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Research Article
Anti-diabetic Activity of Endophytic Fungi, *Penicillium* Species of *Tabebuia argentea*; *in Silico* and Experimental Analysis

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Abstract

Background and Objective: The plant and microbial phytochemicals possessing many biological activities with less toxic effects. Hence, present research was aimed to identify phytochemicals in *Penicillium* species extract and their role in diabetic activity. **Materials and Methods:** The methanolic extract of endophytic fungi *Penicillium* species of *Tabebuia argentea* was used to analyse phytochemical constituents by Gas Chromatography Mass Spectrometry (GC-MS). The same extract was used to evaluate the *in vitro* anti-diabetic activity. The phytochemicals profile obtained from GC-MS was used for *in silico* anti-diabetic activity against 21 different diabetic proteins/enzymes and ADMET (Absorption, Dissolution, Metabolism, Excretion and Toxicity). The analysis of variance was used to determine the significance of difference between treatment groups two-way (ANOVA) followed by SPSS2 (MRX version). **Results:** The methanol extract of *Penicillium* species consisted of 18 different phytochemicals and they inhibited the activity of α-amylase, β-glucosidase and dipeptidyl peptidase IV at maximum level. Out of 18 phytochemicals, the octadecanoic acid methyl ester and 3 phthalates have shown more interaction with all the 21 diabetic proteins/enzymes tested. The octadecanoic acid has shown more interaction with 1dhk, 1nu6, 2wy1, 4y14, 3i2m, 3k35, 4j5t and 5td4. The di-isobutyl isophthalate, dioctyl phthalate and bis-2-ethylhexyl phthalate have shown high interaction with 1m1j, 1ogs and 4acd. The overall observation of present study showed that octadecanoic acid is responsible for inducing anti-diabetic activity and the compound has the ability to interact with all the diabetic proteins and inactivate their activity. The *in silico* investigation clearly indicates how tested compounds interact with different diabetic proteins/enzymes, their role was identified and they were non-toxic and non-carcinogens. **Conclusion:** The *Penicillium* species represented potent bioactive compounds in their extract and are responsible for significant *in vitro* and *in silico* anti-diabetic activity.

Key words: *Penicillium* species, phytochemicals, anti-diabetic, molecular docking, ADMET

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

World is having problems in disease management in related to pathogens resistance, usage of same drugs and cost. The scientists are working on finding available drugs in cheaper cost or finding new drugs at less cost. A search for newer and more effective agents to deal with disease problems is now under way and endophytes are a novel source of potentially useful medicinal compounds. Endophytes comprise a large but little-explored share of fungal diversity[1,2].

The endophytes may provide protection and survival conditions to their host plant by producing a plethora of substances which, once isolated and characterized, may also have potential for use in industry, agriculture and medicine[3]. At present, endophytes are producing biologically important bioactive compounds using to manage many infectious and non-infectious diseases.

Endophytic fungi are of biotechnological interest due to their potential as a source of secondary metabolites that have been proven useful for novel drug discovery[4].

Endophytic fungi have been shown to produce several pharmacologically important compounds such as antimycotics steroid 22-triene-3b-ol[5], anticancer cajanol[6], podophyllotoxin and kaempferol[7], anti-inflammatory ergoflavin[8], antioxidant lectin[9], insecticidal heptelidic acid[10], immunosuppressive sydoxanthone A, B[11] and cytotoxic radicicol[12].

Plants used in traditional medicine have played a very important role in the search for new bioactive strains of endophytic fungi, as it is possible that their beneficial characteristics are a result of the metabolites produced by their endophytic community[13,14].

Tabebuia argentea (Bignoniaceae) is an extensive and yellow blossoming tree and have turned out to be a rich wellspring of numerous natural mixes, particularly, of phenolic and polyphenolic nature. The plant is able to produce anticancer agent, lapachol, it has the ability to interfere with the bioactivities of enzymes known as, topoisomerases, a group of enzymes that are critical for DNA replication in cells[15]. The antitumor activity of Lapachol may be due to its interaction with nucleic acids and the interaction of the naphthoquinone moiety between base pairs of the DNA helix occurs with subsequent inhibition of DNA replication and RNA synthesis[16]. Other biological activities of Lapachol are antimetastatic activity[17], anti-microbial and antifungal[18], antiviral[19], anti-inflammatory[20], antiparasitic[16], leishmanicidal[21] and molluscicidal activity[22]. Only three reports are available in the identification of Lapachol producing endophytes of *Tabebuia argentea* from our lab research[22-24].

Some research works believed to produce pharmacologically important bioactive compounds, in this context, the aims of the present study were to characterize the phytochemical profile of fungal endophyte, *Penicillium* species associated with *Tabebuia argentea* and to detect anti-diabetic activities and *in silico* prediction.

MATERIALS AND METHODS

Collection and extraction of phytochemicals from endophytic fungi, *Penicillium* species: The endophytic fungi, *Penicillium* species of *Tabebuia argentea* were collected from stock culture unit of Department of Biotechnology, Shridevi Institute of Engineering and Technology, Tumakuru, Karnataka, Bengaluru in September, 2016 and grown in 250 mL Erlenmeyer flask containing 100 mL of rose Bengal-yeast extract-sucrose broth for 2 weeks at 26+2°C with periodical shaking at 150 rpm. After the incubation period, the culture was separated from the broth and was extracted using methanol as organic solvent. Extraction was done using the mycelial mat for the metabolites with methanol. Added the equal volume of the solvent to the filtrate, mixed well for 10 min and kept for 5 min till the two clear immiscible layers formed. The upper layer of the solvent containing the extracted compounds was separated using separating funnel. Evaporated the solvent and the resultant compound was dried in rotator vacuum evaporator to yield the crude metabolites[25]. Then, the extract was dissolved with dimethyl sulphoxide at 1 mg mL^{-1} of concentration and kept at 4°C.

Phytochemical analysis: The preliminary phytochemical analysis of the crude extracts of *Penicillium* species was done to know alkaloids, flavonoids, tannins, phenols, saponins, terpenoids and carbohydrates using standard methods[25,26].

Detection of bioactive compounds by GC-MS analysis: The methanol crude extract was subjected to GC-MS analysis to identify the bioactive compounds. The GS-MS analysis of the crude extract was carried out in a Shimadzu GC-MS-QP 2010 Plus fitted with RTX-5 (60 m×0-25 mm×0.25 μm) capillary column in IISc, Bengaluru. The instrument was set to an initial temperature of 70°C and maintained at this temperature for 2 min. At the end of this period, the oven temperature was rose up to 2800°C, at the rate of an increase of 50°C min^{-1} and maintained for 9 min. An injection port as 1 mL mi^{-1}. The ionization voltage was 70 eV. The sample was injected as 10:1. Mass spectral scan range was set at 45-450 (m/z). The identification of bioactive compounds present in the extracts was performed by comparing the mass spectra with data from NIST05 (National Institute of Standards and Technology, US)

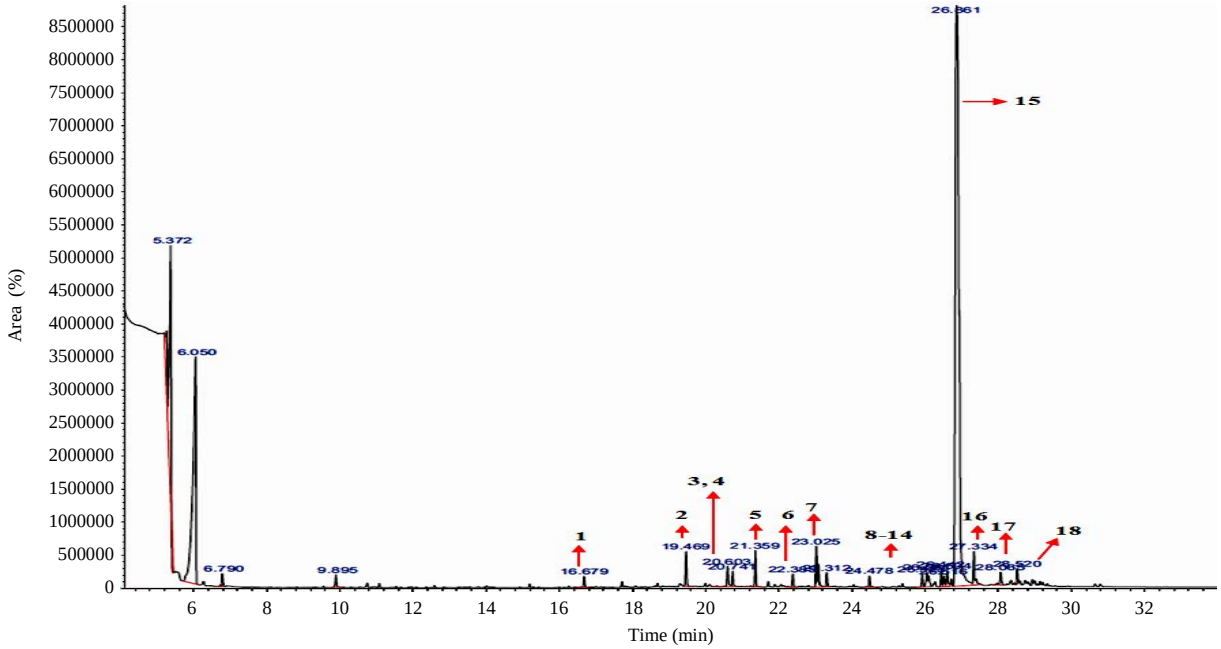

Fig. 1: GC-MS analysis showing different phytochemicals identified based on retention time in methanol extract of endophytic fungi *Penicillium* species

library. The name, molecular weight and structure of the components of the test material were ascertained based on retention time.

Anti-diabetic activity

α-glucosidase activity: A 36 µL of phosphate buffer solution, 30 mL sample solution with various concentrations (10, 25, 50, 100 and 150 µg mL^{-1}) and 17 µL of 4-nitrophenyl-α-D-glycopyranoside (PNPG) substrate as the concentration of 5 mM were put in 37°C for 5 min. After 5 min, 17 µL of α-glucosidase solution 0.15 U mL^{-1} was added to each well to obtain a total volume of 100 mL. The mixture was incubated for 15 min, the reaction was spotted by adding 100 µL of sodium carbonate 200 mM. Absorbance was measured at 405 nm using a microplate reader. Each test was repeated thrice[27]. The calculation was done based on Elya *et al.*[28].

