Antidiabetic Activity of Chemical Constituents in Elaeocarpus Tectorius Fruits - An In Silico Study

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Abstract:

Diabetes is a metabolic disorder with an increasing global prevalence and incidence. The drugs that are used to treat diabetes are often associated with side effects and adverse reactions. Plants are considered a high esteemed source of medicine since ancient times and could be used as an alternative source for the treatment of diabetes. The present study aims to explore the phytochemical constituents and the antidiabetic potential of Elaeocarpus tectorius fruits using Gas-chromatography- mass spectrometry (GC-MS) analysis and molecular docking studies. The ethanolic extracts of Elaeocarpus tectorius fruits were analysed to determine the total phenol and flavonoid contents and then subjected to GC-MS analysis to identify the bioactive compounds. The selected bioactive compounds were then docked to some therapeutic targets involved in the pathogenesis of type 2 diabetes using Autodock 4.2.6. The drug-likeness and toxicity of the ligands were obtained from SwissADME, Protox-II and admetSAR online server tools. The GC-MS analysis revealed the presence of 28 compounds in the ethanolic extracts of E. tectorius fruits. Molecular docking studies highlighted three potential antidiabetic compounds, dibutyl phthalate, diethyl phthalate and phytol that exhibited good binding affinity with target proteins, PPAR- γ, PTP-1B, glucokinase and IL-1β. This study thus suggests that the fruits of E.tectorius have a good potential as a source of antidiabetic compounds.

Keywords:

Diabetes, Autodock, Phytochemicals, Medicinal plants, *Elaeocarpus tectorius*

Introduction

Diabetes Mellitus is a metabolic disorder that occurs due to insulin secretion, action or both. According to the World Health Organization (WHO) about 422 million people live with diabetes worldwide [1]. Of the two major types of diabetes, Type 2 diabetes mellitus is particularly an expanding global health problem and is closely linked to the epidemic of

obesity. It is characterized by high plasma glucose levels and is primarily caused due to environmental factors like diet, physical activity, obesity, lifestyle and genetic factors [2] [3]. Individuals with type 2 diabetes are at a greater risk for developing complications like retinopathy, nephropathy, neuropathy, cardiovascular diseases and skin conditions owing to hyperglycemia and insulin resistance [4].

Type 2 diabetes mellitus (T2DM) is a complex disease involving different cellular pathways like carbohydrate absorption, insulin secretion and insulin resistance. Some of the proteins like peroxisome proliferator-activated receptor gamma (PPAR- γ), glucokinase, interleukin 1 beta (IL- 1β) and protein tyrosine phosphatases are identified to play an important role in the development of T2DM. The medical strategies that are developed to help fight T2DM involves dietary modifications, exercises, antidiabetic and antiobesity medications. However, these medications produce various side effects like nausea and hypoglycaemia [5].

Herbs are used since ancient times for alleviating ailments and for the maintenance of general health and they remain as a major natural resource in the world [6]. The biologically active metabolites of medicinal plants and their possible therapeutic potential has become a subject of active investigation [7]. Many plants are known for their hypoglycaemic properties in folk medicines of different cultures and they have been found to be useful in the treatment of diabetes. Although various synthetic drugs have been developed for treating diabetes, their use is still limited because of their side effects and cost. Plant based medicines are becoming popular because of their lesser toxicity and side effects. The phytoconstituents like flavonoids, glycosides, steroids and alkaloids exhibit hypoglycaemic effects by various mechanisms like increasing the secretion of insulin, increasing insulin sensitivity, inhibiting the production of glucose in liver, enhancing the glucose uptake in muscle and adipose tissues and by inhibiting the intestinal absorption of glucose [8] [9]. Treatment of T2DM with plant based medication has been observed to protect β cells and has effects on β cell proliferation [10]. Molecular docking analysis is a computational approach which is used to predict the binding affinity between two or more molecules. Docking of therapeutic proteins with ligands of interest has therapeutic applications in modern structure-based drug designing [11].

Elaeocarpus tectorius (Lour.) Poir belongs to the Elaeocarpaceae family. It is one of the tree species that bear edible fruits and is scarcely explored. Pharmacological studies on other trees of Elaeocarpus species showed that they exhibit anti-inflammatory, antimicrobial, anti-anxiety, analgesic, antidepressant, anti-asthmatic, antidiabetic, antitumor, and antihypertensive properties. The fruit extracts of *E.tectorius* exhibit significant antioxidant and antimicrobial activities [12]. The present study was aimed to investigate the bioactive compounds and antidiabetic potential of fruits of *Elaeocarpus tectorius* by GC-MS analysis and *in silico* molecular docking to some of the target proteins involved in the pathogenesis of T2DM.

