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Antidiabetic Activity of *Curcuma longa* Ethanolic Extract

Thiruvengadam Abarna[1*], Thirunethiran Karpagam[2], Angappan Shanmuga Priya[3], Badrinarayanan Varalakshmi[4], Vasudevan Suganya[5] and Abinaya Muthukumar[6]

[1,2,3,4,6]Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu, India
[5]PG Department of Biochemistry, Holy Cross College, Tiruchirappalli, Tamil Nadu, India

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ABSTRACT The increasing prevalence of diabetes is a significant threat to public health globally. Conventional therapy for diabetes enhances insulin sensitivity, insulin secretion, and reducing blood glucose levels. Nevertheless, the effects of medication therapy are not always adequate to keep blood glucose levels within acceptable ranges. Many medicinal plants are known to be beneficial for diabetes, and have been used as a base material for the preparation of drugs for diabetes. The current study focused on the phytochemical screening, GCMS, *in vitro* antioxidant, in *vitro* antidiabetic properties and *in silico* drug docking assay using ethanol solvent derived biocompound of *Curcuma longa* for its anti-diabetic properties. Nine phytochemicals were found in preliminary screening of ethanolic extract derived from *Curcuma longa*. The ethanolic solvent derived biocompound of *Curcuma longa* exhibited 35 peaks in the GC-MS analysis, with bis(2-ethylhexyl) terephthalate at 14.4 percent showing the highest peak at 17. Cyclohexasiloxane, dodecamethyl showed the second-highest peak at 14.2 percent at 3, and bis(2-propylpentyl) phthalate at 12.25 percent showing the peak at 14. The antioxidant activity was assessed using FRAP and DPPH free radical-scavenging activity. *Curcuma longa* ethanolic extracts at varying doses (500, 250, 100, 50 and 10µg/ml) showed DPPH inhibition percentages of 61.28 percent, 52.19 percent, 46.16 percent, 35.17 percent and 29.28 percent, respectively. For FRAP assay at varying concentrations (500, 250, 100, 50, and 10µg/ml), the corresponding inhibition percentages were 30.43 percent, 27.65 percent, 19.11 percent, 16.78 percent and 13.42 percent. The antidiabetic efficacy of *Curcuma longa* was assessed using the alpha amylase, alpha glucosidase, and β-galactosidase inhibitory tests. With regard to α-amylase, α-glucosidase, and β-galactosidase, the corresponding IC50 values was 97.28 µg/ml, 52.01 µg/ml and 54.51 µg/ml. bis(2-propylpentyl) phthalate (Pubchem id: SSS19194), alpha-amylase (human pancreatic) complexed with nitrite and acarbose demonstrated a binding affinity of -6.6 KCal/mole for *in silico* investigations.

INTRODUCTION

Hyperglycemia is a hallmark of diabetes, a group of chronic metabolic diseases. It is commonly referred to as diabetes mellitus (DM). Lack of insulin production, action, or both is a hallmark of diabetes mellitus. This leads to persistent hyperglycemia and disturbances in most of the body's metabolic processes. The pancreas produces the hormone insulin, which helps to transport glucose from the bloodstream into the cells. In 2019, the global prevalence of diabetes was estimated at 9.3 percent, affecting 463 million people (Saeedi et al. 2019). This is expected to increase to 578 million by 2030 and reach 700 million by 2045 (IDF 2019). India currently has the highest number of diabetic patients worldwide and has been notoriously dubbed the 'diabetic capital of the world' (Mukhtar et al. 2020). Diabetes contributes to early mortality and chronic ill health, causing nearly one death every 10 seconds. Furthermore, it is the leading cause of death worldwide each year (Kaul 2013).

Plants are frequently employed in many traditional medical systems to prevent diabetes and offer a possible source of hypoglycemic medications as a replacement to prescription drugs. Implementation of specific medicinal herbs has proven beneficial for a number of diabetes patients. Through a variety of methods, these plants can correct metabolic imbalances and potentially avoid diabetic complications. Furthermore, many phytoconstituents with antidiabetic qualities have been discovered in recent years (Jarald et al. 2008). Many patients have a lack of response to allopathic treatments, which have their own set of issues. Sulfonylureas lose their effectiveness in 44 percent of patients within 6 years. Sulphonylureas may exacerbate heart disease, thiazolidinediones may cause

*Address for correspondence:
Dr. T. Abarna
E-mail: <abarnarajkumar@gmail.com>, <Karpagam@sigc.edu>, <sppriyaharsh83@gmail.com>, <varalakshmi@sigc.edu>, <srisugan20@gmail.com>, <abarnat@sigc.edu>

liver toxicity, and lower blood sugar levels may cause weight gain. These drugs can also have adverse or even toxic side effects. In olden times, the active compounds from pharmacological plants used therapeutically. Metformin, is a biguanide and an efficient oral glucose-lowering drug used to treat diabetes, was made from *Galega officianalis* (Daniel and Norman 2001).