α-amylase assay: A 250 mL of 500 µg mL^{-1} extract, 250 µL of starch 2.0% (w.v) and 250 µL of 1 U mL^{-1} α-amylase solution was homogeneously mixed into a test tube. After incubated at 20°C for 3 min, 500 µL of color reagent (dinitrosalicylic acid) was added to stop the enzymatic reaction. The mixture was kept into boiled water and 250 µL of 1 U mL^{-1} α-amylase was added immediately. The mixture was heated up to 15 min. Further, the solution was removed from the heating process and cooked at room temperature (-26+20°C) for 3 min. A 4500 µL aqua dest was added to obtain a total volume of 6000 µL. The solution was homogenized using a vortex. The α-amylase activity was determined at 540 nm using spectrophotometry to measure product absorbance (maltose) which reduces DNS. The produced absorbance was compared with a blank. Percent inhibition was calculated using the equation of Elya *et al.*[28].

Dipeptidyl peptidase IV assay: A 25 µL extract was added to 50 µL Dipeptidyl peptidase (DPP-IV) (500 µg mL^{-1}). The mixture was incubated at 37°C for 5 min. A 100 µL Gly-Pro-P-Nitroanilide (GPPN) (2 mM) was added to the wells containing extract and enzyme. Incubation was contained for 15 min. The reaction was terminated by adding 25 µL glacial acetic acid (25%). The absorbance was measured at $\lambda = 405$ nm[29].

***In silico* antidiabetic activity**

Bioactive compound preparation: Most of the 3D (3 Dimensions) structures of drug molecules identified in the methanol extract of endophytic fungi, *Penicillium* species were downloaded from PubChem Compound section of National Center for Biotechnology Information (NCBI)[30]. Ligands during this process also being checked for Torsion count to detect currently active bonds with default settings. Importantly, amide bonds were checked and treated as non-rotatable. Ligands were also utilized to merge non-polar hydrogens. The 2D structures of 18 ligands are illustrated in Table 1 and Fig. 1. The 3D structures of these 18 ligands were elucidated.

Table 1: Identified phytochemicals in *Penicillium* species extract and their synonymous, identified based on retention time in GC-MS

Peak No.	Retention time (min)	Extracted ionic peaks	Identified compound name		Synonyms
1	16.681	191.1, 57, 206.1, 192.1, 41	Phenol, 2,4-bis (1,1-dimethylethyl)-	1.	Phenol, 2,4-di-tert-butyl-
				2.	2,4-Di-tert-butylphenol
				3.	2,4-di-t-Butylphenol
				4.	1-Hydroxy-2,4-di-tert-butylbenzene
				5.	2,4-Bis (1,1-dimethylethyl) phenol
				6.	2,4-Bis (tert-butyl) phenol
				7.	2,4-tert-butylphenol
				8.	2,4-bis (1,1-dimethylethyl) phenol
2	19.469	121, 91, 41, 93, 77	12-Azabicyclo [9.2.2] pentadeca-1(13), 11,14-trien-13-ylamine	1.	12-azabicyclo[9.2.2]pentadeca-1(13),11,14-trien-13-amine
3	20.601	164, 208, 165, 190, 78	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-	1.	(3R)-8-Hydroxy-6-methoxy-3-methyl-3,4-dihydro-1H-isochromen-1-one
				2.	(3R)-8-Hydroxy-6-méthoxy-3-méthyl-3,4-dihydro-1H-isochromén-1-one
				3.	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (3R)-
				4.	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-
				5.	Isocoumarin, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-(-)-
				6.	(3R)-8-Hydroxy-6-methoxy-3-methyl-3,4-dihydro-1H-isochromen-1-one
				7.	(R)-(-)-6-methoxymellein
				8.	(R)-6-methoxymellein
				9.	2,4-Dihydro-8-hydroxy-6-methoxy-3-methyl-1H-2-benzopyran-1-one
4	20.744	148.9, 57, 41, 150, 104	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	1.	Phthalic acid, diisobutyl ester
				2.	Diisobutyl phthalate; Hexaplas M/1B
				3.	Isobutyl phthalate; Palatinol IC
				4.	Diisobutylester kyseliny ftalove
				5.	1,2-Benzenedicarboxylic acid, di (2-methylpropyl) ester
				6.	1,2-Benzenedicarboxylic acid, 1,2-bis (2-methylpropyl) ester
				7.	Bis (2-methylpropyl) phthalate
				8.	Isobutyl-o-phthalate
				9.	di-2-methylpropyl phthalate
5	21.358	74, 87, 43, 55, 41	Hexadecanoic acid, methyl ester	1.	Palmitic acid, methyl ester
				2.	n-Hexadecanoic acid methyl ester
				3.	Methyl hexadecanoate
				4.	Methyl n-hexadecanoate
				5.	Methyl palmitate
				6.	Acide hexadecanoique methyl ester
6	22.387	163, 148, 70, 181, 77	Phthalic acid, methyl octyl ester	1.	1,2-Benzenedicarboxylic acid methyl octyl ester
				2.	1,2-Benzenedicarboxylic acid, octyl methyl ester
				3.	Methyl octyl phthalate
7	23.027	67, 81, 55, 95, 41	10,13-Octadecadienoic acid, methyl ester	1.	Methyl (10E,13E)-10,13 octadecadienoate
				2.	Methyl (10E,13E)-octadeca-10,13-dienoate
8	23.312	74, 87, 43, 55, 41	Octadecanoic acid, methyl ester	1.	Methyl stearate
				2.	Methyl octadecanoate
				3.	Octadecanoic acid, methyl ester
				4.	Stearic acid, methyl ester
				5.	Stearic acid methyl ester
				6.	Methyl n-octadecanoate
				7.	n-Octadecanoic acid methyl ester

Table 1: Continue

Peak No.	Retention time (min)	Extracted ionic peaks	Identified compound name	Synonyms
9	24.476	87, 148.9, 41, 45, 43	Unknown compound	Unknown
10	25.925	287.1, 302.1, 209, 105, 165	Unknown compound	Unknown
11	26.054	287.1, 302.1, 209, 105, 165	Phenol, 2,4-bis(1-phenylethyl)-	1. 2,4-Bis (1-phenylethyl) phenol 2. 2,4-Bis (α-methylbenzyl) phenol 3. Phenol, 2,4-bis(1-phenylethyl)- 4. 2,4-Bis (1-phenylethyl) phenol
12	26.443	148.9, 57, 166.9, 71, 43	Bis (2-ethylhexyl) phthalate	1. Phthalic acid, Bis (2-ethylhexyl) ester 2. Bis (2-ethylhexyl) 1,2-benzenedicarboxylate 3. Di (ethylhexyl) phthalate 4. Di (2-ethylhexyl) phthalate 5. Dioctyl phthalate 6. Octyl phthalate; 2-Ethylhexyl phthalate 7. Phthalic acid di (2-ethylhexyl) ester 8. di-iso-Octyl phthalate 9. Di (2-ethylhexyl) o-phthalate 10. Di-sec-octyl phthalate 11. Di (2-ethylhexyl) orthophthalate 12. Bis (2-ethylhexyl) o-phthalate 13. 1,2-Benzenedicarboxylic acid, Bis (2-ethylhexyl) esterBis (2-ethylhexyl) ester phthalic acid 14. 1,2-Benzenedicarboxylic acid, 1,2-Bis (2-ethylhexyl) ester
13	26.514	287.1, 302.1, 105, 288.1, 209	Phenol, 2,4-Bis (1-phenylethyl)-	1. 2,4-Bis (1-phenylethyl) phenol 2. 2,4-Bis (α-methylbenzyl) phenol 3. Phenol, 2,4-Bis (1-phenylethyl)- 4. 2,4-Bis (1-phenylethyl)phenol
14	26.604	95, 81, 91, 55, 107	Phenol, 2,6-Bis (1,1-dimethylethyl) -4-[(4-hydroxy-3,5-dimethylphenyl) methyl]-	1. Mesitol, 2. alpha.4-(3,5-di-tert-butyl-4-hydroxyphenyl)-, 3. 4-[(3,5-ditert-butyl-4-hydroxyphenyl) methyl]-2,6-dimethylphenol
15	26.863	149, 167, 57.1, 71.1, 43.1	1,2-Benzenedicarboxylic acid, diisooctyl ester	1. Di-isooctyl phthalate; Hexaplas M/O 2. Iso-octyl phthalate 3. Flexol plasticizer diop 4. Phthalic acid, Bis (6-methylheptyl) ester 5. Phthalic acid, diisooctyl ester 6. Bis (6-methylheptyl) phthalate
16	27.335	55, 95, 81, 41, 43	Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta., 7.beta.,8a.alpha.)]-	1. 4.beta.H,5.alpha.-Eremophilane 2. 10.alpha.-Eremophilane 3. 7-Isopropyl-1,8a-dimethyldecahydronaphthalene 4. 1,8a-dimethyl-7-(propan-2-yl) decahydronaphthalene 5. 1,8a-dimethyl-7-propan-2-yl-2,3,4,4a,5,6,7,8-octahydro-1H-naphthalene 6. Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)- 7. [1R-(1.alpha.,4a.beta.,7.beta.,8a.alpha.)]-
17	28.066	95, 194.1, 81, 149, 55	5-diethylamino-2-nitrosophenol	1. 5-(Diethylamino)-2-nitrosophenol
18	28.519	149, 43, 55, 122, 95	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8, 8a-hexahydro-1H-naphthalen-2-one	1. 6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone 2. 4,8a-dimethyl-6-prop-1-en-2-yl-1,4a,5,6,7,8-hexahydronaphthalen-2-one

