7.24	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
28	31.608	31.567	31.667	TIC	4457	0.34	883	0.37	5.05	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
29	31.758	31.667	31.8	TIC	7363	0.56	1109	0.47	6.64	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-
30	31.817	31.8	31.85	TIC	2674	0.2	914	0.39	2.93	3-Methylsalicylic acid, 2TMS derivative
31	31.892	31.85	31.933	TIC	4882	0.37	1284	0.54	3.8	4-Cyclopentene-1,3-dione
32	31.983	31.933	32.075	TIC	8743	0.66	1361	0.57	6.42	2(1H)-Pyrazinone
33	32.108	32.075	32.142	TIC	4034	0.31	1074	0.45	3.76	4H-Pyran-4-one
34	32.175	32.142	32.267	TIC	7163	0.54	1094	0.46	6.55	2-Fluoro-3-trifluoromethylbenzoic acid, 8-chlorooctyl ester
35	32.364	32.267	32.4	TIC	13058	0.99	2116	0.89	6.17	Isoindole-4-carboxylic acid, 2,3-dihydro3-oxo-2-phenyl-, ethyl ester
36	32.453	32.4	32.483	TIC	10631	0.8	2516	1.06	4.23	2,3,8-Trimethyl-1H-pyrrolo[3,2-H]quinoline-7-carboxylic acid ethyl ester
37	32.608	32.483	32.633	TIC	24515	1.85	3092	1.31	7.93	1-Propanone, 1,1'-(2,4,6-

										trihydroxy-m-phenylene)di-
38	32.683	32.633	32.733	TIC	21459	1.62	4073	1.72	5.27	2-Fluoro-5-trifluoromethylbenzoic acid, pentyl ester
39	32.796	32.733	32.825	TIC	21721	1.64	4546	1.92	4.78	Valeramide, 2-methyl-N-(2-phenylethyl)-N-octadecyl-
40	32.879	32.825	32.942	TIC	33053	2.5	5141	2.17	6.43	Cyclotrisiloxane, hexamethyl-
41	32.983	32.942	33.008	TIC	19037	1.44	5025	2.12	3.79	6-Fluoro-2-trifluoromethylbenzoic acid, ethyl ester
42	33.058	33.008	33.1	TIC	27196	2.06	5082	2.15	5.35	4,6-Bis(formyloxymethyl)-2,5-dimethoxytropone
43	33.144	33.1	33.192	TIC	28021	2.12	5353	2.26	5.23	2-Bromo-4,6-difluoroaniline
44	33.269	33.192	33.3	TIC	31940	2.42	5244	2.21	6.09	3-Hydroxy-2-(3,4-methylenedioxyphenyl)-4H-chromen-4-one
45	33.342	33.3	33.367	TIC	20427	1.54	5328	2.25	3.83	1,3-Benzoxazole-5-carboxylic acid, 7-amino-2-(phenylmethyl)-, methyl ester
46	33.4	33.367	33.425	TIC	18200	1.38	5303	2.24	3.43	Thebaol, O-ethyl-
47	33.48	33.425	33.517	TIC	31764	2.4	6116	2.58	5.19	Isonipecotic acid, N-(2-fluoro-6-trifluoromethylbenzoyl)-, undecyl ester
48	33.558	33.517	33.625	TIC	35914	2.72	5943	2.51	6.04	Trimethylsilyl-di(timethylsiloxy)-silane
49	33.657	33.625	33.692	TIC	19429	1.47	5014	2.12	3.87	N-Isopropyl-3-phenylpropanamide
50	33.733	33.692	33.767	TIC	19946	1.51	4470	1.89	4.46	3-Methylbutyl N-(heptafluorobutyryl)norleucinate
51	33.831	33.767	33.892	TIC	28822	2.18	4154	1.75	6.94	Propanoic acid, 2,2-dimethyl-, cesium salt
52	33.926	33.892	33.958	TIC	13342	1.01	3546	1.5	3.76	Butanamide, N-(2-methylphenyl)-3-oxo-
53	34.052	33.958	34.167	TIC	44562	3.37	4335	1.83	10.28	9-Phenanthrenemethyl 2,6-dimethylbenzoate
54	34.192	34.167	34.233	TIC	11825	0.89	3052	1.29	3.87	4-Ethylbenzoic acid, 2-formyl-4,6-dichlorophenyl ester
55	34.263	34.233	34.317	TIC	13653	1.03	3001	1.27	4.55	trans-4'-Ethyl-4-(methylthio)chalcone
56	34.383	34.317	34.433	TIC	13498	1.02	2192	0.93	6.16	Cyclotrisiloxane, hexamethyl-
57	34.475	34.433	34.517	TIC	4025	0.3	961	0.41	4.19	Cyclotrisiloxane, hexamethyl-
58	34.621	34.517	34.667	TIC	7905	0.6	1306	0.55	6.05	Thebaol, O-ethyl-