Indian traditional herbs have been used for a number of medical applications since ancient times, which have demonstrated efficacy and safety. Numerous chemical components found in these ayurvedic plants and its parts make them appropriate for use in medicine. Antioxidants, which are used to stop the production of free radicals, are also abundant in these herbs. *Aloe vera*, *Murraya koeingi*, *Allium cepa*, *Mucuna pruriens*, *Mormodica charantia*, *Caesalpinia bonducella*, *Allium satium*, *Coccinia indica*, and *Eugenia jambolana* are a few examples. Using ethnobotanical data, almost 800 Indian botanicals were found to have potential as antidiabetic drugs. In numerous ways, *C. longa* is a natural detoxifier for the heart, liver, muscle, tissue, and other organs and cells to work normally. Because of its naturally cool and lovely yellow color, the rhizome is used as a natural colouring agent and is approved to be used in dietary supplements and preservatives for both human and animal consumption. Turmeric has many medicinal uses, such as lowering gas, improving digestion, getting rid of worms, managing menstruation, easing arthritis, getting rid of gallstones, and increasing overall body energy. Additionally, it promotes wound healing and helps repair damaged skin tissue. Because it is non-toxic, the plant is used locally to treat inflammatory skin disorders like psoriasis.

C. longa is a functional food due to its immunomodulatory, anticancer, and antioxidant qualities. The *C. longa* has the ability to scavenge free radicals and boost antioxidant enzymes such as catalase and superoxide dismutase (Krup et al. 2013). Lipid peroxidation is also suppressed. Turmeric's capacity to scavenge free radicals is facilitated by its high flavonoid and phenolic component content, known as curcumin (Gupta et al. 2013). *Curcuma longa*, a member of the Zingiberaceae family, is native to tropical and subtropical areas of the world (Prasad and Aggarwal 2011). The main factor contributing to turmeric's curative properties for a number of ailments, like diabetes, cancer, and neurological, hepatic, and renal damage, is its curcuminoid concentration. Curcumin improves metabolic profile and reduces associated secondary diseases (Rivera-Mancía et al. 2018).

Pancreatic alpha-amylase, a crucial digestive enzyme, catalyses the initial stage of starch hydrolysis into maltose and then to glucose. Higher postprandial hyperglycemia (PPHG) is the result of this dietary starch's rapid degradation. Since it has been shown that an increase in postprandial glucose levels is correlated with Human Pancreatic Alpha-Amylase (HPA) activity, controlling this enzyme is essential for the treatment of diabetes. High-affinity for HPA activity inhibitors generated from plants is therapeutically important (Ponnusamy et al. 2011).

In silico docking is an essential technique for assessing the mechanism of interactions of molecules. Docking helps to identify phytocompounds and its correlation to the target enzyme alpha-amylase (Mater et al. 2022).

Objectives

Investigating and confirming *Curcuma longa*'s antidiabetic and antioxidant properties, with a special emphasis on its active component curcumin, is the main goal of the study. This entails comprehending the modes of action of curcumin, the main bioactive ingredient in turmeric, and enhancing its bioavailability for improved therapeutic effects.

MATERIALS AND METHODS

Procurement and Extraction

Curcuma longa (turmeric) was sourced from departmental stores in Tiruchirapalli, powdered using a mixer, and stored in a sterile, sealed container for analysis. The extraction of *Curcuma longa* was prepared via soxhlet apparatus. Ethanol was used as a solvent.

Qualitative Screening of Phytochemicals

Screening was carried out using the extracts made from ethanol following the method of Trease and Evans (1989).

Analytical Study for Bioactive Compound

Following the protocol established by Kumar and Rajakumar (2016), the GC-MS analysis was performed.

In vitro Antioxidant Activity

The antioxidant activity of *C. longa* was assessed using two methods, that is, the Ferric Reducing Antioxidant Power (FRAP) assay, following the procedure outlined by Benzie and Strain (1999), and the Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, as described by Braca et al. (2002).

In vitro Anti Diabetic Activity

To assess the antidiabetic activity of *Curcuma longa in vitro* inhibitory activities was assessed for α-amylase following Dong et al. (2012), α-glucosidase as per Shukla et al. (2016), and β-galactosidase following Seddigh and Darabi (2014). These enzymes can break carbohydrates.