Table 2: List of enzymes selected for docking studies

PDB name	Name
1dhk	Structure of porcine pancreatic alpha-amylase
1hny	The structure of human pancreatic alpha-amylase at 1.8 angstroms resolution
1m1j	Crystal structure of native chicken fibrinogen with two different bound ligands
1nu6	Crystal structure of human Dipeptidyl Peptidase IV (DPP-IV)
1ogs	Human acid-beta-glucosidase
1v4t	Crystal structure of human glucokinase
1xu7	Crystal Structure of the Interface Open Conformation of Tetrameric 11b-HSD1
1y7v	X-ray structure of human acid-beta-glucosidase covalently bound to conduritol B epoxide
2jfe	The crystal structure of human cytosolic beta-glucosidase
2oox	Crystal structure of the adenylate sensor from AMP-activated protein kinase complexed with AMP
2p8s	Human dipeptidyl peptidase IV/CD26 in complex
2zj3	Isomerase domain of human glucose:fructose-6-phosphate amidotransferase
3ctt	Crystal complex of N-terminal Human Maltase-Glucoamylase with Casuarine
3k35	Crystal Structure of Human SIRT6
3l2m	X-ray Crystallographic Analysis of Pig Pancreatic Alpha-Amylase with Alpha-cyclodextrin
3no4	The crystal structure of the alpha-glucosidase (family 31) from Ruminococcus obeum ATCC 29174
3wy1	Crystal structure of alpha-glucosidase
4acd	GSK3b in complex with inhibitor
4y14	Structure of protein tyrosine phosphatase 1B

Selection of receptors: The receptors were chosen in light of their capacity in the pathway of diabetes. The 3D structure of 1DHK, 1HNY, 1M1J, 1NU6, 1OGS, 1V4T, 1XU7, 1Y7V, 2JFE, 200X, 2ZJ3, 3CTT, 3K35, 3L2M, 3NO4, 3W37, 3WY1, 4ACD, 4J5T, 4Y14 and 5TD4. The receptors selected for present study have appeared in Table 2. The 3D structures of these receptors were accessible in their local shape in PDB database. The 3D directions of the receptors were obtained from PDB database. To verify the capacity of the model in reproducing experimental observation with a new ligand, all these structures were analyzed again at the binding site.

Docking simulations: The iGEMDOCKv2.1 was employed for binding affinity measurement between selected ligands and targeted proteins of diabetes. The content of configure file was determined as position of receptor file and ligand file.

ADME TEST: ADME/Toxicity parameters compliance was evaluated by screening through ADMET-SAR, a commercial tool. The ADMET-SAR is system pharmacology or system chemical biology and toxicology platform designed for the assessment of would be therapeutic indications, off target effects and potential toxic end points of natural products. In the studied work, this database/tool was used to predict and evaluate the human metabolism compliance, toxicity risk assessment and mode of action by using standard experimental data.

Statistical analysis: Analysis of variance two- way (ANOVA) of SPSS2 (Statistical Package for the Social Sciences) (MRX version) was used to determine the significance of difference between treatment groups (<0.05). Means between treatment groups were compared for significance using Duncan's new Multiple Range post-test[22].

RESULTS AND DISCUSSION

From qualitative phytochemical analysis of *Penicillium* species methanol extract exhibited potent bioactive compounds. The *Penicillium* species have shown the bioactive phytochemicals such as phenols, flavonoids, terpenoids, tannins, carbohydrates, alkaloids and saponins. Similar results were reported by Sharma *et al.*[31] from *Pestalotiopsis neglecta* and Bhardwaj *et al.*[25] from *Penicillium frequentans*.

The partially purified crude extract of *Penicillium* species was subjected to GC-MS analysis. Total 18 compounds were identified based on retention time and area percentage, molecular formula and weight were identified (Table 1, Fig. 1). The highest amount of 1,2-Benzenedicarboxylic acid, diisooctyl ester was noticed in GC-MS as a high peak. The endophytic fungi, *Colletotrichum gloeosporioides* of *Phlogacanthus thyrsiflorus* have yielded the phenol, 2,4-bis(1,1-Dimethyl ethyl), 1-Hexadecane, 1-Hexadecanol, hexadecanoic acid, octadecanoic acid methyl ester and 1-nonadecane[26]. Bis(2-ethylhexyl) phthalate, Pentanoic acid, Melamine, 4H-Pyran-4-one, 2,3-Dihydro-3,5-dihydroxy-6-methyl-, Dodecane, Nonadecane, 5-Hydroxymethylfurfural, 1,2,3-Propanetriol, 1-Acetate, Heptose, Triacetin, 2,3-Dihydroxypropanal, 1-Cycloheptene, D-Allose, Pentadecane, 1,5-Anhydrohexitol, 3-Deoxy-D-mannoic lactone, Tetradecane, Heneicosane, 4-Oxo-, 1,2-Benzenedicarboxylic acid and

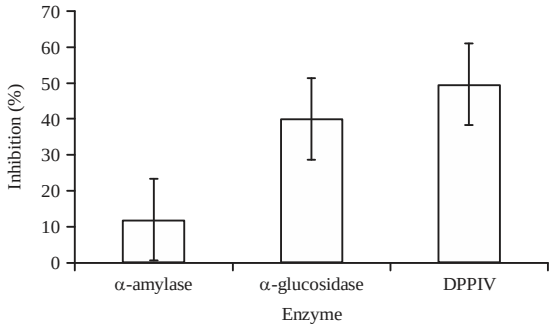

Fig. 2: Enzymes inhibition assay of *Penicillium* species extract
The percent inhibition of α-amylase, α-glucosidase and DPP IV by *Penicillium* species methanol extract. The values followed by Mean+SEM remained significantly different at $p \leq 0.05$

Bis (2-ethylhexyl) phthalate[31]. Papitha *et al.*[32] have identified the similar bioactive compounds from the plant, *Tinospora cordifolia*. Many microbes have shown that the secondary metabolites have the ability to bind with active sites and enzymes, receptors and proteins. Phthalic acid, methyl-octyl ester and Bis (2-ethylhexyl) phthalate were identified in the extract and have reported as antimicrobial agents[26,31,32]. Authors have found Phenol, 2,4-bis(1-phenylethyl)-[26] and Phenol, 2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl)methyl]-[33] from endophytic fungi, *Colletotrichum gloeosporioides* and *Fusarium solani* of different plants. The Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)-, R-(1. alpha.,4a. beta.,7. beta.,8a. alpha.)]- were identified from *Phoma herbarum*[34] and *Aquilaria sinensis*[35]. The octadecanoic acid methyl ester was identified from fungal endophytes of *Ocimum sanctum* and exhibiting many biological activities[36]. The results confirm that *Penicillium* species produce important secondary metabolites and which are exhibiting many biological activities. Comparing to earlier reports, the study supports the evidence that bioactive compounds produced by fungal endophytes may not be involved in the host-endophytic relationship but may also have industrial applications. The endophytic fungal species are exploiting for their important bioactive compounds and they having novel medical applications. The results of present study confirms that endophytic fungal species are able to produce biologically important medicinal bioactive compounds. Hence, further studies are required to explore the secondary metabolites of *Tabebuia argentea* and its endophytic fungal species and they can be used for management of different diseases.

In vitro antidiabetic activity, the extract of *Penicillium* species has potentially inhibited the activity of α-amylase, α-glucosidase and dipeptidyl peptidase IV listed in Fig. 2. The obtained result clearly indicates that, the inhibition of enzymes is concentration dependent. The α-glucosidase was inhibited more by bioactive compounds of endophytic fungal species and the result was less than positive control standard drug acarbose. Similar results were reported by many scientist using endophytic extracts against α-glucosidase[37-39]. The same extract was inhibited the activity of α-glucosidase at maximum level compared to standard drug acarbose. The extract inhibited the activity of DPP-IV and it was lower than standard drug diprotin as a positive control. No reports on endophytic fungal extracts showing the inhibitory action of dipeptidyl peptidase IV. The present study is the first study on inhibition of dipeptidyl peptidase IV using fungal extract. There are some reports say that, the plant extracts have the ability to inhibit the activity of dipeptidyl peptidase IV[40-42].

The results concluded that, the endophytic fungal extract has shown potent antidiabetic activity by *in silico* assay. Molecular docking was performed on 21 different diabetic target proteins and with all 18 endophytic bioactive compounds using iGEMDock2.1. The binding interactions of these ligands with target proteins were selected on the basis of binding energy or total energy, VDW and hydrogen bonding interaction. These values along with the hydrogen bond forming residues are presented in Table 3. From the analysis, the pancreatic α-amylase has shown more interaction with octadecanoic acid methyl ester followed by Di-isooctyl phthalate, Bis-2 ethylhexyl phthalate, Phenol 2,6, bis-2 hydroxy-5-methyl Benzenedicarboxylic acid. The octadecanoic acid methyl ester binds with the amino acids of human pancreatic α-amylase followed by Diisooctyl phthalate, 2,4-Bis(1-phenylethyl)phenol, Phenol 2,6, bis-2 hydroxy-5-methyl. Native chicken fibrinogen amino acids more interact with Diisobutyl isophthalate followed by Methoxymellein, 5-diethylamino, Methyl palmitate. Out of 18, 7 bioactive compounds able to inhibits the Human Dipeptidyl Peptidase IV by interacting with different amino acids. The octadecanoic acid methyl ester is able to interact with DPP-IV enzyme followed Diisooctyl phthalate, Phenol 2,6, bis-2 hydroxy-5-methyl, Dimethyl phenol, Bis-2ethylexyl phthalate, Benzenedicarboxylic acid, 2,4-Bis(1-phenylethyl)phenol (Table 3) (Fig. 3).

The Diisooctyl phthalate have showed highest binding affinity with human β-glucosidase followed by octadecanoic acid methyl ester, Phenol 2,6-bis-2 hydroxy-5-methyl, Benzenedicarboxylic acid, Diisobutyl isophthalate, Dimethyl phenol, Methoxymellein and Bis-2ethylhexyl phthalate. The octadecanoic acid methyl ester is able to interact with human glucokinase enzyme with more binding energy followed by Phenol 2,6-bis-2 hydroxy-5-methyl, Dimethyl phenol, Diisooctyl phthalate and Methyl palmitate.