In-vitro Anti Diabetic Activity:

Alpha Amylase Inhibitory Assay:

TABLE 13: OD VALUE AND PERCENTAGE INHIBITION OF STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) FOR ALPHA AMYLASE INHIBITORY ASSAY

Concentration (µg/mL)	Standard (Acarbose) – Mean ± SD (% OD)	Sample (*Piper longum*) – Mean ± SD (% OD)	Standard (Acarbose) – Mean ± SD (% Inhibition)	Sample (*Piper longum*) – Mean ± SD (% Inhibition)
Control	1.533 ± 0.00212	1.532 ± 0.00158	0.0 ± 0.0	0.0 ± 0.0
250	0.559 ± 0.00292	0.875 ± 0.00361	63.5 ± 1.17	42.9 ± 1.25
500	0.414 ± 0.00381	0.754 ± 0.00265	73.0 ± 1.25	50.8 ± 1.15
1000	0.235 ± 0.00212	0.633 ± 0.00265	84.7 ± 1.15	58.7 ± 1.15

IC50 Value-Standard: 220.059 µg/mL (Calculated using ED50 Plus V 1.0 Software). IC50 Value-sample: 940.068 µl/mL (Calculated using ED50 Plus V 1.0 Software).

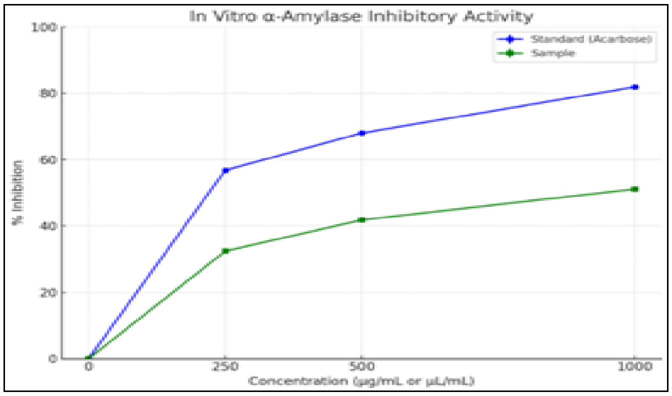

FIG. 24: LINE GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF ALPHA AMYLASE INHIBITORY ASSAY

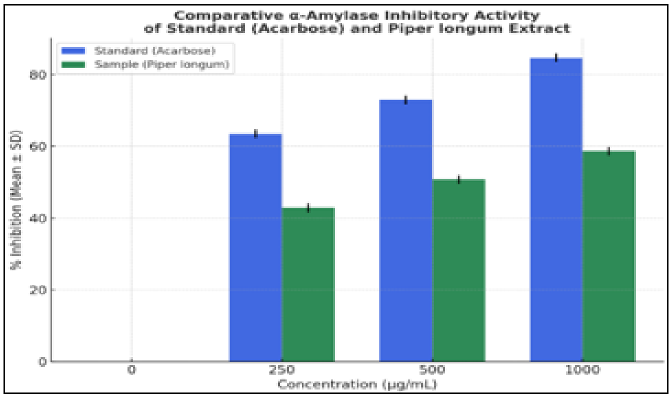

FIG. 25: BAR GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF ALPHA AMYLASE INHIBITORY ASSAY

Alpha Glucosidase Inhibitory Assay:

TABLE 14: OD VALUE AND PERCENTAGE INHIBITION OF STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) FOR ALPHA GLUCOSIDASE INHIBITORY ASSAY

Concentration (µg/mL)	Standard (Acarbose) – Mean ± SD (% OD)	Sample (Piper longum) – Mean ± SD (% OD)	% Inhibition Standard (Acarbose) Mean ± SD	% Inhibition Sample Mean ± SD
Control (0)	0.536 ± 0.012	0.536 ± 0.012	0.0 ± 0.0	0.0 ± 0.0
250	0.244 ± 0.006	0.385 ± 0.009	54.8 ± 2.9	31.3 ± 1.2
500	0.135 ± 0.003	0.310 ± 0.006	74.7 ± 1.6	44.6 ± 1.6
1000	0.077 ± 0.002	0.241 ± 0.005	85.6 ± 1.8	56.9 ± 1.9

IC_{50} Value –Standard –229.45 µg/mL (Calculated using ED_{50} PLUS V1.0 Software). IC_{50} Value –sample –717.393 µl/mL (Calculated using ED_{50} Plus V1.0 Software).

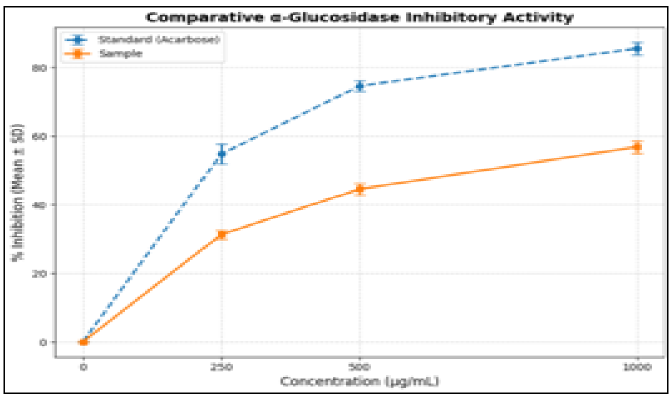

FIG. 26: LINE GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF ALPHA GLUCOSIDASE INHIBITORY ASSAY

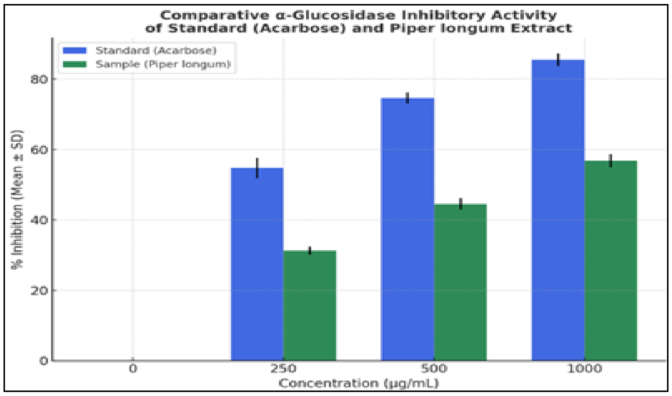

FIG. 27: BAR GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ACARBOSE) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF ALPHA GLUCOSIDASE INHIBITORY ASSAY

In-vitro Anti Inflammatory Activity:

Inhibition of Albumin Denaturation:

TABLE 15: OD VALUE AND PERCENTAGE INHIBITION OF STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) FOR INHIBITION OF ALBUMIN DENATURATION ASSAY

S. no.	Tested Sample (µg/ml)	Mean ± SD (% OD)	Mean ± SD (% Inhibition)
1	Control	2.394 ± 0.1485	0.0 ± 0.0
2	500	1.5677 ± 0.0220	34.3958± 3.15
3	250	1.7497 ± 0.0639	26.6455± 6.86
4	100	1.8153 ± 0.0707	23.8883± 7.24
5	50	1.862 ± 0.0278	21.9903± 5.88
6	10	1.9753 ± 0.0273	17.2735± 5.62
7	Standard (Aspirin)	1.1747 ± 0.0192	50.8008 ± 3.47

IC50 Value of standard: 84.2 µg/ml, IC50 Value of tested sample: 136.8 µg/ml.