Materials and Methods

Collection of plant materials and Preparation of extracts:

The fruits of *Elaeocarpus tectorius* were collected from Coonoor, The Nilgris district, Tamil Nadu, India. The fruits were washed to remove dust particles; the fruit pulp was separated from the seeds and shade dried. The dried material was finely powdered using a mortar and pestle and stored in an airtight container for further use. Five grams of powdered plant material was macerated with 50 ml of ethanol and incubated for 48 hours in a shaker

incubator at 40ºC. The extracts were then filtered and the solvent was evaporated to get dry extract. The amount of crude extract recovered after drying was weighed and the percentage of extraction yield was calculated [12]. The dried extract was stored at -20°C for further use.

$$\text{Extraction yield (\%)} = \frac{Weight\ of\ the\ dry\ extract\ \ (g)}{Weight\ of\ plant\ \ sample\ \ used\ \ for\ \ extraction\ \ (g)} \times 100$$

Qualitative phytochemical analysis:

Preliminary phytochemical screening was performed to identify the presence of different phytochemical constituents such as alkaloids [13], terpenoids [14], phenolic compounds [15], saponins [16], flavonoids [14], steroids [17] and glycosides [18] using standard methods.

Determination of total phenolics:

The total phenolic content of *E. tectorius* fruit extract was estimated using Folin-Ciocalteau method [19]. 10mg of extract was diluted with 2mL of methanol. 0.1mL of the extract was taken in test tubes and made up to 1 ml with distilled water. Then 0.5 mL of Folin- Ciocalteau phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20% Na_2CO_3) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm using a UV–VIS spectrophotometer against a reagent blank. The analysis was performed in triplicates and the results were based on the calibration curve prepared using gallic acid as a standard. The total phenolic content of the extract was expressed as milligrams gallic acid equivalent (GAE) per gram of extract.

Determination of total flavonoids:

The total flavonoid content of the extract was investigated using the aluminium chloride method with slight modifications [20]. 10mg of extract was diluted with 2mL of methanol. 0.5mL of extract was taken in a test tube and added 0.1 mL of 10% aluminium chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 minutes and the absorbance of the mixture was measured at 415 nm using a UV–VIS spectrophotometer against a reagent blank. The analysis was performed in triplicates and the results were based on the calibration curve prepared using quercetin as a standard. The total flavonoid content of the extract was expressed as milligrams quercetin equivalent (QE) per gram of extract.

Statistical analysis:

The estimation of total phenolics and flavonoids were performed in triplicates and expressed as means ± standard deviation (SD).

Gas chromatography-mass spectrometry (GC-MS) analysis:

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. The following conditions were employed. Column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1µ Mdf, composed of 100% dimethyl poly siloxane), operating in electron impact mode at 70eV; Helium (99.999%) was used as carrier gas at a constant flow of 1ml /min and an injection volume of 1 ml was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C / min, to 200°C then 5°C / min, to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and

fragments from 45 to 450 Da. Interpretation of mass spectrum GC-MS was conducted using the database of the National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown compound was compared with the spectrum of the known compounds stored in the NIST library.

Molecular docking studies:

Molecular docking studies were employed to predict the binding energies between the target proteins and selected ligands. Docking studies were carried out using Autodock 4.2.6 and the interactions between proteins and the ligands were analysed using Discovery Studio Visualizer 2020.

Protein preparation:

Docking calculations were performed for four target proteins involved in the pathogenesis of T2DM. The crystalline structure of proteins was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). All non-protein molecules and the water molecules were removed and hydrogen atoms were added to the protein molecules and converted to PDBQT file format (.pdbqt) using Autodock tools.

Ligand preparation:

The selected ligands to be docked with the target proteins were downloaded from the PubChem database of the National Centre for Biotechnological Information (NCBI) (https://pubchem.ncbi.nlm.nih.gov/) and converted to PDB file format using CORINA classic (https://www.mn-am.com/online_demos/corina_demo). Metformin and Pioglitazone were used as controls. Gasteiger charges were added to the ligands and converted to PDBQT file format (.pdbqt) using Autodock tools.