Table 3: *In silico* anti-diabetic activity of *Penicillium* species phytochemicals

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
2,4-Bis (1-phenylethyl)phenol				
1dhk	-80.58	-73.58	-7.00	Gln5, gln5, gln5, thr6, gln7, ser8
1hny	-86.38	-86.38	0.00	tyr67, tyr67, glu181, tyr182, his185
1m1j	-80.53	-70.06	-10.47	Pro69, ser70, gly98, arg137, tyr68, tyr68, lys72, gln73, gln73
1nu6	-86.47	-79.79	-6.69	Glu699, gln761, his754, his757, his757, glu699, asp729, asp729, gln731
1ogs	-77.03	-70.47	-6.56	Ala1, pro3, ala1, arg2, pro3, ser25, phe26, asp27
1v4t	-79.57	-76.57	-2.50	Asp363, phe316, his317, his317, glu319, arg358, arg358, pro359
1xu7	-93.89	-78.21	-15.68	Gly41, ser43, ser43, lys44, lys44, ala65, arg66, his120, his120, ile121
1y7v	-81.49	-75.60	-5.89	Gln362, his365, arg285, leu314, asp315, phe316, ala318, pro319, gln362, his365
2jfe	-78.81	-68.43	-10.37	Arg312, phe225, val227, his250, phe334, trp345
2oox	-86.20	-78.15	-8.04	Phe529, asp250, his453, arg457, arg459, cys528, phe529
2zj3	-75.01	-71.51	-3.50	Gly508, glu504, glu504, leu507, arg511, his638, ser639
3ctt	-91.30	-85.30	-6.00	Gly258, asp495, asn100, arg108, asp163, leu463, tyr494, asp495
3k35	-89.43	-77.64	-11.79	Asp61, his66, gly67, thr55, pro60, asp61, his66, his66, lys79, tyr255
3l2m	-82.32	-76.32	-6.00	Gln5, gln5, gln5, gln5, thr7, ser8
3no4	-88.10	-85.80	-2.31	Phe330, pro340, gln344, lys346, gln344, lys346
3w37	-79.98	-74.00	-5.98	Gly288, arg103, arg103, asp191, tyr521, asn522
3wy1	-83.91	-78.86	-5.05	Ala349, his348, his348, lys352, asn443, asn443, his515, his515, phe516
4acd	-75.70	-73.20	-2.50	Pro294, ser66, phe67, phe67, leu88, gly202
4j5t	-81.31	-77.22	-4.09	Asp690, asp690, asp690, asn754, asn757, asn757, asn758, lys761
4y14	-78.59	-73.66	-4.93	His208, gln78, arg79, arg79, ser80, pro206, pro210
5td4	-90.04	-75.92	-14.12	His305, ala307, gly308, gly309, arg303, gly304, his305, gly306, gly308, gly309, phe348
Di methyl				
1dhk	-62.81	-55.81	-7.00	Arg421, thr11, pro332, arg398, asp402
1hny	-65.49	-62.08	-3.41	Lys178, tyr67, tyr67, glu181, tyr182, his185
1m1j	-74.10	-65.60	-8.50	Thr21, thr22, thr22, pro20, thr21, thr21
1nu6	-61.87	-54.87	-7.00	Arg356, arg382, arg356, arg358, ser360, ile374
1ogs	-70.13	-67.63	-2.50	Asp24, arg2, pro3, asp24, ser25, phe26, met49
1v4t	-60.65	-54.03	-6.62	Arg327, lys296, tyr297, gly299, glu300, gly328
1xu7	-71.17	-64.17	-7.00	Arg252, gln21, arg198, val208, ser209, ser209, ile210, arg252, glu254
1y7v	-68.00	-65.50	-2.50	Asp24, arg2, pro3, asp24, ser25, phe26
2jfe	69.09	-62.09	-7.00	His120, trp425,phe121, glu165, phe225, glu165, phe225, tyr309, trp345, trp417, glu424, trp425
2oox	-73.59	-70.09	-3.50	Ala276, phe574, asn269, asn269, val274, phe296, glu92, glu92
2zj3	-69.14	-54.82	14.32	Ser420, gln421, ser422, ser422, thr375, gln421, gln421
3ctt	-69.98	-69.98	0.00	Val184, pro206, leu540, leu540, trp552
3k35	-64.62	-53.57	-11.05	Asp61, his66, gly67, pro60, his66, his66
3l2m	-63.12	-59.83	-3.30	Gly112, ile49, val51, thr52, ala108, gly112
3no4	-70.47	-63.47	-7.00	Tyr558, glu559, tyr558, glu559, arg565, arg565, tyr566
3w37	-74.13	-67.07	-7.06	Gly700, tyr659, arg699, asn758, gly791, glu792, glu792
3wy1	-61.02	-57.52	-3.50	Arg456, arg457, arg457, his459, pro460, phe463, phe463
4acd	-70.32	-66.82	-3.50	Gly262, arg223, ser261, gln265, arg223, gln265
4j5t	-71.94	-68.06	-3.89	Tyr66, pro59, pro59, tyr66, tyr768, his785
4y14	-63.83	-53.55	-10.28	Glu75, glu76, ala77, met74, glu76, val249, glu252
5td4	-97.29	-54.63	-12.66	Arg195, asp197, his299, asn300, trp58, tyr62, asp197, asn300

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
5-diethylamino				
1dhk	-76.41	-44.63	-31.78	Arg195, asp197, glu233, his299, asp300, tyr62, leu162, asp197, glu233, asp300
1hny	-70.90	-51.18	-19.71	Gly309, ala310, ile312, thr314, arg303, gly304, ala310, ile312
1m1j	-84.95	-64.89	-20.06	Cys81, cys46, thr22, ser48, gly49, cys46, pro47, ser48, thr22
1nu6	-75.89	-65.90	-9.98	Pro475, val558, ser511, lys512, lys512, ile529, phe559, arg560
1ogs	-83.81	-72.13	-11.67	Thr30, tyr40, phe31, phe31, tyr40, phe426, leu493
1v4t	-83.10	-65.81	-17.29	Phe23, arg377, his380, phe23, gln24, gln24, ser373, thr376, thr376, his380
1xu7	-87.06	-62.06	-25.00	Asn127, leu128, phe129, his130, asp132, ala181, ser125, asn127, asn127, leu128, his130, his135
1y7v	-76.97	-68.72	-8.25	Asp24, arg2, pro3, asp24, ser25, phe26, arg49, tyr418
2jfe	-71.39	-42.50	-28.89	Gln17, his120, glu373, trp417, glu424, trp425, phe121, trp345, trp417, trp425
2zj3	-85.47	-59.43	-26.04	Cys373, ser420, gln421, ser422, ser422, thr425, thr428, ser420, gln421, gln421
3ctt	-80.28	-54.57	-25.71	Trp391, asn393, asn393, asp414, asn417, val487, trp391, gln488, his489, his489
3k35	-85.74	-69.64	-16.10	Gln111, his131, phe62, gln111, gln111, his131
3l2m	-84.08	-71.69	-12.39	Tyr155, asp159, cys160, lys142, thr143, asp153, tyr155, tyr155, gln156, gln156
3no4	-78.97	-67.83	-11.14	Arg58, leu435, asn57, leu430, leu430, asn431, lys433
3w37	-73.27	-57.05	-16.22	Trp229, asp232, asn496, ser505, lys506, ala231, ile233, lys506, lys506
3wy1	-76.55	-57.56	-18.99	Asp48, arg456, arg457, arg456, arg457, arg457, phe463
4acd	-80.50	-65.63	-14.87	Val208, glu211, asn213, leu207, arg209, arg209, gly210, asp233, tyr234
4j5t	-73.19	-43.32	-29.87	Leu53, arg54, his55, phe56, asp61, phe56, th58, arg209
4y14	-70.28	-51.19	-19.09	Gly93, glu136, asp137, asp137, ile134, phe135, phe135
5td4	-72.48	-50.56	-21.92	Asp236, ser244, ser244, ser245, phe286, val287, val287
Methoxymellein				
1dhk	-70.02	-58.22	-11.80	Gly334, arg398, pro332, gly334, arg398, asp402
1hny	-79.91	-56.49	-23.41	Ser3, gln7, gly9, asp402, pro4, thr6, gln8, arg10, asp402
1m1j	-91.86	-78.96	-12.90	Cys46, cys81, pro47, ser48, gly49, ser48, ser48, gly49, cys81
1nu6	-82.97	-66.11	-16.86	Arg125, arg125, asp709, asn710, gly741, trp124, arg124, arg125, glu205, asn710, asp739, his740, his740
1ogs	-86.90	-72.70	-14.19	Ser38, his451, ser455, his495, phe31, phe31, leu493, leu493, trp494, his495
1v4t	-78.09	-73.17	-4.92	Arg422, gln24, gln24, glu372, ser373, thr376
1xu7	-92.10	-75.24	-16.86	Met93, glu94, thr122, arg66, glu94, his120, ile121, thr122, val142
1y7v	-79.36	-74.43	-4.92	Ser26, pro3, asp24, ser25, phe26, met49, glu50
2jfe	-70.56	-61.30	-9.26	Asn319, glu323, gln317, glu323, gln328, gln328, asp329, asp329, ile332
2oox	-88.79	-71.30	-17.49	Gly244, thr270, gln271, ala95, thr270, thr270, leu272, gly273, val274, phe296
2zj3	-77.73	-64.47	-13.26	Gln421, ser422, ser376, ser420, gln421
3ctt	-81.85	-63.66	-18.19	Asp667, his669, gln670, lys715, val668, val668, his669, gln670
3k35	-85.48	-63.07	-22.41	Asp61, phe62, arg63, gly64, thr213, ala51, asp61, arg63, ser214, gln240
3l2m	-79.56	-64.13	-15.43	Tyr62, his101, asp197, trp58, trp59, tyr62, tyr62, val163, asp197
3no4	-92.75	-69.81	-22.94	Asp544, arg565, asp544, tyr558, glu559, arg565, tyr565, tyr566, tyr566, tyr566
3w37	-85.41	-68.78	-16.63	Asp232, asn496, ala231, ile233, asn496, ser505, lys506, lys506
3wy1	-91.41	-61.83	-19.58	His348, lys352, gln438, thr445, thr445, leu348, leu433, asp441, ala444
4acd	-74.75	-58.76	-15.99	Arg209, gly210, glu211, val208, arg209, arg209, gly210, asp233, asp233, asp233, tyr234
4j5t	-71.56	-64.20	-7.35	Asn129, asp202, lys203, val205, val205, trp206, glu435
4y14	-81.03	-69.47	-11.56	Arg79, his208, gly209, pro210, arg79, arg79, ser80, leu204, ser205, pro206, pro206, po210
5td4	-75.7	-62.13	-13.57	Lys200, glu233, ile235, tyr151, leu162, ala198, lys200, his201, glu233, ile235, ile235