In silico Analysis

A molecular docking study of bis(2-propylpentyl) phthalate with human pancreatic α-amylase complexed with nitrite and acarbose was performed, by referencing the works of Kesari et al. (2022). Analysis were performed using AutoDock 4.2.6 (MGL Tools), AutoDock Vina, and BIOVIA Discovery Studio, through which the phyto compound bis(2-propylpentyl) phthalate (PubChem ID: 191964) (Fig.1) was visualised and its ligand preparation completed.

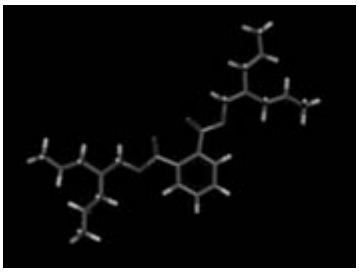

Fig. 1. 3D structure of bis(2-propylpentyl) phthalate

Six aromatic carbons were discovered when 36 non-polar hydrogens were combined by Gasteiger charges. bis(2-propylpentyl) phthalate, included sixteen rotatable linkages.

Target Protein Preparation

The target protein for this study was human pancreatic α-amylase complexed with nitrite and acarbose (PDB ID: 2QV4). It was identified using X-ray diffraction with a resolution of 1.97 Å, consisting of a single chain designated as A. The protein structure was obtained from the RCSB PDB database, and BIOVIA Discovery Studio software was utilised for visualisation and optimisation (Fig.2). During the optimisation process, heteroatoms and water molecules were removed, and polar hydrogens were added. The active site of the protein was identified, and the coordinates were recorded as xyz (13.758000, 58.583058, and 22.465406). The molecular docking investigation was subsequently conducted on the optimised target protein.

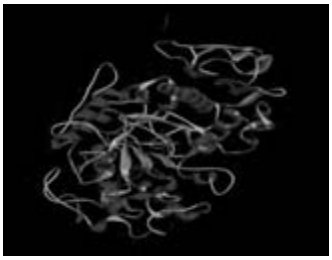

Fig. 2. Structure of target protein

Molecular Docking Study

AutoDock Vina software was employed for the molecular docking investigation, which examined the binding between human pancreatic alpha-amylase (PDB ID: 2QV4), complexed with nitrite, and acarbose, in conjunction with the optimised ligand bis(2-propylpentyl) phthalate (PubChem ID: 191964) (Fig.3). Configuration file (conf file) was generated and uploaded as part of the docking process to complete the workflow.

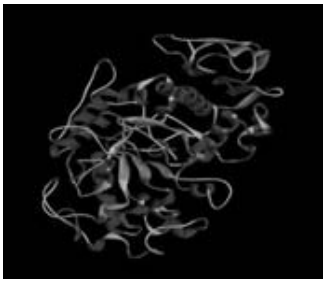

Fig. 3. Structure of optimised protein

Fig. 4. Molecular docking

The Lamarckian genetic method was used to explore molecular docking analysis. Molecular docking simulations were conducted using AutoDock Vina via command-line execution. BIOVIA Discovery Studio was utilised to interpret the topmost conformation of the docked complex (Fig. 4).

Statistical Analysis

Every test was conducted in triplicate, and the given data was expressed as mean ± standard deviation (Zar 2010). Analysis of correlation was performed.

RESULTS AND DISCUSSION

Diabetes mellitus is a long-term metabolic condition that can have major repercussions, including retinal, neurological, renal, and cardiovascular failure.

Qualitative screening of *C. longa* ethanolic extract showed good results for nine medicinally active components (Table 1). Nearly 14 secondary metabolites were analysed. Among these substances flavonoids, steroids, phenols and flavonglycosides are significant secondary metabolites that are primarily responsible for the plant's therapeutic benefits.

Table 1: Qualitative phytochemicals screening of ethanolic extract of *Curcuma longa*

S. No.	*Phytochemical constituents*	*Curcuma longa in ethanolic extract*
1	Resins	- (absence)
2	Carboxylic acid	+ (presence)
3	Carbohydrates	+
4	Flavonoids	+
5	Saponins	-
6	Tannins	-
7	Alkaloids	-
8	Steroids	+
9	Glycosides	-
10	Proteins	+
11	Fat	+
12	Gum	+
13	Phenol	+
14	Flavonglycosides	+

GC MS Analysis of *Curcuma longa* (Ethanol as Solvent)

Bio-active-chemicals from the *Curcuma longa* (ethanolic extract) were identified using GC MS analysis (Fig.5). Peaks and their RT were displayed in the GC-MS chromatogram, and the identified compounds, chemical formula, and retention time were displayed in Table (Table 2). The GC MS analysis of *C.longa* ethanolic extract revealed 35 peaks in total, with the bis(2-ethylhexyl) terephthalate at 14.4 percent showing the highest peak at 17. Cyclohexasiloxane and dodeca methyl had the second-highest peak at number three, at 14.2 percent, while bis(2-propylpentyl) phthalate had the third-highest peak at number fourteen, at 12.22 percent. Numerous ester compounds that are extremely beneficial and curative were found in these results.