Molecular docking:

Autogrid program available from Autodock tools was used for the preparation of grid maps. The Lamarckian genetic algorithm was chosen to search for the best conformers. The process was carried out with the default parameters of Autodock. The population size was set to 150, maximum number of evaluations to 2,500,000, maximum number of generations 27,000, maximum number of top individual that automatically survived 1, gene mutation rate 0.02 and crossover rate 0.8. The results were visualized using Discovery Studio Visualizer 2020.

Drug-likeness of the ligands:

SwissADME, a free web tool was used to generate the physicochemical and drug-likeness properties of the ligands.

Toxicity of the ligands:

The toxicity profile of the ligands was predicted using free *in silico* toxicity predictor softwares, Protox-II server and admetSAR.

Results and Discussion

Extraction yield:

Extraction is the first and main step to recover and isolate the desired phytochemicals from plant materials [21]. The extraction yield of *E.tectorius* fruits using ethanol as solvent was found to be 18.3 %. The extraction efficiency is influenced by the

chemical nature of phytochemicals, the method used, sample particle size and nature of the solvent [22].

Qualitative phytochemical analysis:

The preliminary phytochemical screening of ethanolic fruit extract of *E. tectorius* revealed the presence of phytochemicals like alkaloids, terpenoids, phenolic compounds, flavonoids, steroids and glycosides. The screening also revealed that saponins were absent in the extract (Table 1).

Table 1: Phytochemical screening of *E. Tectorius* fruit extracts

Phytochemical constituents	*E.tectorius* fruits
Alkaloids	+
Terpenoids	+
Phenolic compounds	+++
Saponins	-
Flavonoids	+++
Steroids	++
Glycosides	+

((+): Presence of chemical compound, (−): Absence of chemical compound (+: Low intensity of characteristic colour, ++: Medium intensity of characteristic colour, +++: Higher intensity of characteristic colour))

The phytochemicals produced by plants provide protection against various medical conditions like oxidative stress, diabetes and hypertension [23]. Alkaloids have a wide range of pharmacological effects and are medically known as analgesics, anticancer drugs, antimalarial and antihypertensive agents [24]. Terpenoids which constitute a largest class of natural products have numerous biological activities including analgesic, anti-inflammatory, antioxidant, anticancer and antimicrobial activities [25]. Phenolic compounds and flavonoids that hold an aromatic ring with at least one hydroxyl group has been reported in several medicinal plants are widely known for their antidiabetic, antioxidant, anticancer, cardio protective and skin protective roles [26]. Steroids are biologically important secondary metabolites produced by plants known for their antioxidant, antibacterial and immunostimulant activities [27]. Glycosides are organic molecules that have great therapeutic potential including analgesic, antimicrobial and anticancer activities [28]. These phytochemicals might contribute to the therapeutic potential of the plant**.**

Determination of total phenolics and flavonoid content:

The total phenolic content of the ethanolic extract of *E.tectorius* fruits, calculated from gallic acid calibration curve ($y = 0.009x + 0.074$, $R^2 = 0.995$) was found to be 239.63 ± 12.85 milligram gallic acid equivalents (GAE) /gram extract and the total flavonoid content calculated from quercetin standard curve ($y = 0.032x + 0.220$, $R^2 = 0.997$) was 124.53 ± 0.94 milligram quercetin (QE)/ gram extract. Phenolic compounds are strong antioxidants that have the ability to prevent tissue damage caused by free radicals. They exhibit antidiabetic activity by improving impaired insulin sensitivity and by up regulation of glucose transport

[29, 30]. Flavonoids are a group of hydroxylated phenolic compounds that show antidiabetic activity by enhancing insulin secretion and promoting proliferation of pancreatic β cells [31]. Since the fruit extract contains significant amount of total phenolic and flavonoid compounds, it might be responsible for the antidiabetic activity.

Gas-chromatography mass spectrometry (GC-MS) analysis:

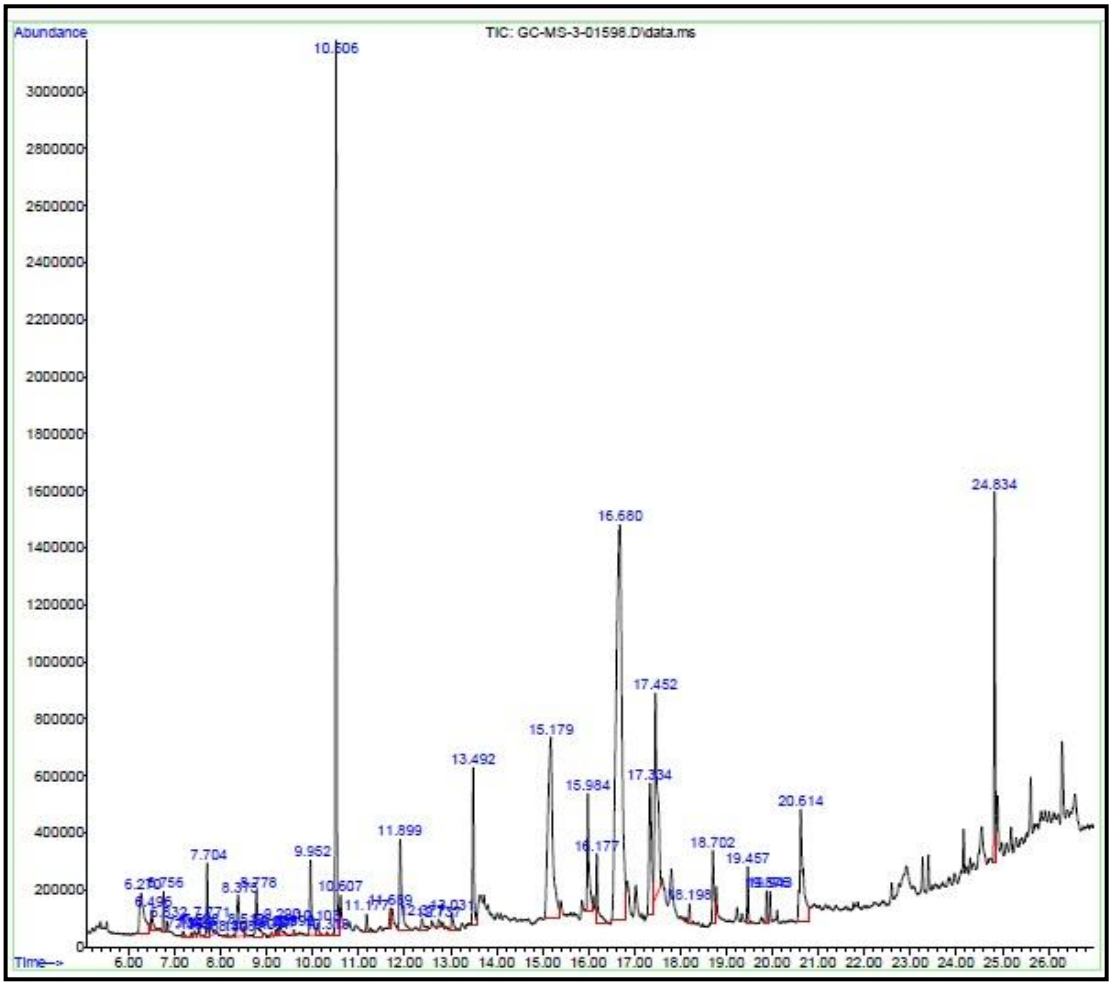
The GC-MS analysis of the ethanolic extracts of *E. tectorius* fruits revealed the presence of 28 different compounds (Fig.1). The identified phytochemicals with their retention time, molecular formula and molecular weight are given in Table 2. Among the identified phytochemicals dibutyl phthalate, 2, 3-dihydro-3, 5- Dihydroxy-6-methyl-4H-Pyrane-4-one, n-Hexadecanoic acid was found to have anti-inflammatory, antidiabetic, antimicrobial and antioxidant activities [32-34]. The compound, 2,3-dihydro-3,5- Dihydroxy-6-methyl-4H-Pyrane-4-one was shown to have anti-proliferative and pro-apoptotic effects [35]. 2-oxepanone is reported to exhibit antimicrobial, insecticidal, anticancer, antioxidant and anti- inflammatory activities [36]. The compound, 1, 2 cyclopentanedione has antioxidant activity [37]. Diethyl phthalate is reported to exhibit antimicrobial, acetylcholinesterase, and neurotoxic activity [38].