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
16				
1dhk	-67.61	-67.61	0	Asn393, trp396, lys457, his491
1hny	-57.44	-57.44	0	Pro4, thr6, thr6, gln8, arg10, thr11, asp402
1m1j	-79.49	-79.49	0	Thr21, thr22, thr83, thr21, thr22
1nu6	-64.28	-64.28	-0	Gly424, pro426, lys523, lys523, gln586, gln586
1ogs	-71.54	-71.54	0	Ala1, arg2, pro3, ser25, phe26, met49
1v4t	-59.71	-59.71	0	Asp78, arg85, arg85, val86, met87, his105
1xu7	-75.97	-75.97	0	Ala65, arg66, his120, ile121, thr122, val142
1y7v	-69.79	-69.79	0	Arg2, pro3, ser25, phe26, phe26, met49
2jfe	-60.32	-60.32	0	Ala246, his256, ile352, phe334
2oox	-73.22	-73.22	0	Glu92, ala268, asn269, asn269, thr270, val274, phe296
2zj3	-61.63	-61.63	0	Gly613, arg614, arg614, pro615, lys631, lys631, arg632
3ctt	-69.41	-69.41	0	Arg29, tyr46, val77, phe78, lu160, phe165
3k35	-62.23	-62.23	0	Arg63, arg63, gly212, thr213, ser214
3l2m	-66.12	-66.12	0	Trp58, trp58, tyr62, tyr62, val163
3no4	-71.56	-71.56	0	Ala237, pro278, phe289, asn309, asn381
3w37	-62.60	-62.60	0	Arg773, arg773, ser774, ser774, gln839, arg840, trp841
3wy1	-65.64	-65.64	0	Asp510, leu511, pro512, thr517, ala518, phe534
4acd	-60.82	-60.82	0	His173, his173, ser236, thr330
4j5t	-59.38	-59.38	0	Lys203, gly433, glu435
4y14	-68.11	-68.11	0	Tyr46, phe182, arg221, gln262
5td4	-59.60	-59.60	0	Tyr2, leu211, leu211, lys227, pro228, ile230
18				
1dhk	-60.12	-54.25	-5.86	Asn3, leu237, ala260, lys261, asp21, asp21
1hny	-61.74	-58.24	-3.50	Lys178, tyr67, tyr67, val129, glu181
1m1j	-67.82	-65.78	-2.04	Gly49, pro82, thr83, cys46, pro47, ser48, thr22
1nu6	-62.15	-53.65	-8.50	Arg596, asp678, ile319, gln320, asn321, arg596, gly572
1ogs	-75.11	-64.61	-10.50	Arg285, trp312, trp312, tyr313, tyr313, phe316, leu317
1v4t	-68.03	-56.85	-11.19	Gly299, thr332, thr332, lys296, tyr297, glu300, glu300, arg327, gly328, glu331
1xu7	-63.10	-56.10	-7.00	Arg252, gln21, asn207, val208, ser209, arg252
1y7v	-62.94	-59.09	-3.85	Pro3, ser25, phe26, met49, glu50
2jfe	-60.63	-60.63	0.00	Phe121, tyr309, glu373, trp417, glu424, trp425
2oox	-73.54	-60.57	-12.97	Thr270, gln271, leu272, asn269, asn269, val274, phe296
2zj3	-67.80	-53.62	-14.18	Ser420, gln421, ser422, ser422, gln421, gln421
3ctt	-72.40	-68.09	-4.32	Met567, ala285, phe522, phe535, ala536, ala537
3k35	-74.24	-60.10	-14.14	Asp61, arg63, gly64, ala51, phe62, phe62, arg63, thr213, ser214
3l2m	-65.05	-65.05	0.00	Ile453, ile465, ile479, glu484, glu484, asp485, asp485, ile488
3no4	-68.24	-64.74	3.50	Gln545, leu569, phe606, phe606
3w37	-66.99	-60.99	-6.00	Tyr659, gly700, tyr39, arg699, arg699, asn758
3wy1	-65.17	-63.73	-1.44	Asp510, leu511, pro512, phe516, ala518, ala522, phe534
4acd	-65.92	-61.63	-4.29	Gly262, arg223, ser261, arg223
4j5t	-61.42	-54.67	-6.75	Tyr66, gly383, pro59, pro59, phe384, tyr768, glu784
4y14	-71.78	-60.11	-11.67	Cys215, arg221, arg221, tyr46, asp181, phe182, ser216, ala217, arg221
5td4	-59.71	-54.17	-5.55	Asn5, pro4, thr6, gln7, gln8, gln8, arg10

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
Eremophilane				
1dhk	-67.38	-67.38	0	Lys35, asn393, trp396, lys457
1hny	-57.95	-57.95	0	Pro4, thr6, thr6, gln8, thr11, phe335, asp402
1m1j	-68.61	-66.11	-2.5	Thr21, pro20, thr21, thr83, pro20
1nu6	-68.44	-59.97	-8.47	Arg596, asp678, asn321, arg596, gly572
1ogs	-71.20	-71.20	0	Pro3, ser25, phe26, arg48, met49, glu50
1v4t	-58.91	-58.91	0	Asp78, arg85, val86, met87, his105
1xu7	-65.72	-65.72	0	Gly41, lys44, lys44, asn119, his120, ile121
1y7v	-72.24	-72.24	0	Pro3, ser25, phe26, met49, glu50
2jfe	-60.38	-60.38	0	Phe179, phe225, trp345, glu424, asn426, gln427
2oox	-75.54	-75.54	0	Asn269, asn269, glu92, gly92
2zj3	-59.46	-59.46	0	Val609, arg614, arg614, pro615, thr630, lys631, lys631, arg632
3ctt	-70.28	-70.28	0	Ala285, leu286, leu286, arg520, his645, lys776, lys776, asp777
3k35	-72.70	-72.70	0	Ala51, phe62, arg63, gln111, his131
3l2m	-65.08	-65.08	0	Trp396, val457, lys457, lys457, his491, glu493
3no4	-75.39	-75.39	0	Pro278, phe289, asn306, phe378, asn381
3w37	-61.09	-61.09	0	Lys421, lys421, met513, his514, tyr515
3wy1	-64.03	-64.03	0	Asp510, leu511, pro512, val513, phe516, thr517, ala518, phe534
4acd	-65.52	-65.52	0	Arg223, gln265, arg223
4j5t	-56.99	-56.99	0	Ser317, ile318, asn757, asn757, tyr760, lys761, lys761, glu764
4y14	-60.07	-60.07	0	Arg79, ser80, ser80, leu204, ser209, pro210, pro210
5td4	-57.55	-57.55	0	Trp58, trp59, trp59, tyr62, tyr62, asn300
Benzenedicarboxylic acid				
1dhk	-87.56	-73.29	-14.27	Ser270, ser26, ser26, trp269, ser270, ser270, lys13, ser25, ser26, gln31
1hny	-78.32	-71.32	-7.00	Gln63, trp59, tyr62, gly104, ala106, leu165
1m1j	-77.74	-69.95	-7.80	Ser48, val79, trp34, ser48, ser48
1nu6	-88.83	-84.16	-4.67	Arg356, glu347, arg356, arg356, arg358, ser360, ile374, ile375, ile375, ser376
1ogs	-92.60	-88.09	-4.52	Cys4, arg2, pro3, ser25, phe26, arg48, met49, glu50
1v4t	-82.61	-60.96	-21.65	His317, his317, ser360, asp363, phe316, his317, pro359, se360
1xu7	-95.43	-83.56	-11.87	Tyr257, arg269, asn270, glu254, glu255, tyr257, arg269, arg269, asn270, arg273, lys274
1y7v	-93.40	-86.11	-7.28	Cys4, arg2, pro3, cys4, pro6, ser25, phe26, gly46, arg48, aarg48, met49
2jfe	-80.27	-76.77	-3.50	Arg312, glu165, phe225, arg312, phe334, trp345
2oox	-77.01	-70.03	-6.98	Asp250, asp250, val27, leu28, pro29, val160, arg165
2zj3	-78.96	-70.76	-8.20	Ser454, his462, arg342, gly354, cys459, gly460, val461
3ctt	-77.53	-75.73	-1.80	Ala285, ala509, glu510, arg520, phe535, phe535, lys770
3k35	-78.99	-67.02	-11.97	Trp188, trp186, leu184, asp185, trp186, trp186
3l2m	-79.05	-73.14	-5.91	Trp388, thr376, thr377, trp388, trp388, gln390
3no4	-86.80	-74.14	-12.66	Ser499, ser499, asn502, ser499, asn502, arg506, pro580, gly582, ala618
3w37	-87.45	-75.78	-11.67	Arg840, ser774, thr775, gln839, arg840, arg840, trp841, trp841
3wy1	-93.65	-77.45	-16.50	His348, lys352, thr445, his348, gln438, asn443, ala444
4acd	-81.76	-77.19	-4.57	Arg209, val208, arg209, arg209, gly210, glu211, asp233, asp233, tyr234
4j5t	-87.48	-70.48	-17.46	Gln308, thr494, asn495, arg304, met463, thr494, asn495, asn495
4y14	-86.98	-78.73	-8.25	Arg79, ser80, arg79, arg79, ser80, ser205, pro206, pro206
5td4	-79.32	-72.56	-6.76	Gln63, trp59, tyr62, gly104, ala106, leu165