The results show that *C. longa* has the ability to scavenge free radicals (Table 3). The standard used was ascorbic acid. The ethanolic extract of *Curcuma longa* effectively suppressed free radicals, with higher doses leading to a greater reduction in radical activity. The inhibition percentages of ethanolic extract of *Curcuma longa* at various doses (500, 250, 100, 50 and 10µg/ml) were 61.28 percent, 52.19 percent, 46.16 percent, 35.17 percent and 29.28 percent, respectively.

Mashhadi et al. (2024) synthesised a novel magnetic nanocomposite, CoFe2O4-SiO2@PC-Cu, by immobilising phycocyanin onto magnetised silica, followed by the incorporation of Cu (II) ions using copper (II) chloride. This resulted in the formation

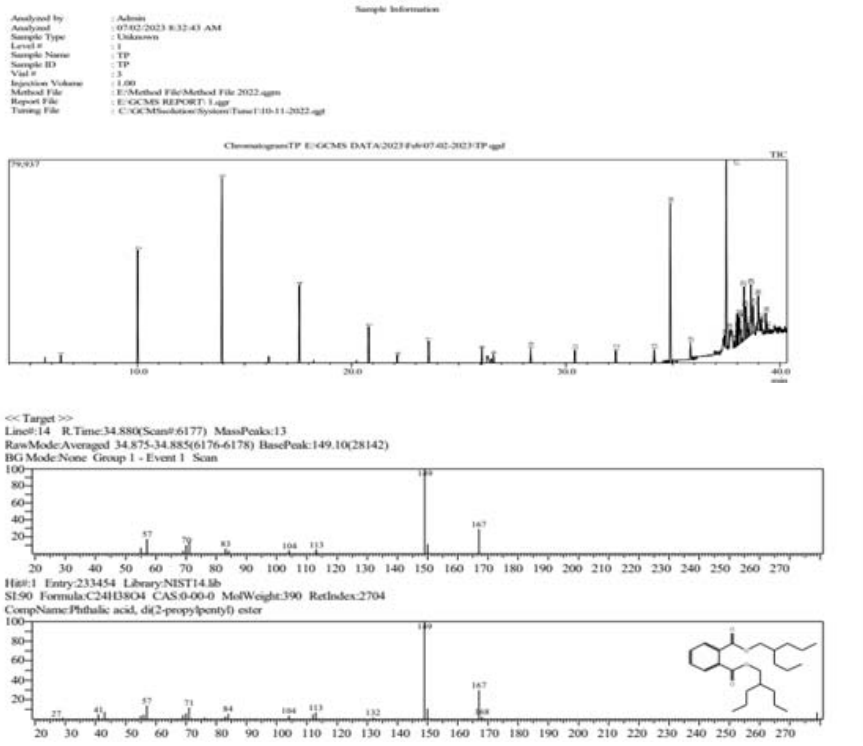

Fig. 5. GC-MS Chromatogram showing the Phytochemicals present in the *Curcuma longa* ethanolic extract

of the CoFe2O4-SiO2@PC-Cu nanocomposite. The nanocomposite was then evaluated for its antioxidant activity using the DPPH assay and compared with quercetin, a standard antioxidant. The compound demonstrated superior antioxidant activity.

Oxidative stress is a major health risk factor since it causes serious conditions like diabetes. The FRAP values for the aqueous turmeric extract was 646.67 to 1015.52 µM Fe [II]/100 g of sample (Table 4). A higher FRAP value suggests stronger antioxidant qualities in *C.longa*.

Antidiabetic Activity of the *Curcuma longa* Extract

Analysis of Alpha-amylase, Alpha-glucosidase, and β-galactosidase Suppressive Activity

An *in vitro* investigation assessing the percentage of alpha-amylase, alpha-glucosidase, and β-galactosidase suppression was carried out, and the IC_{50} values were computed.

Alpha-amylase Suppressive Activity

The ethanolic extract of *Curcuma longa* at various doses (500, 250, 100, 50 and 10µg/ml) showed suppressive activity at 39.64 percent, 34.80 percent, 26.05 percent, 14.71 percent, and 9.68 percent, respectively (Table 5). The IC_{50} value for alpha-amylase was 97.28 µg/ml.