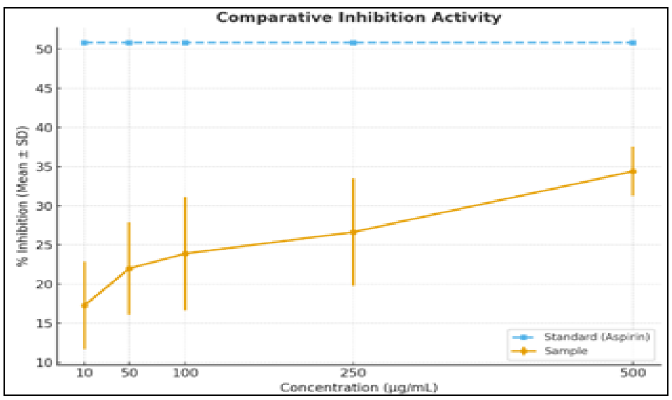

FIG. 28: LINE GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF INHIBITION OF ALBUMIN DENATURATION ASSAY

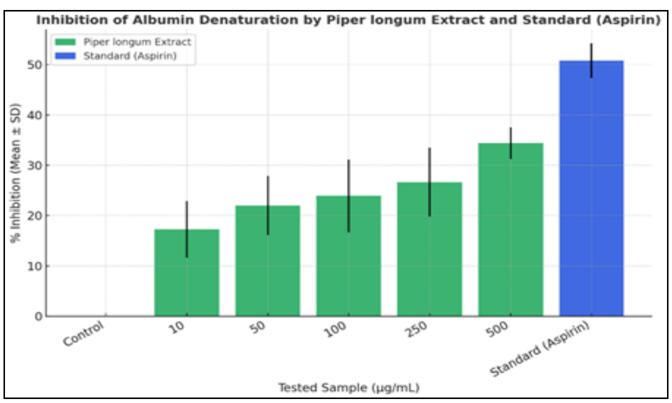

FIG. 29: BAR GRAPH COMPARISON OF PERCENTAGE INHIBITION BETWEEN THE STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) OF INHIBITION OF ALBUMIN DENATURATION ASSAY

HRBC Membrane Stabilization Method:

TABLE 16: OD VALUE, PERCENTAGE OF HEMOLYSIS AND PERCENTAGE OF PROTECTION OF STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) FOR HRBC MEMBRANE STABILIZATION METHOD

Tested Sample (µg/mL)	Mean ± SD (% OD)	Mean Hemolysis (%) ± SD	Mean Protection (%) ± SD
Standard (Aspirin)	3.2803 ± 0.0539	0.00 ±0.00	100.00 ± 0.00
Negative control	0.1553 ± 0.0357	100.00 ± 0.00	0.00 ± 0.00
10	0.3153 ± 0.0107	90.54 ± 0.33	9.46 ± 0.33
50	0.5517 ± 0.0374	83.34 ± 1.14	16.66 ± 1.14
100	1.0187 ± 0.0035	69.10 ± 0.11	30.90 ± 0.11
250	2.795 ± 0.1181	14.95 ± 3.60	85.05 ± 3.60
500	3.114 ± 0.0190	5.23 ± 0.58	94.77 ± 0.58