Table 2: Bioactive compounds identified in the ethanolic extract of *E.tectorius* fruits

Name of the Compound	Retention time (min)	Molecular formula	Molecular weight (g/mol)
2-amino-3-methyl-1-butanol	6.270	$C_5H_{13}NO$	103.16
1,2-Cyclopentanedione	6.496	$C_5H_6O_2$	98.1
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	6.756	$C_6H_8O_4$	144.13
2-ethyl 1- hexanol	6.832	$C_8H_{18}O$	130.23
3,4-dihydro-2H pyran	7.184	C_5H_8O	84.12
Methyl 3-hydroxytetradecanoate	7.352	$C_{15}H_{30}O_3$	258.4
3,4Dehydro-dl-proline	7.427	$C_5H_7NO_2$	113.1
Butyl 23-hydroxy-3,6,9,12,15,18,21-heptaoxatricosan-1-oate	7.704	$C_{20}H_{40}O_{10}$	440.53
Benzyl alcohol	8.140	C_7H_8O	108.14
2-Oxepanone	8.375	$C_6H_{10}O_2$	114.14
4,5-Diamino-2-	8.778	$C_4H_6N_4O$	126

hydroxypyrimidine			
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	9.952	$C_6H_8O_4$	144.12
Azulene	10.506	$C_{10}H_8$	128.17
3,3,6-trimethyl-1,5-Heptadien-4-ol	11.689	$C_{10}H_{18}O$	154.25
5-Hydroxymethylfurfural	11.899	$C_6H_6O_3$	126.11
2-Methoxy-4-vinylphenol	12.377	$C_9H_{10}O_2$	150.17
5-Methyl-5-formylamino-6-imino-hexahydro-2-thioxopyrimidin-4-one	13.031	$C_6H_8N_4O_2S$	200
1-chloro-2-methoxy- benzene	13.492	C_7H_7ClO	142.583
Diethyl Phthalate	16.177	$C_{12}H_{14}O_4$	222.24
5-O-hexyl-d- galactitol	17.344	$C_{12}H_{26}O_6$	266.33
1-O-hexyl- lyxitol	17.452	$C_{11}H_{24}O_5$	236.31
1-Nonadecene	18.198	$C_{19}H_{38}$	266.50
n-Hexadecanoic acid	18.702	$C_{16}H_{32}O_2$	256.42
Tetradecanoic acid	18.702	$C_{14}H_{28}O_2$	228.37
Dibutyl phthalate	19.876	$C_{16}H_{22}O_4$	278.34
Phytol	19.943	$C_{20}H_{40}O$	296.5310
Oleic Acid	20.614	$C_{18}H_{34}O_2$	282.4614
Cyclotetracosane	24.834	$C_{24}H_{48}$	336.6379

Figure 1: GC-MS chromatogram of ethanolic fruit extract of *Elaeocarpus tectorius*



Phytol, a diterpene alcohol exhibits antidiabetic activity by regulating the enzymes involved in maintaining insulin levels and thus helps in the management of type 2 diabetes. It also has antimicrobial, anticancer and antidiuretic properties [39]. 2-Methoxy-4-vinylpehnol is reported to have antimicrobial, antioxidant and anti-inflammatory activities [40].

Molecular docking studies:

Molecular docking studies are routinely carried out in modern drug designing for understanding drug-receptor interactions and it is used to study the interactions between a small molecule and a protein at atomic level [41]. The selected bioactive compounds from *E. tectorius* fruit extract were docked with T2DM target proteins using Autodock 4.2.6 and their binding energies are illustrated in Table 3. Among the selected compounds, diethyl phthalate, dibutyl phthalate and phytol showed good binding affinity in kcal/mol. Phytol with PPAR- γ (-7.27 kcal/mol) and dibutyl phthalate with PTP- 1B (-7.27 kcal/mol) showed highest binding affinity among the ligands. Dibutyl phthalate had a binding affinity of -6.57 kcal/mol with PPAR- γ and -5.8 kcal/mol with glucokinase. Diethyl phthalate had a binding affinity of -6.48

kcal/mol with PPAR- γ and -6.18 kcal/mol with PTP- 1B. Phytol had a binding affinity of about -6.09 kcal/mol with glucokinase.

Table 3: Molecular docking of selected bioactive compounds from *E. tectorius* fruits with T2DM target proteins