Acarbose, an oligosaccharide, inhibits alpha-glucosidase that breaks down carbohydrates (Wadkar et al. 2008). For obese diabetics, it can be used in conjunction with a diet plan because it exhibits a minimal hypoglycemic effect. Curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, exhibit dose-dependent inhibitory activity on pancreatic lipase—with IC_{50s} in the mg/mL range—and on ?-glucosidase-with IC_{50s} in the µM range; furthermore, certain curcumin-derived synthetic compounds significantly inhibit both α-amylase and α-glucosidase in vitro (He et al. 2024).

Table 2: GC-MS chromatogram table showing phytochemicals present in the *Curcuma longa* ethanolic extract

Peak	*Retention time*	*Start time*	*End time*	*Area*	*Area %*	*Height*	*Height %*	*Name*
1	6.425	6.405	6.445	4426	0.39	3074	0.6	4-(Benzoylmethyl)-6-methyl-2H-1,4-benzoxazin-3-one
2	10.01	9.975	10.045	74138	6.47	44495	8.66	Cyclopentasiloxane, decamethyl-
3	13.942	13.905	13.985	132415	11.55	72947	14.2	Cyclohexasiloxane, dodecamethyl-
4	17.557	17.525	17.595	53341	4.65	30559	5.95	3 Butoxy-1,1,1,7,7,7 -hexamethy l-3,5,5-tris(trimethylsiloxy) tetrasiloxane
5	20.797	20.765	20.83	26403	2.3	14256	2.77	1-(3,4-ditrimethylsiloxyphenyl)-2-isopropylaminoethanol
6	22.126	22.11	22.15	5168	0.45	3066	0.6	2,2-Dimethyl-propyl2,2-dimethyl-propanesulfinyl sulfone
7	23.595	23.565	23.63	17000	1.48	8692	1.69	Silane, (phenyloxiranylidene) bis[trimethyl-
8	26.086	26.055	26.115	11402	0.99	5749	1.12	Silane, (phenyloxiranylidene) bis[trimethyl-
9	26.627	26.61	26.65	5495	0.48	3544	0.69	3-buten-2-ol, formate
10	28.36	28.33	28.39	9911	0.86	5725	1.11	Silane, (phenyloxiranylidene) bis [trimethyl-
11	30.425	30.39	30.455	10747	0.94	5006	0.97	Silane, (phenyloxiranylidene)bis [trimethyl-
12	32.333	32.305	32.37	8464	0.74	4123	0.8	Silane, (phenyloxiranylidene)bis [trimethyl-
13	34.129	34.1	34.18	10378	0.91	4379	0.85	Disulfide, (1-methyl-1,2-ethanediyl) bis [2-propenyl
14	34.879	34.835	34.935	137371	11.98	62817	12.22	Phthalic acid, di (2-propylpentyl) ester
15	35.82	35.775	35.87	15658	1.37	6648	1.29	Disulfide, (1-methyl-1,2-ethanediyl)bis [2-propenyl
16	37.406	37.375	37.445	10454	0.91	4105	0.8	Per(trimethylsilyl)-raffinose
17	37.492	37.45	37.55	171906	14.99	74006	14.4	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
18	37.647	37.62	37.675	10423	0.91	4870	0.95	Di-isodecyl phthalate
19	37.888	37.87	37.92	4402	0.38	2422	0.47	n-allyloxymethylacrylamide
20	37.965	37.92	37.99	25184	2.2	9264	1.8	Di-isodecyl phthalate
21	38.005	37.99	38.025	22765	1.99	12032	2.34	Di-isodecyl phthalate
22	38.039	38.025	38.07	20860	1.82	11162	2.17	4,7-dimethyldeca-2,3,7,8-tetraene
23	38.11	38.07	38.16	35433	3.09	10677	2.08	Di-isodecyl phthalate
24	38.18	38.16	38.21	7483	0.65	5305	1.03	Tri(2-ethylhexyl) acetylcitrate
25	38.323	38.21	38.38	74516	6.5	21109	4.11	Di-isodecyl phthalate
26	38.418	38.38	38.46	43186	3.77	12600	2.45	Di-isodecyl phthalate

Table 2: Contd...