IC50 Value of standard: 82 µg/ml, IC50 Value of tested sample: 140.2 µg/ml

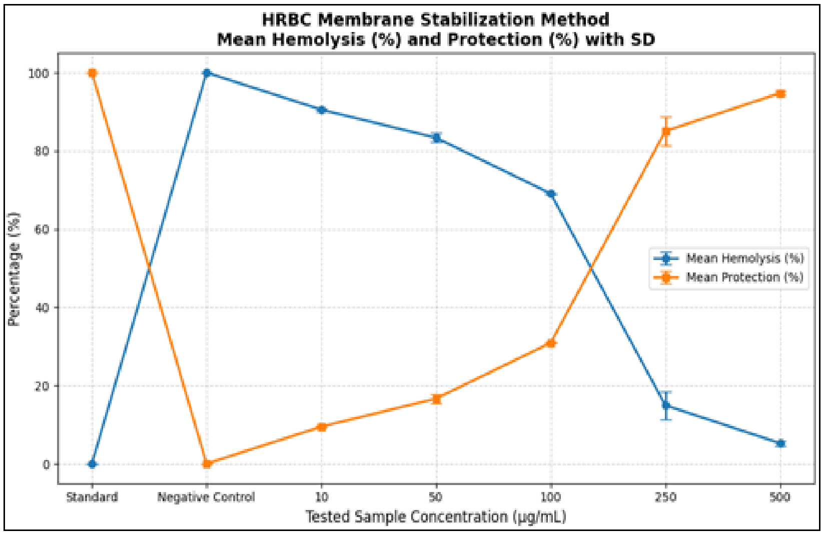

FIG. 30: LINE GRAPH COMPARISON OF PERCENTAGE PROTECTION AND PERCENTAGE HEMOLYSIS OF STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) HRBC MEMBRANE STABILIZATION METHOD

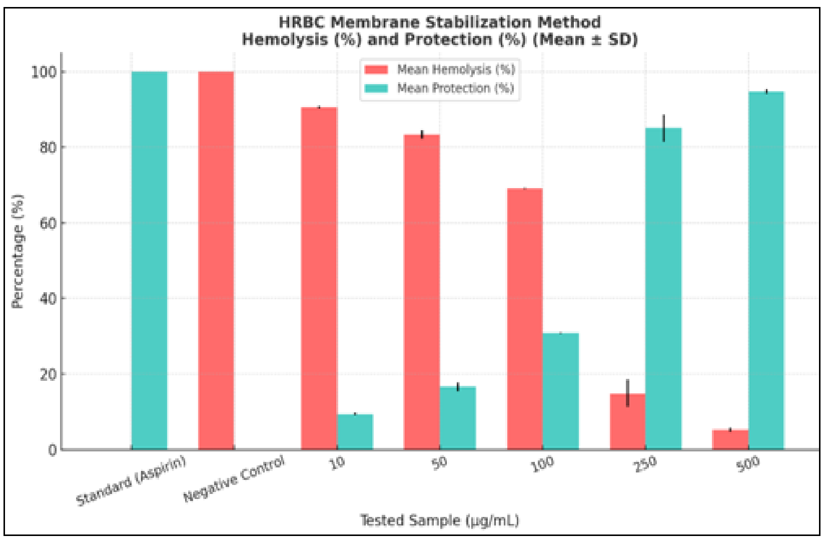

FIG. 31: BAR GRAPH COMPARISON OF PERCENTAGE PROTECTION AND PERCENTAGE HEMOLYSIS OF STANDARD (ASPIRIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) IN HRBC MEMBRANE STABILIZATION METHOD

In-vitro Anti Microbial Activity:
Anti Fungal Activity:
Agar Well Diffusion Method:

TABLE 17: MEANS ± SD OF ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (AMPHOTERICIN B) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST *CANDIDA ALBICANS* AND *ASPERGILLUS NIGER*

S. no.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC (Amphotericin B)
1.	*Candida albicans*	*Piper longum*	24.1±0.14	23.45±0.35	19.1±0.56	9.1±0.14	20.1±0.14
2.	*Aspergillus niger*	leaves	12.6±0.14	0	0	0	17.35±0.21

SD – Standard Deviation, *Significance - $p < 0.05$

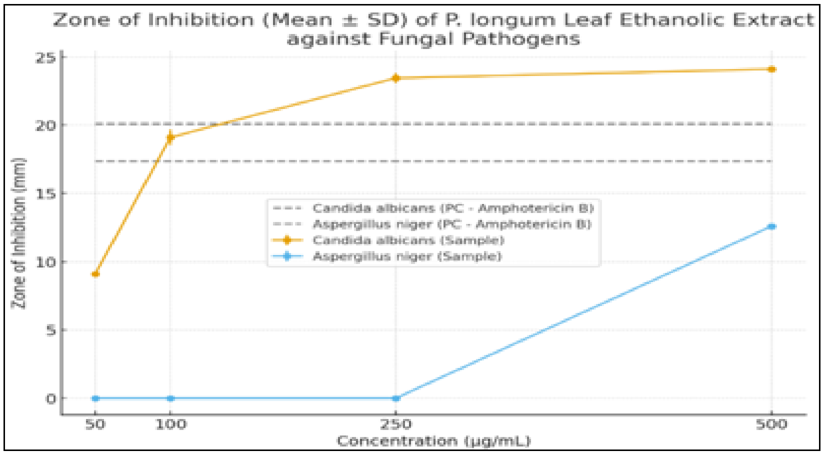

FIG. 32: LINE GRAPH COMPARISON BETWEEN ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (AMPHOTERICIN B) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST *CANDIDA ALBICANS* AND *ASPERGILLUS NIGER*