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
Bis-2ethylhexyl phthalate				
1dhk	-92.46	-83.94	-8.53	Ser270, trp269, trp269, ser270, thr23, val24, ser25, ser26, ser26
1hny	-80.56	-80.56	0.00	Tyr67, glu181, glu181, asn184, asn184, his185, his185, his215
1m1j	-83.71	-80.86	-2.85	Lys138, gln134, arg137, lys138, tyr68, tyr68, lys72, gln73, gln73
1nu6	-89.57	-89.57	0.00	Glu452, arg453, arg453, pro475, gly476, gly476, ser511, lys512, lys512, phe559
1ogs	-85.12	-76.81	-8.31	Ala1, asp27, pro3, cys4, arg48, met49, glu50, leu51, leu51
1v4t	-77.52	-62.98	-14.54	Asp217, his218, his218, gln219, his218, his218, gln219, arg403
1xu7	-84.26	-70.97	-13.29	Arg252, gln21, asn162, asn162, asn207, asn207, arg252
1y7v	-84.09	-76.45	-7.64	Ala1, ala1, arg2, pro3, pro3, phe26, phe26, asp27, arg262
2jfe	-80.75	-72.56	-8.20	Arg445, val51, val51, tyr429, tyr429, phe440, pro443, pro443, arg445
2oox	-80.30	-80.30	0.00	Leu272, phe42, lys44, lys99, lys99, leu272, lys44
2zj3	-86.64	-81.98	-4.67	His462, thr448, thr448, val449, his462, asn464, gly466, pro467, pro467
3ctt	-87.24	-84.23	-3.01	Phe109, arg108, arg108, phe109, phe109, glu110, asp495
3k35	-100.24	-98.37	-1.87	Phe62, his131, leu184, leu184, ile217
3l2m	-83.64	-76.19	-7.46	Trp59, asp356, trp59, gln63, val163, val354, trp357
3no4	-85.27	-80.77	-4.50	Leu600, glu46, arg63, arg563, arg563, asp597, ala599, leu600, leu600, asn601
3w37	-74.11	-74.11	0.00	Glu301, thr681, thr681, arg699, his755, glu756, gly757, val787
3wy1	-93.07	-85.72	-7.35	Pro230, lys225, leu227, ala229, pro230, glu231, asn301, tyr389, arg400
4acd	-101.80	-93.48	-8.31	Asn301, gly228, ala220, pro230, glu231, phe297, asn301, met302, asp333, asp333, val334
4j5t	-96.06	-84.50	-11.56	Asn471, his804, his805, asn471, asn471, arg801, his803, his804, his805, his806, his806, his807
4y14	-80.31	-85.33	-4.98	Ser80, lys73, glu75, ser80, gln102, pro206, his208, gly209, pro210, pro210
5td4	-82.53	-81.66	-0.88	Gln302, arg303, gly304, arg346, phe348, asn352, asp353
Diisobutyl isophathalate				
1dhk	-82.76	-74.75	-8.01	Val135, ser197, asn65, asn65, phe66, glu134, glu134
1hny	-72.19	-58.01	-14.19	Ser132, asp135, lys172, pro130, tyr131, tyr131, trp134, asp135, lys172, tyr174
1m1j	-94.06	-90.24	-3.82	Ser48, ser48, ser48, gly49, val79, pro47, ser48, ser48, gly49
1nu6	-84.06	-73.06	-11.00	Ser349, thr351, aso588, his592, met348, ser349, thr351, ser376, ser376, glu378, glu379, gly380, asp588
1ogs	-89.69	-82.50	-7.19	Cys4, arg2, pro3, cys4, asp24, ser25, phe26, arg48, glu50
1v4t	-82.94	-71.57	-11.37	Cys230, gly258, gln287, gly229, asn231, glu256, glu256, gln287, glu290
1xu7	-82.55	-68.30	-14.25	Lys174, tyr257, asn270, arg273, lys174, glu174, glu255, asn270, arg273, lys274
1y7v	-86.40	-82.90	-3.50	Cys4, pro3, cys4, phe26, arg48, met49, glu50, glu50
2jfe	-80.20	-67.20	-13.00	Arg432, asp439, phe440, glu441, gln47, asp380, tyr429, phe440, glu441
2oox	-99.07	-89.91	-9.16	Thr270, gln271, ala95, val570, phe574, asn269, gln271, leu272, gly273, val274, leu274, leu275, phe296
2zj3	-70.76	-59.01	-11.75	Thr425, ala426, asp427, val677, cys426, asp427, val677, glu680
3ctt	-77.66	-68.16	-9.50	Asp649, arg653, tyr636, arg649, asp649, arg653, pro676, glu767
3k35	-95.59	80.94	-14.64	Arg63, arg63, phe62, phe62, gln111, hos131, ile217
3l2m	-76.03	-67.54	-8.50	Arg389, arg392, thr376, thr377, trp388, trp388, arg389, arg392
3no4	-91.75	-79.89	-11.85	Tyr525, phe526, tyr141, asn439, asn439, asp523, phe531, gly549
3w37	-83.27	-78.98	-4.29	Trp841, arg773, ser774, thr775, gln839, gln839, arg840, arg840, trp841, val842, val842
3wy1	-88.18	-81.50	-6.68	Arg400, tyr65, phe166, gly228, ala229, asp333, asp333, tyr389, phe397, arg400
4acd	-93.31	-86.94	-6.37	Pro212, asn213, leu207, val208, arg209, gly210, glu211, asp233, asp233, tyr234
4j5t	-82.16	-60.60	-21.56	Arg799, arg801, ser802, ser802, his803, arg727, arg727, arg799, phe800, arg801, ser802, his803
4y14	-77.97	-65.52	-8.56	Arg33, ser146, glu147, asp148, asp148, val155, val155, lys197
5td4	-82.58	-74.79	-7.78	Asp353, asp356, arg303, gly304, phe348, asn252, asn252, asp353

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
Diisooctyl phthalate				
1dhk	-110.85	-103.50	-7.33	Ser270, ser26, trp269, trp269, ser270, gly309, thr23, ser25, gln25, asn35
1hny	-90.84	-87.34	-3.50	Lys200, tyr151, leu162, lys200, his201, glu233, ile235, ile235, glu240
1m1j	-88.54	-76.09	-12.45	Asn266, gly268, arg419, asn266, asn266, phe267, gly268, tyr382, lys400, gly403
1nu6	-103.65	-97.92	-5.73	Asp588, glu347, met348, ser349, thr351, ile375, ser376, ser376, asp588
1ogs	-106.31	-100.60	-5.73	Pro3, cys4, arg2, pro3, cys4, asp24, phe26, arg48, met49, glu50
1v4t	-87.15	-78.83	-8.32	Val101, lys102, gly94, glu95, ser100, val101, lys102, lys458, lys459, cys461
1xu7	-91.13	-83.62	-7.62	Asn270, lys174, glu255, tyr257, arg269, arg269, asn270, pro271, arg273
1y7v	-87.52	-84.96	-2.56	His365, trp312, pro319, his365, thr369, arg463
2jfe	-75.18	-70.18	-5.18	Gln427, met178, trp345, glu424, asn426, asn426, gln427
2oox	-84.69	-70.56	-14.12	Asn230, arg260, met200, asn230, gly254, leu257, leu257, leu258, arg260, gly266
2zj3	-83.86	-78.70	-5.16	His596, arg511, arg594, his596, tyr598, ser639, val640, asp641
3ctt	-82.00	-79.81	-2.19	Ser664, ala693, tyr694, tyr694, lts715, lys715, val716
3k35	-103.84	-99.57	-5.27	His131, phe62, arg63, his131, met155, leu184, asp185, trp186, trp186, ile217
3l2m	-99.40	-90.39	-9.01	Lys200, his201, trp59, tyr151, val163, lys200, his201, glu233, ile235, glu240
3no4	-90.52	-84.02	-6.50	Arg153, gly152, arg153, tyr155, arg412, asp522
3w37	-98.38	-89.21	-9.17	Arg102, arg102, trp104, glu105, ile106, pro107, pro107, arg113, gly516
3wy1	-101.62	-86.61	-15.01	Ala514, his515, phe516, leu511, pro512, pro512, val513, ala514, his515, ala518
4acd	-85.92	-81.06	-4.86	Thr232, val208, arg209, gly210, pro212, pro212, thr232, asp233, asn287
4j5t	-92.88	-85.88	-7.00	His803, ile734, phe800, arg801, his803, his804, his806, his807
4y14	-90.64	-70.18	-20.45	Arg24, arg254, gln262, tyr20, arg24, ala27, arg254, gly259
5td4	-97.31	-81.07	-16.24	Lys322, arg389, gln390, lys322, arg343, trp388, trp388, arg389, glu484
Dimethyl phenol				
1dhk	-58.96	-53.96	-5.00	Ser270, val24, trp269, ser25, gln31
1hny	-62.16	-56.16	-6.00	Val129, lys178, tyr67, tyr67, glu181, glu181, tyr182, tyr182, his185
1m1j	-70.66	-62.16	-8.50	Cys46, cys81, thr21, thr22, pro82, thr83
1nu6	-92.88	-89.37	-3.51	Thr365, thr365, leu366, asn369, tyr388, thr411, ser412
1ogs	-86.13	-80.82	-5.31	Leu51, cys4, phe26, arg48, glu50, leu51, leu51, arg211
1v4t	-90.13	-84.63	-5.50	Arg422, gln22, gln24, leu25, gln26, glu27, arg369, glu372, thr376, his380
1xu7	-65.53	-56.03	-9.50	Thr92, met93, glu94, arg66, his120, ile121
1y7v	-64.34	-55.51	-8.83	Arg496, gln497, asp453, asp453, gly454, his495, gln497, gln497
2jfe	-95.45	-90.85	-4.60	Gln317, asn319, lys321, glu323, ile326, gln328, asn329, asn329
2oox	-71.94	-59.10	-12.85	Asp245, tyr246, ser247, asn269, asn269
2zj3	-60.86	-47.32	-13.55	Gln421, ser422, ser422, thr425, gln421, gln421
3ctt	-69.62	-56.55	-13.07	Arg520, thr775, thr778, leu286, leu286, arg520, his645, lys776, lys776, asp777
3k35	-71.66	-63.39	-8.27	Asp61, gly64, gln240, asp61, phe62, arg63, thr213, ser214, ser214
3l2m	-62.12	-52.92	-9.20	Ser199, lys200, lys200, his201, glu233, ile235
3no4	-68.57	-58.49	-10.07	Asn561, asn61, glu543, trp425, gln560, gln560, arg58, arg58, gly59
3w37	-63.25	-56.54	-6.71	Gly700, tyr659, arg699, gly791, glu792
3wy1	-65.32	-52.84	-12.49	Asn241, asn241, asn495, gly498, asp439, asp539, leu240, asn495
4acd	-62.04	-50.19	-11.85	Phe293, gln295, arg92, arg278, lys292,lys292, phe293, phe293
4j5t	-55.87	-49.64	-6.23	Gln358, glu361, glu361, ile362, ile362, gly556
4y14	-66.05	-63.55	-2.50	Tyr46, tyr46, asp181, phe182, ala217, arg221
5td4	-61.84	-49.47	-12.37	Arg398, arg421, arg398, asp402