Peak	*Retention time*	*Start time*	*End time*	*Area*	*Area %*	*Height*	*Height %*	*Name*
27	38.515	38.46	38.545	16980	1.48	5742	1.12	4-isoxazolamine, 5-(1-methylethyl)- n-[(1-methylethyl)carbonimidoyl]-
28	38.643	38.545	38.705	67076	5.85	19889	3.87	Phthalic acid, cyclobutyl tridecyl ester
29	38.752	38.705	38.81	36258	3.16	11024	2.15	Di-isodecyl phthalate
30	38.98	38.935	39.055	41981	3.66	13743	2.67	Di-isodecyl phthalate
31	39.095	39.075	39.11	4388	0.38	3299	0.64	Furo[3,4-b]furan-2,6(3h,4h)-dione,4.beta.-ethyl-3a.alpha., 6a.alpha.,-dihydro-3-methylene-, (-)-
32	39.124	39.11	39.14	5646	0.49	4674	0.91	Acetic acid, trifluoro-, anhydride with diphenylphosphinic acid
33	39.15	39.14	39.17	1861	0.16	2773	0.54	Cyclohexane, 1-(1,1-dimethylethyl)-4-methylene-
34	39.348	39.335	39.38	14156	1.23	7963	1.55	Phthalic acid, cyclohexylmethyl tridecyl ester
35	39.44	39.38	39.45	9367	0.82	2144	0.42	Furo[3,4-b]furan-2,6(3h,4h)-dione, 4.beta.-ethyl-3a.alpha.,6a.alpha., -dihydro-3-methylene-, (-)-

***In vitro* Antioxidant Activity**

DPPH Assay of Ethanolic Extract of C.longa

Table 3: DPPH of Ethanolic extract *C.longa* at different concentration and standard ascorbic acid

S. No.	*Concentration (µg/ml)*	*% of inhibition standard ascorbic acid*	*% of inhibition test sample*
1	10	14.53±0.23	29.28±0.27
2	50	33.12±0.41	35.17±0.17
3	100	49.02±0.29	46.16±0.24
4	250	67.33±0.05	52.19±0.51
5	500	83.24±0.32	61.28±0.24

Table 4: FRAP of ethanolic extract of *C.longa* at different concentrations and standard ascorbic acid

S. No.	*Concentration (µg/ml)*	*% of inhibition standard ascorbic acid*	*% of inhibition test sample*
1	10	14.12±0.31	13.42±0.27
2	50	28.09±0.21	26.78±0.17
3	100	41.11±0.97	39.11±0.24
4	250	54.76±0.33	52.65±0.51
5	500	75.36±0.84	71.43±0.24

Table 5: Inhibition percentage of alpha-amylase suppressive activity

S. No.	*Test sample concentration (îg/ml)*	*Mean±SD*
1	Acarbose (500 µg/ml)	91.00± 2.840
2	500 µg/ml	39.64± 0.990
3	250 µg/ml	34.80± 1.968
4	100 µg/ml	26.05± 3.784
5	50 µg/ml	14.71± 1.490
6	10 µg/ml	9.68± 3.139

Analysis of Alpha-glucosidase Suppressive Activity

The *Curcuma longa* (ethanolic extract) exhibited 87 percent, 85 percent, 71 percent, 45 percent and 6 percent activity at 500 µg/ml, 250µg/ml, 100 µg/ml,50 µg/ml 10 µg/ml respectively, in the alpha-glucosidase suppression experiment (Table 6). The IC_{50} value of alpha-glucosidase was 52.01 µg/ml.

Widowati et al. (2018) reported that *Curcuma longa* inhibited alpha-amylase, alpha-glucosidase, and β-galactosidase.

Table 6: Inhibition percentage of alpha-glucosidase suppressive activity

S. No.	*Test sampleconcentration (ìg/ml)*	*Mean ±SD*
1	Acarbose (500 µg/ml)	95.80± 1.101
2	500 µg/ml	87.91± 0.590
3	250 µg/ml	85.70± 0.363
4	100 µg/ml	71.65± 0.971
5	50 µg/ml	45.90± 4.670
6	10 µg/ml	6.11± 3.981

Analysis of â-galactosidase Suppressive Activity

The β-galactosidase activity of the *Curcuma longa* (ethanolic extract), had shown 79.24 percent, 77.37 percent, 70.77 percent, 52.81 percent and 31.45 percent of activity at 500µg/ml, 250µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml, respectively (Table 7). The IC_{50} value for the β-galactosidase suppressive ability was 54.51 µg/ml. According to EI Omari et al. (2019), suppressing β-galactosidase can result in intestinal hydrocarbon reduction, which in turn lowers the glucose level. The IC_{50} value for the β-galactosidase suppressive ability was larger than 5 mg/ml for all extracts. In ν-irradiated rats, orally administered curcumin nanoparticles (10 mg/kg) significantly reduced brain β-galactosidase activity and improved mitochondrial function, as evidenced by increased complex I and II activity and higher ATP production (Moselhy et al. 2025).