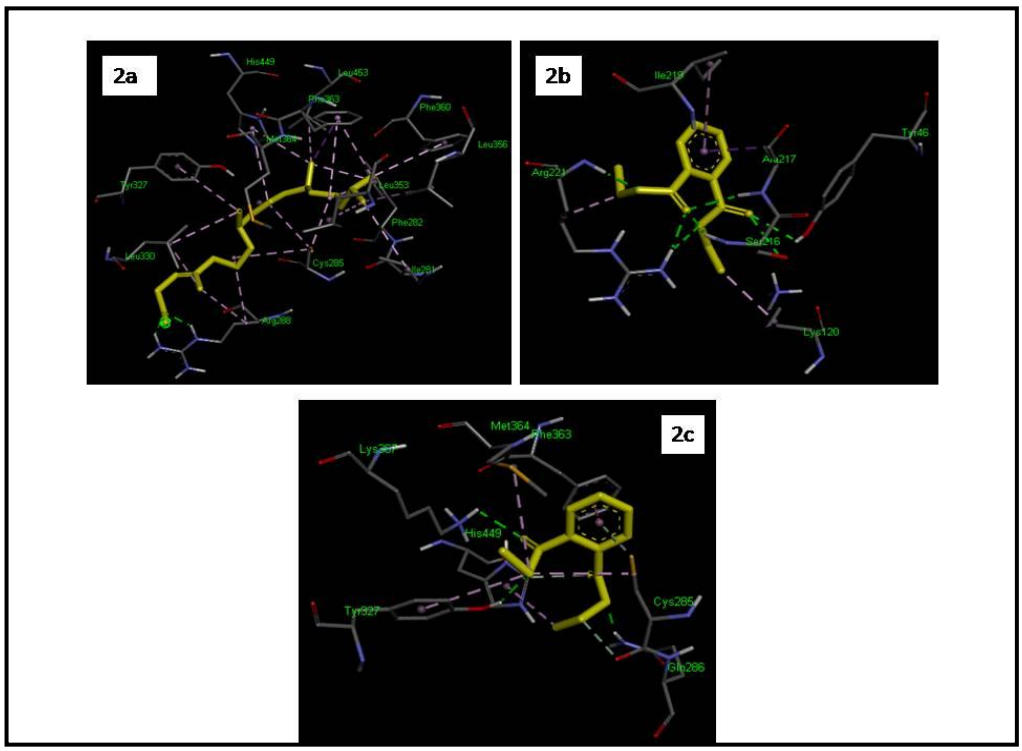
Ligands	Estimated free energy of binding (Kcal/mol)			
	PPAR- γ	**PTP 1B**	**GK**	**IL- 1β**
1,2-Cyclopentanedione	-4.48	-4.6	-4.68	-4.45
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	-5.29	-5.07	-5.48	-4.67
2-Oxepanone	-5.08	-5.21	-5.25	-4.33
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	-5.6	-6.05	-4.91	-4.53
2-Methoxy-4-vinylphenol	-5.28	-5.46	-5.27	-5.15
Diethyl Phthalate	-6.48	-6.18	-5.79	-4.92
1-Nonadecene	-5.7	-3.72	-5.82	-3.69
Tetradecanoic acid	-5.72	-5.52	-4.71	-4.4
Dibutyl phthalate	-6.57	-7.27	-5.8	-4.91
Phytol	-7.27	-5.03	-6.09	-4.92
Metformin	-5.84	-5.98	-7.06	-5.24
Pioglitazone	-8.53	-7.31	-8.27	-7.26

(PPAR- γ- Peroxisome proliferator activated receptor gamma, PTP 1B- Protein tyrosine phosphatase 1B, GK- Glucokinase, IL- 1β- Interleukin 1 beta)

The docking results were visualized using Discovery Studio Visualizer 2020 for the analysis of the interactions between the proteins and ligands. Dibutyl phthalate had strong interaction with the target proteins. Dibutyl phthalate complex interacted with PPAR- γ forming two hydrogen bonds (TYR 327, LYS 367) and with PTP- 1B forming four hydrogen bonds (TYR 46, SER 216, ALA 217, ARG 221). Diethyl phthalate interacted with IL- 1β forming one hydrogen bond (LEU 62) and with PPAR- γ forming three hydrogen bonds (GLN 286, TYR 327, LYS 367). Diethyl phthalate interacted with PTP-1B forming one hydrogen bond at ARG 79. Phytol interacted with PPAR- γ by forming one hydrogen bond (ARG 288) and with PTP-1B by forming two hydrogen bonds (GLU 75, GLN 78).