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
Methyl palmitate				
1dhk	-81.60	-81.60	0.00	Ala260, lys261, trp269, ser25, ser26, gln31
1hny	-67.45	-58.22	-9.22	Arg392, trp388, trp388, arg389
1m1j	-90.30	-86.80	-3.50	Gly84, gly49, cys81, thr83, pro47, ser48
1nu6	-70.74	-61.74	-9.07	Asp192, tyr195, gln123, asn151, trp168, glu191, tyr195
1ogs	-82.90	-80.40	-2.50	Tyr40, cys4, phe26, asp27, tyr40, arg48, glu50, leu51
1v4t	-85.13	-75.38	-9.75	Gly258, ala259, gln287, gly227, gly229, cys230, asn231, glu256, gln287, glu290, gly410
1xu7	-73.80	-65.42	-8.38	Ser170, lys187, ser169, tyr177, leu215, gly216, tyr280
1y7v	-84.54	-78.62	-5.92	Asp27, tyr40, cys4, phe26, asp27, arg48, glu59, leu51
2jfe	-77.06	-64.06	-13.00	Arg432, asp439, phe440, glu441, gln47, gln47, tyr429, phe440, phe440, glu441
2oox	-82.81	-79.45	-3.36	His453, glu92, asp98, asp98, lys99
2zj3	-66.99	-56.21	-10.78	Thr446, thr446, val461, gly444, ser454, ser454, gly460, val461
3ctt	-74.10	-69.22	-4.87	Ile523, ala285, phe522, ala536, ala537, met567
3k35	-70.97	-63.76	-7.20	Asp61, gln240, asp61, phe62, arg63, thr213, ser214, ser214
3l2m	-64.80	-59.15	-5.65	Lys142,lys142, asp153, tyr155, tyr155, gln156, gln156
3no4	-72.24	-63.37	-8.87	Tyr200, gln202, thr208, tyr200, phe204, lys205, asp206
3w37	-64.92	-55.76	-9.17	Val842, ser774, arg840, arg840, trp841
3wy1	-63.10	-50.29	-12.81	Lys398, asp401, gly402, glu377, val380, lys398, lys398
4acd	-72.60	-58.38	-14.22	Gly262, tyr216, cys218, arg223, arg223, ser261, arg223
4j5t	-68.01	-60.20	-7.80	Tyr29, ser414, tyr29, gly371, phe373, glu374, glu417
4y14	-72.33	-58.82	-13.51	Gly262, tyr216, cys218, arg223, arg223, ser261, arg223
5td4	-71.66	-61.15	-10.51	Thr376, arg392, trp388, trp388, arg389
Methyl stearate				
1dhk	-67.60	-67.60	0.00	Asn350, glu352, val354, his73, ag74, gln131
1hny	-69.62	-62.88	-6.74	Asp135, lys172, tyr118, asp125, tyr131, asp135, tyr174
1m1j	-87.36	-81.21	-6.15	Gly84, cys81, pro82, thr83, lys32, pro47, ser48
1nu6	-81.26	-74.76	-6.50	Arg125, arg125, glu205, trp627, trp629, his740, his740
1ogs	-78.33	-72.17	-6.16	Tyr313, ala318, leu341, tyr284, tyr244, gln284, tyr313, tyr313, phe316, leu317
1v4t	-83.86	-74.38	-9.48	Gly258, ala259, gln287, gly227, gly229, cys230, asn230, asn231, glu256, glu260, gly410
1xu7	-79.38	-75.88	-3.50	Lys274, arg269, asn270, pro271, pro271, lys274, arg269, asn270, pro271, lys272
1y7v	-84.87	-78.98	-5.89	Asp27, tyr40, cys4, phe26, phe26, arg48, glu50, leu51
2jfe	-71.78	-71.78	0.00	Val168, met172, phe225, ala246, phe249, his250, ile332, trp345
2oox	-83.07	-80.42	-2.65	His453, glu92, asp98, lys99
2zj3	-68.87	-68.87	0.00	Leu605, arg614, arg614, pro615, thr630, arg632
3ctt	-71.84	-66.63	-5.21	Ilr523, ala509, arg520, phe555, phe555, lys776
3k35	-70.42	-62.63	-7.79	Gln145, asp194, gln145, thr182, ser189, asp194
3l2m	-70.69	-58.66	-12.03	Arg392, thr376, thr377, trp388, trp388
3no4	-85.64	-74.54	-11.09	Arg63, arg459, phe491, arg63, arg117
3w37	-78.11	-75.21	-2.90	Arg773, arg773, ser774, thr775, gln839, arg840, trp841, val842
3wy1	-95.25	-81.48	-13.77	His105, gln170, asp202, phe147, phe166, ala229, asp333, tyr389, arg400
4acd	-79.58	-74.34	-5.25	Ser236, ser236, leu207, leu207, val208, arg209, gly210, glu211, pro212, asp233, thr235
4j5t	-66.89	-56.00	-10.89	Arg209, phe58, arg209, phe384, lys439
4y14	-70.47	-63.54	-6.93	Arg79, arg79, gln102, gln102, pro206, pro206, gly209
5td4	-71.86	-68.36	-3.50	Arg20, arg20, tyr52, arg72, ser73, gly74, glu78, ser112, ser113, thr114

Table 3: Continue

PDBs	Binding energy	VDW	H-bond	Interacting amino acids
Octadeconic acid methyl ester				
1dhk	-117.35	-93.33	-24.02	Ser55, asn362, asn362, ser55, trp44, arg346, trp367, ile358, gly359, pro361, asn362, asp381
1hny	-102.76	-83.72	-19.04	Ser3, asn5, gln8, thr84, asn5, thr6, gln7, gln7, thr84, asn88, asn220, trp221
1m1j	-104.91	-82.36	-22.55	Ser447, trp448, gly1, his2, his2, arg3, trp389, thr435, thr435, asp436,trp448, gly1, his2, his2, arg3, arg3, pro4, pro4
1nu6	-116.96	-98.72	-18.25	Tyr372, tyr386, ser412, tyr372, tyr386, asp413, asp413, tyr414, leu436, ser437, leu445, asn487
1ogs	-102.49	-86.73	-15.77	Ala438, ser439, ser465, ser465, gln440, lys441, lys441, asn442, asn442, asp443, ser464, lys466
1v4t	-105.32	-82.83	-22.49	Arg63, asn247, glu248, leu47, glu48, tyr61, glu67, glu245, glu248, glu248
1xu7	-107.04	-60.77	-46.27	Gln21, ser202, val206, asn207, val208, ile210, gln253, glu254, asn24, gln21, arg198, ser202
1y7v	-109.91	-92.24	-17.67	Arg131, tyr135, thr138, asn146, his495, thr134, asp137, asp137, thr138, thr138, pro139
2jfe	-104.37	-90.77	-13.60	Gln125, asn196, lys199, asp129, ser135, glu136, pro181, his195
2oox	-105.58	-67.15	-38.42	Trp452, leu241, thr245, gln251, arg33, leu52, asn53, arg449, trp452, asn244, asn244, arg33
2zj3	-114.57	-78.06	-36.51	Cys373, thr375, ser376, gln421, ser422, ser473, ala674, lys675, leu404, gln421, asp427, tyr434, ser676, val677
3ctt	-106.02	-74.94	-31.08	His115, arg283, arg283, his645, ser646, ala780, gln117, gln117, phe119, ser120, asn122, asn122
3k35	-111.68	-90.75	-20.94	Arg124, arg193, arg124, gln145, tyr146, arg148, arg162, thr165, glu281, gln41, thr292
3l2m	-113.67	-101.80	-11.81	Gly112, thr113, val50, thr52, trp59, gly106, ala108, val163
3no4	-107.29	-84.87	-22.42	His339,ala321, lys324, glu325, gly328,lys332, ala331, his339, gly336, his339, asp274, ala321, lys324, lys324, glu325, glu325
3w37	-98.61	-69.18	-29.44	Ala202, gln203, ala205, his206, gln219, his222, thr739, gln203, his206,gln219, gly690, tyr740
3wy1	-118.62	-87.37	-31.25	Asp344, his515, phe516, ala529, gln531, gln439, ala343, asp346, ala349, lys352, phe516, tyr530, gln631, asp440
4acd	-101.61	-78.14	-23.47	Pro346, val348, thr356, his361, thr356, phe360, phe360, pro372, ala382
4j5t	-113.49	87.00	-26.49	Glu463, glu470, gln617, asp724, arg727, ser802, his803, hlu463, lys669, lys669, arg727, tyr728, pro731, his803
4y14	-116.56	-86.31	-30.24	Arg105, arg105, glu170, ser201, ser203, his208, arg105, glu161, gln167, glu167, thr168, glu170, glu170, leu172, his208
5td4	-117.70	-96.94	-20.76	gly36, lys322, trp388, arg389, gln390, lys35, Lys35, asp377, trp388, arg389, ile396, asp456
Phenol 2,6-bis-2 hydroxy-5-methyl				
1dhk	-88.03	-80.36	-7.67	Asn35, leu237, lys257, ala260, lys261, trp269, ser311, gly20, asp21, thr23, gln31
1hny	-84.58	-65.04	-19.54	Arg343, cys378, asp381, trp382, thr377, gly379, asp381, trp382, trp388
1m1j	-87.64	-77.54	-10.10	Arg51, asp66, val79, ser48, arg51, asp66, ser48, ser48, val79
1nu6	-101.11	-87.42	-13.69	Met348, asp588, ser349, thr351, ser376, ser376, glu378, asp588, asp588, lys589, his592
1ogs	-99.39	-83.63	-15.75	Ala1, asp27, atg47, ala1, arg2, pro3, pro3, cys4, ser25, phe26, asp27, arg48, met49, glu50
1v4t	-91.98	-83.05	-8.93	Leu25, his380, gln24, gln24, leu25, gln26, glu27, glu27, ser373, thr376, thr376, his380
1xu7	-97.11	-85.75	-11.86	Thr124, ser126, ser125, asn127, asn127, asp132, his134, his135, val180
1y7v	-97.69	-84.21	-13.47	Ala1, asp27, arg48, arg2, pro3, pro3, cys4, ser25, phe26, asp27, met49, glu50
2jfe	-85.31	-70.89	-14.42	Arg203, his206, lys199, arg203, arg203, his206, met296, met296
2oox	-89.24	-80.42	-8.81	Arg33, arg449, trp452, asn244, asn244, thr245, arg33, asn53
2zj3	-84.72	-81.61	-3.11	Arg511, leu515, glu518, lys621, glu622, his638, his638, ser639
3ctt	-99.52	-81.38	-18.14	Ser47, ser139, pro158, tyr46, lys48, pro137, pro137, ser155, gly157, pro158
3k35	-88.16	-78.15	-10.01	Val256, asp257, gly52, thr55, thr55, tyr255, tyr255, val256, asp257
3l2m	-92.32	-87.14	-5.18	Asn53, ile49, val51, thr52, asn53, ala107, gly110, gly112
3no4	-89.84	-85.97	-3.87	Tyr558, tyr558, trp425, trp425, ser426, tyr558, glu79, trp425, trp425, ser426, tyr558
3w37	-91.28	-86.40	-4.89	Arg853, asp785, ser819, ser819, gly820, leu851, leu851, lys852, arg853, phe908
3wy1	-89.26	-81.92	-7.33	Asn447, asp346, pro437, his438, asp440, asp441, pro442, pro442, asn447
4acd	-86.78	-77.15	-9.62	Ser66, leu88, ser66, phe67, gly68, val87, val87, leu88, leu88, asn95
4j5t	-89.36	-82.92	-6.44	Glu402, glu463, arg467, asp724, arg727, arg727, tyr728, his803
4y14	-91.49	-83.99	-7.50	Tyr46, asp48, gln262, tyr46, asp48, val49, asp181, phe182, asg221, gln262
5td4	-88.83	-80.33	-8.50	Tyr151, his305, trp58, tyr151, lys200, his201, glu233, ile235, asn300, his305, gly306