Molecular Docking Study of Bis(2-propylpentyl) Phthalate Against Human Pancreatic Alpha-amylase Complexed with Nitrite and Acarbose

In the current research, bis(2-propylpentyl) phthalate was selected for drug docking studies due to its interactions from previous studies with target proteins that play a crucial role in diabetes. By investigating the potential binding interactions of this compound with the specific targets, it can offer important insights regarding its therapeutic potential for diabetes treatment. While the other compounds lack reported mechanisms of action in

Table 7: Inhibition percentage of β-galactosidase suppressive activity

S. No.	*Test sample concentration (μg/ml)*	*Mean ±SD*
1	Acarbose (500 μg/ml)	87.26± 0.74
2	500 μg/ml	79.24± 0.92
3	250 μg/ml	77.37± 0.31
4	100 μg/ml	70.77± 3.43
5	50 μg/ml	52.81± 6.24
6	10 μg/ml	31.45± 6.69

relation to diabetes, which led the researchers to prioritise bis(2-propylpentyl) phthalate for further investigation (Fig.5).

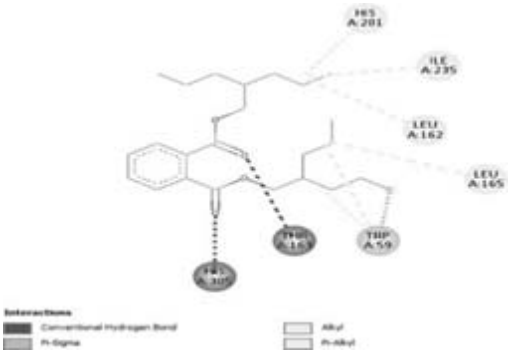

Fig. 5. Two-dimensional interaction of the target protein with ligand

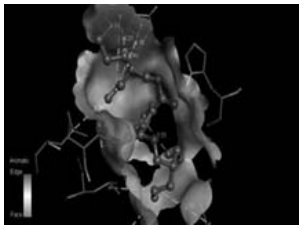

Fig. 6. Target protein's aromatic ring with ligand

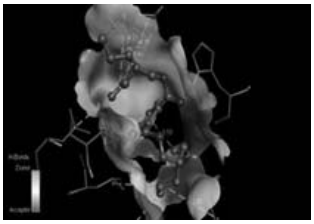

Fig. 7. Target protein and ligand's H-bond donor and acceptor region

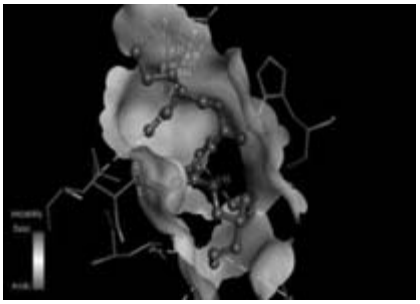

Fig. 8. Target protein and ligand's hydrophobicity region

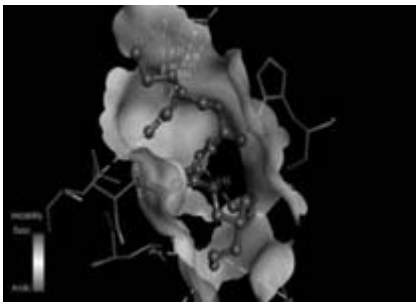

Fig. 9. Target protein and ligand's ionisability region

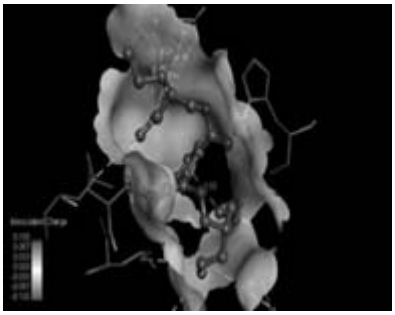

Fig. 10. Target protein and ligand's interpolated charge region

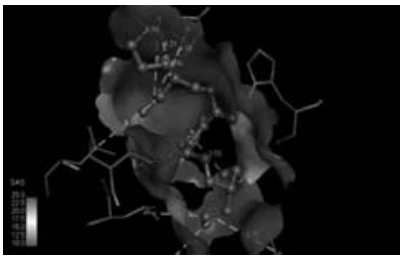

Fig. 11. Target protein and ligand's SAS region

The binding interactions of human pancreatic alpha-amylase complexed with nitrite and acarbose (PDB ID: 2QV4) bis(2-propylpentyl) phthalate (PubChem ID: 191964) demonstrated the binding affinity of -6.6 kcal/mole and characterised by the formation of two hydrophobic pi-sigma bonds with TRP 59 (A) at a distance of 3.99 Å and two conventional hydrogen bonds with THR 163 (A) and HIS 305 (A) at distances of 2.59 Å and 2.37 Å. LEU 162 (A), ILE 235 (A), and LEU 165 (A) form three hydrophobic alkyl bonds at distances of 4.790 Å, 5.30 Å, and 5.15 Å (Fig.6).