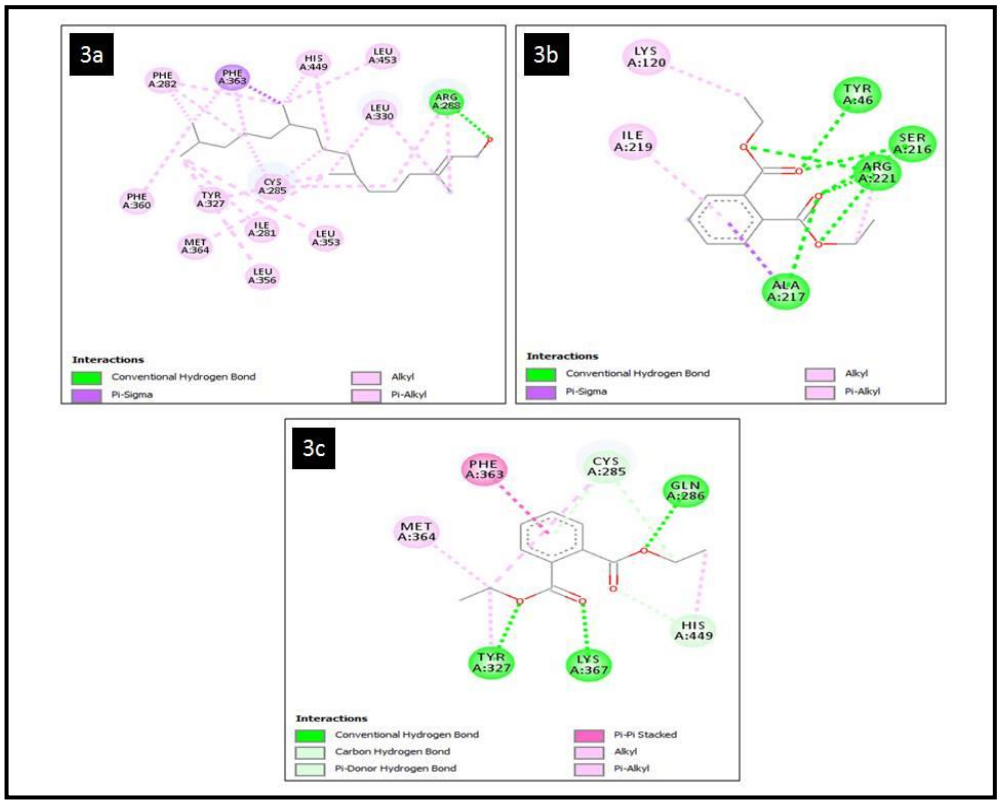
Molecular docking results suggest that dibutyl phthalate and diethyl phthalate could inhibit protein tyrosine phosphatase 1B which plays important roles in the cell growth and differentiation through phosphorylation and dephosphorylation of tyrosine residues [42]. The inhibitors of PTP 1B enhance the sensibility of insulin receptor and have favourable curing effect on insulin resistant conditions associated with T2DM [43]. Phytol, dibutyl phthalate and diethyl phthalate showed good binding interaction with PPAR- γ, which is a potential metabolic regulator of peripheral organs and tissues, such as adipose tissue. Upregulation of the expression of PPAR- γ has been reported to improve insulin sensitivity and glucose uptake [44].

Figure 2: 3D display of interactions between ligands and protein. 2a: phytol and PPAR-γ, 2b: dibutyl phthalate with PTP- 1B, 2c: diethyl phthalate and PPAR- γ



Binding of peroxisome proliferator-activated receptor-gamma (PPAR- γ) to agonists stimulate genes that favours the storage of triglycerides, thereby lowering the circulating free fatty acid concentrations. This causes a shift from using free fatty acids to glucose as a fuel substrate and it is one of the mechanisms whereby they improve insulin sensitivity in peripheral tissues [45]. The ligands also exhibited good binding affinity with glucokinase that plays an important role in glucose homeostasis and acts as glucose sensor for insulin secretion by pancreatic β cells [46]. The binding energies between IL- 1β and the ligands were also appreciable. IL- 1β is a pro-inflammatory cytokine that plays an important role in the destruction of pancreatic β cells. Interleukin 1-receptor antagonist, a natural competitive inhibitor of IL- 1β protects pancreatic β cells from the IL-1β induced apoptosis. However, the expression of interleukin 1-receptor antagonist is found to be decreased in the β cells of patients with T2DM [47]. Hence natural products that can inhibit IL-1β would be useful in the treatment of T2DM.

Figure 3: 2D display of interactions between ligands and protein. 3a: phytol and PPAR-γ, 3b: dibutyl phthalate with PTP- 1B, 3c: diethyl phthalate and PPAR- γ



Druglikeness of the ligands:

SwissADME is a free web server which predicts whether a particular compound has the potential to be drug-like by checking various aspects like physicochemical properties such as molar mass, hydrogen donor, acceptor, log *P* value and pharmacokinetic properties like gastro-intestinal absorption and topological polar surface area (TPSA) [48].