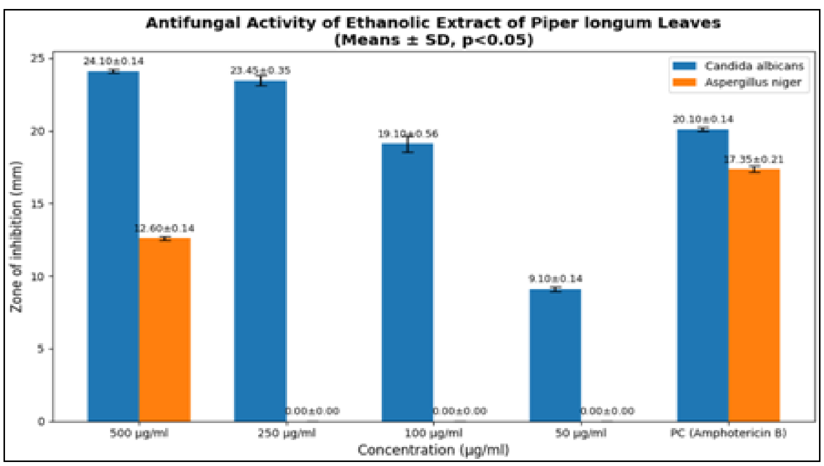

FIG. 33: BAR GRAPH COMPARISON BETWEEN ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (AMPHOTERICIN B) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST *CANDIDA ALBICANS* AND *ASPERGILLUS NIGER*

Anti Bacterial:

Agar Well Diffusion Method:

TABLE 18: MEANS ± SD OF ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (GENTAMICIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST TEST ORGANISMS

S. no.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) Mean±SD				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC (Gentamicin)
1.	*S. aureus*	*Piper*	13.45±0.35	13.35±0.21	12.75±0.35	11.1±0.14	15.85±0.21
2.	*P. acnes*	*longum*	21.25±0.35	13.6±0.14	10.85±0.21	0	15.6±0.14
3.	*E. coli*	leaves	13.85±0.21	9.85±0.21	0	0	16.85±0.21
4.	*E. faecalis*		10.75±0.35	9.75±0.35	0	0	14.85±0.21

SD – Standard Deviation, *Significance - $p < 0.05$.

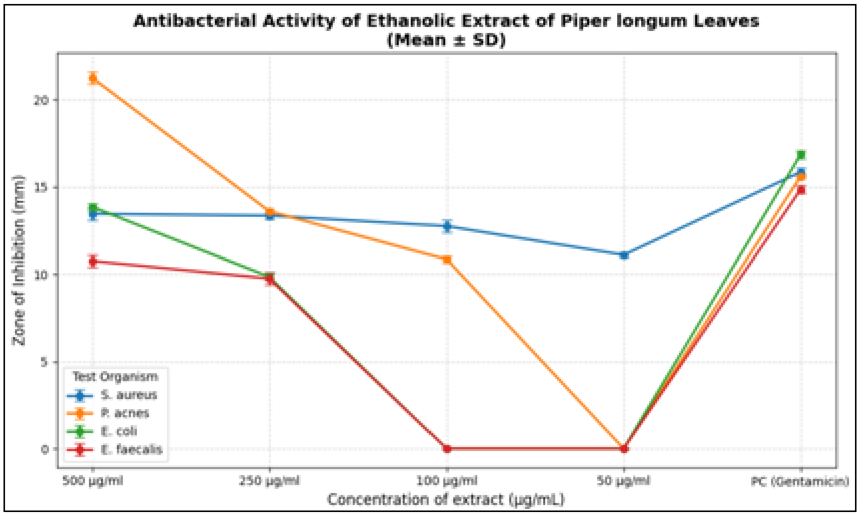

FIG. 34: LINE GRAPH COMPARISON BETWEEN ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (GENTAMICIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST *S. AUREUS*, *P. ACNES*, *E. COLI* AND *E. FAECALIS*