The distances of the five hydrophobic pi-alkyl bonds with HIS 201 (A) and TRP 59 (A) are 4.98 and 4.10 Å, 5.39 Å, 4.21 Å, 5.49 Å and 4.98 Å, respectively. Three hydrophobic bonds are located between the aromatic face and edge, five hydrophobic pi-alkyl bonds are located on the aromatic face side, and two conventional hydrogen bonds are located on the aromatic edge side. The hydrogen donor group includes both conventional hydrogen bonds (Fig.7). THR 163 (A) is located in the hydrophobic zone between -1.00 and 0.00, while HIS 305 (A) is located in the hydrophobic region of -3.00 (Fig.8). The hydrophobic region of -2.00 contains hydrophobic pi-alkyl and alkyl linkages. The hydrophobic region 2.00 is where ILE 235 (A) is located. Other bonds are located in between the acidic and basic regions, with THR 164 (A) and HIS 305 (A) located in the acidic and basic ionisation regions, respectively (Fig.9). The interpolated charges for alkyl, pi-alkyl, hydrophobic pi-sigma, and conventional hydrogen bonds are -0.033 and 0.000, respectively (Fig. 10). The SAS range of 22.5 to 25.00 includes hydrophobic alkyl and hydrophobic pi-alkyl bonds as well as conventional hydrogen bonds (Fig.11).

The binding interactions of human pancreatic alpha-amylase complexed with nitrite and acarbose (PDB ID: 2QV4) reveal a binding affinity of -6.6 kcal/mole, characterised by the formation of two hydrophobic pi-sigma bonds with tryptophan 59 (TRP 59) and two conventional hydrogen bonds with threonine 163 (THR 163) and histidine 305 (HIS 305). The spatial arrangement of these interactions includes hydrophobic alkyl bonds formed by leucine 162 (LEU 162), isoleucine 235 (ILE 235), and leucine 165 (LEU 165), alongside multiple hydrophobic pi-alkyl bonds associated with HIS 201 and TRP 59, indicating a complex interplay of hydrophobic and hydrogen bonding interactions that stabilise the enzyme-substrate complex. The interpolated charges of these bonds, along with their specific distances, provide insights into the molecular dynamics and thermodynamic stability of the interactions, which are critical for understanding the enzyme's catalytic mechanism and potential inhibition by compounds like acarbose.

Zhang et al. (2024) noted that nitrite modifies HPA structure and function, which has direct implications for designing next-generation α-amylase inhibitors with improved specificity under oxidative stress conditions. Akshatha et al. (2021) reported that molecular docking research on compounds from *S. longisporoflavus* and its endophyte revealed that alkaloids and flavonoids inhibit α-amylase by forming hydrogen bonds with its amino acids. This study, which used the human pancreatic α-amylase structure (PDB ID 2QV4) complexed with acarbose, aligns with previous findings.

CONCLUSION

Elevated incidence of diabetes is posing a risk to health to all in the ecosystem. In addition to its many therapeutic applications in traditional medicine, *Curcuma longa* also contains antidiabetic properties. The current study examined the *Curcuma longa*'s (ethanolic extract) antidiabetic properties. The *Curcuma longa* (ethanolic extract) exhibited 35 peaks in the GC-MS analysis, with bis(2-ethylhexyl) terephthalate at 14.4 percent showing the highest peak at 17. The alpha-amylase, alpha-glucosidase, and β-galactosidase inhibitory assays were used to determine Curcuma longa's antidiabetic properties. The IC_{50} values for α-amylase, α-glucosidase, and β-galactosidase in the antidiabetic inhibitory assay were 97.28 µg/ml, 52.01 µg/ml and 54.51 µg/ml, respectively. Ethanolic extracts of *Curcuma longa* at various dosages demonstrated DPPH inhibition percentages of 61.28 percent, 52.19 percent, 46.16 percent, 35.17 percent and 29.28 percent, respectively. The FRAP assay conducted at these same concentrations, the observed inhibition percentages were 30.43 percent, 27.65 percent, 19.11 percent, 16.78 percent and 13.42 percent. For the *in silico* analysis, the human pancreatic alpha-amylase complexed with nitrite and acarbose (PDB ID: 2QV4) alongside bis (2-propylpentyl) phthalate (Pubchem ID: 19194) exhibited a binding affinity of -6.6 KCal/mol.

RECOMMENDATIONS

By virtue of its positive properties and biologically active compounds, *C. longa* can be used to develop medicinal products. Clinical trials and quality control are necessary to determine safe dosages and the effectiveness of *C. longa* in treating diabetes. To determine the mechanism by which C. longa and its bioactive components provide protection against diabetes, more research is also necessary. This information would help in the creation of efficient treatments that target diabetes.

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