Table 4: Druglikeness of the selected ligands predicted by SwissADME

Ligand	Physicochemical Properties and drug-likeness						
	Molar mass (g/mol)	HBD	HBA	Log P	TPSA (Å²)	GIA	Drug-likeness
Dibutyl phthalate	278.34	0	4	3.69	52.60	High	Yes

Diethyl phthalate	222.24	0	4	2.29	52.60	High	Yes
Phytol	296.53	1	1	6.22	20.23	low	Yes

(HBD- Hydrogen bond donor, HBA- Hydrogen bond acceptor, TPSA- topological polar surface area, GIA- Gastro-intestinal absorption)

From the docking studies, dibutyl phthalate, diethyl phthalate and phytol was selected for druglikeness and toxicity analysis. The SMILES notation of the three compounds, obtained from PubChem database was submitted to the SwissADME server to analyse their drug-likeness properties and the results are summarized in Table 4. Toxicity issues or undesirable pharmacokinetic properties are the main reasons for the failure of drug candidates at the clinical trials [49]. The concept of drug-likeness has become an important consideration in the selection of compounds with desirable bioavailability during the early stages of drug discovery [50]. The ligands were checked for Lipinski's rule of five, Gastrointestinal (GI) absorption and topological polar surface area (TPSA). The Lipinski's filter of five states that for a molecule to be considered drug-like, it should fulfil four different physicochemical parameters (H-bond donors ≤ 5, molecular weight ≤ 500, log $P \leq 5$, H-bond acceptors ≤ 10) [51]. TPSA is an important property for a molecule to be drug-like since it influences the absorption, bioavailability and blood-brain barrier penetration and should be less than 140 $Å^2$ [52]. The ligands exhibited favourable drug likeness properties.

Toxicity of the ligands:

Toxicity assessment is an important step done during the process of drug discovery. The ligands were analysed for their toxic properties using Protox-II and admetSAR and the results are presented in Table 5. Protox-II is a free *in silico* toxicity predictor which predicts the lethal dose 50 (LD_{50}) value in mg/kg body weight, based on which the server has divided the results into six classes. LD_{50} is the dose at which 50% or half of the test population will die upon exposure to a compound [53]. In Protox-II, the compounds belonging to Classes 5 and 6 are generally safe for consumption and non-toxic and specifically compounds with LD_{50} value of above than 5000 mg/kg (Class 6) is labelled as the safest non-toxic class of compounds for oral consumption. admetSAR predicts the absorption, distribution, metabolism, excretion and toxicity profile of a compound and plays an important role in the early steps of drug discovery [54].

Table 5: Toxicity profile of the selected ligands predicted by Protox II and admetSAR

Ligand	Protox-II		admetSAR		
	LD_{50} mg/kg	Toxicity class	BBB permeable	HIA	Carcinogenicity
Dibutyl phthalate	3474	5	Yes	HIA+	No
Diethyl phthalate	6172	6	Yes	HIA+	No
Phytol	5000	5	Yes	HIA+	No

(BBB- Blood Brain Barrier, HIA- Human intestinal absorption)

Diethyl phthalate had LD_{50} value of 6172 mg/kg and belongs to class 6 of Protox classification. Dibutyl phthalate had LD_{50} value of 3474 mg/kg and phytol had LD_{50} value of 5000 mg/kg and both belongs to class 5 of Protox classification and is considered safe. admetSAR results revealed that all compounds pass through blood-brain barrier, have good intestinal absorption and are non- carcinogenic. The ligands passed all the filters of drug-likeness and toxicity and thus can be used as potential antidiabetic agents.

Conclusion

In this study, molecular docking analysis of some proteins involved in the pathogenesis of T2DM was performed using Autodock against selected phytochemicals obtained from the GC-MS analysis of fruit extract of *Elaeocarpus tectorius*. From the docking analysis, it can be concluded that dibutyl phthalate, diethyl phthalate and phytol can be used in the treatment of diabetes. This study suggests that the edible fruits of *Elaeocarpus tectorius* could be a rich source of bioactive compounds that could be used to treat metabolic disorders like diabetes. These compounds were analysed for their drug-likeness and toxic properties and they passed all the filters. *In vitro* and *in vivo* studies are further needed to understand their molecular mechanisms.

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Conflict of interest

The authors declare that there is no conflict of interest.

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