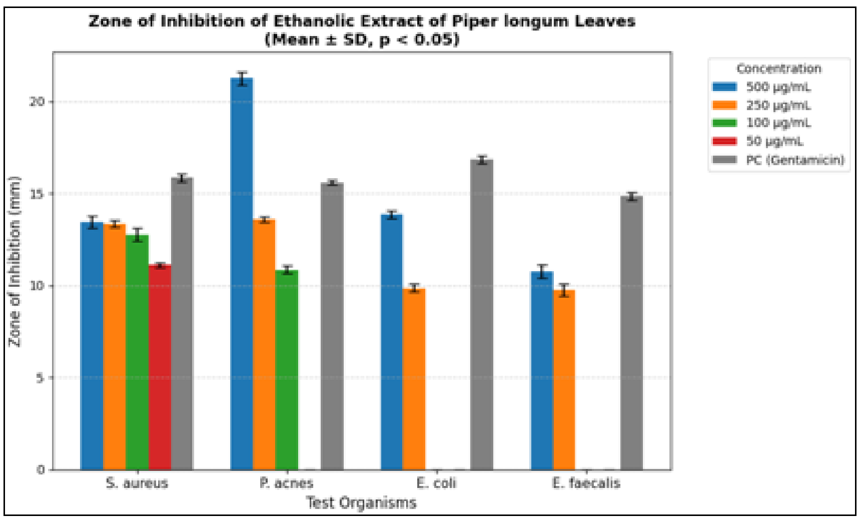

FIG. 35: BAR GRAPH COMPARISON BETWEEN ZONE OF INHIBITION OBTAINED BY POSITIVE CONTROL (GENTAMICIN) AND SAMPLE (*P. LONGUM* L. LEAF ETHANOLIC EXTRACT) AGAINST *S. AUREUS*, *P. ACNES*, *E. COLI* AND *E. FAECALIS*

DISCUSSION: The ethanolic extract of *Piper longum* leaves exhibited potent inhibitory activity against α-amylase and α-glucosidase, indicating its potential to modulate postprandial hyperglycemia. Dual inhibition at both early (α-amylase) and late (α-glucosidase) stages of carbohydrate digestion suggests a synergistic mechanism for effective glycemic control. Bioactive constituents, including piperine, flavonoids, and tannins, may mediate these effects through competitive or noncompetitive enzyme inhibition and conformational modulation. The anti-inflammatory activity of the extract is likely associated with stabilization of cellular membranes and prevention of protein denaturation, thereby limiting the release of inflammatory mediators. Piperine has been reported to downregulate NF-κB, COX-2, and TNF-α signaling, while flavonoids and tannins contribute antioxidant activity and enzyme inhibition, enhancing membrane protection.

The extract also demonstrated broad-spectrum antimicrobial efficacy. Compounds such as piperine, piperlongumine, and β-caryophyllene may disrupt microbial membranes, interfere with nucleic acid synthesis, and induce oxidative stress, inhibiting growth of both Gram-positive and Gram-negative bacteria as well as fungi. These results underscore the therapeutic versatility of *P. longum* leaves, supporting their traditional use and potential development as a natural antimicrobial and anti-inflammatory agent.

CONCLUSION: This study comprehensively evaluated *Piper longum* L. leaves through pharmacognostic, phytochemical, and pharmacological analyses. Key diagnostic features and chemical markers, including piperine and related alkaloids, ensure reliable authentication and standardization. The ethanolic extract exhibited potent antidiabetic, anti-inflammatory, and broad-spectrum antimicrobial activities, supporting its traditional use. The multifaceted pharmacological effects, combined with piperine's bioavailability-enhancing properties, highlight *P. longum* leaves as a promising candidate for development into multi-targeted, plant-based therapeutics.

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CONFLICTS OF INTEREST: Nil

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