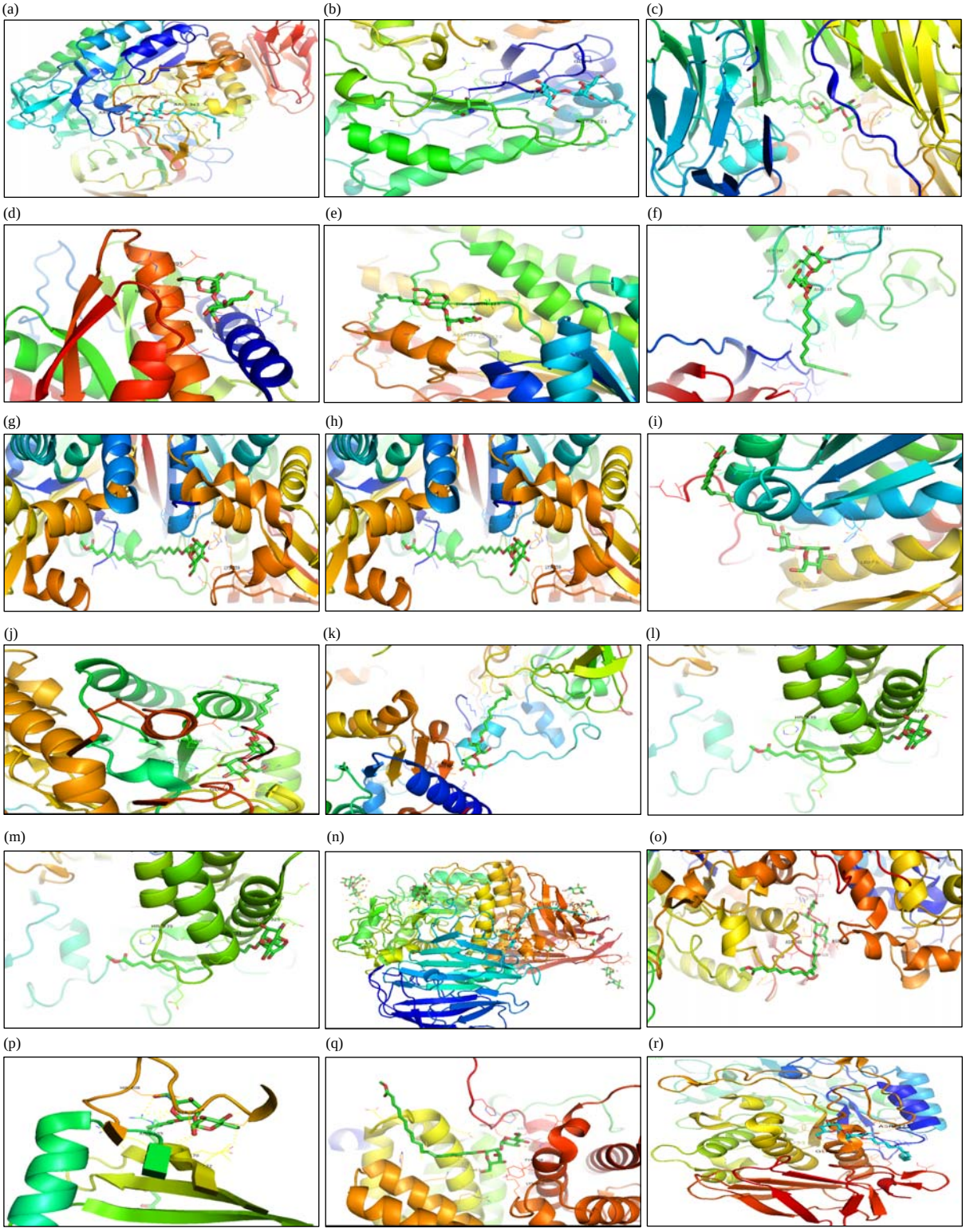

Fig. 3(a-r): Octadecanoic acid methyl ester showing interaction with diabetic enzymes and their binding energy, (a) 1dhk (-117.35), (b) 1hny (-102.76), (c) 1nu6 (-116.96), (d) 1v4t (-102.49), (e) 1xu7 (-104.07), (f) 1y7v (-100.91), (g) 2jfe (-104.37), (h) 200x (-105.58), (i) 2zj3 (-114.57), (j) 3ctt (-106.02), (k) 3k35 (-111.68), (l) 3l2m (-113.67), (m) 3no4 (-107.29), (n) 3w37 (-98.61), (o) 3wy1 (-118.62), (p) 4y14 (-116.56), (q) 4j5t (-113.49) and (r) 5td4 (-117.7)

The Human 11beta-hydroxysteroid dehydrogenase type I have shown more interaction with octadecanoic acid methyl ester followed by Phenol 2,6-bis-2 hydroxy-5-methyl, Benzenedicarboxylic acid, 2,4-Bis (1-phenylethyl) phenol, Methoxymellein, Diisooctyl phthalate, 5-diethylamino (Table 3, Fig. 3).

The octadecanoic acid methyl ester have shown more interaction with human acid-beta-glucosidase followed by Phenol 2,6-bis-2 hydroxy-5-methyl, Benzenedicarboxylic acid, Diisooctyl phthalate, Diisobutyl isophthalate, Methyl palmitate. Human cytosolic β-glucosidase firmly interact with octadecanoic acid methyl ester with high energy followed by Phenol 2,6-bis-2 hydroxy-5-methyl. The AMP activated protein kinase was more interact with octadecanoic acid methyl ester followed by Diisobutyl isophthalate, Phenol 2,6-bis-2 hydroxy-5-methyl, Methoxymellein, 2,4-Bis (1-phenylethyl) phenol and Diisooctyl phthalate. The fructose-6-phosphate amidotransferase was inhibited by octadecanoic acid methyl ester at highest binding energy followed by Bis-2ethylhexyl phthalate, 5-diethylamino, Phenol 2,6-bis-2 hydroxy-5-methyl. Statin HMG-coa reductase enzyme was inhibited by octadecanoic acid methyl ester, Phenol 2,6-bis-2 hydroxy-5-methyl at high binding energy followed by 2,4-Bis (1-phenylethyl) phenol, Bis-2ethylhexyl phthalate (Table 3, Fig. 3).

SIRT6 family member, NAD(+)-dependent protein deacetylases is able to control genomic stability and transcriptional control of glucose metabolism. The octadecanoic acid methyl ester inhibited this enzyme by showing highest binding energy followed by Diisooctyl phthalate, Bis-2ethylhexyl phthalate, Diisobutyl isophthalate, 2,4-Bis (1-phenylethyl) phenol, Phenol 2,6-bis-2 hydroxy-5-methyl, 5-diethylamino, Methoxymellein. The pig pancreatic α-amylase was greatly inhibited by octadecanoic acid methyl ester followed by Diisooctyl phthalate and Phenol 2,6-bis-2 hydroxy-5-methyl. The more interaction was between creatinine amidohydrolase with octadecanoic acid methyl ester by their binding energy followed by Methoxymellein, Diisobutyl isophthalate, Diisooctyl phthalate, Phenol 2,6-bis-2 hydroxy-5-methyl, 2,4-Bis(1-phenylethyl)phenol, Benzenedicarboxylic acid, Methyl Stearate, Bis-2ethylexyl phthalate (Table 3, Fig. 3).

Sugar beet α-glucosidase was inhibited by octadecanoic acid methyl ester at a maximum level compared with Diisooctyl phthalate, Phenol 2,6-bis-2 hydroxy-5-methyl, Benzenedicarboxylic acid, Methoxymellein. α-glucosidase was greatly inhibited its activity by octadecanoic acid methyl ester followed by Diisooctyl phthalate, Methyl Stearate, Benzenedicarboxylic acid, Bis-2ethylhexyl phthalate, Methoxymellein, Phenol 2,6-bis-2 hydroxy-5-methyl, Diisobutyl isophthalate. The glycogen synthase kinase-3 beta was showed more binding energy when it interact with Bis-2ethylhexyl phthalate, octadecanoic acid methyl ester, Diisobutyl isophthalate, Phenol 2,6-bis-2 hydroxy-5-methyl, Diisooctyl phthalate (Table 3, Fig. 3).

Octadecanoic acid methyl ester conjugate protein tyrosine phosphatase 1B exhibits more interaction followed by Phenol 2,6-bis-2 hydroxy-5-methyl, Diisooctyl phthalate, Benzenedicarboxylic acid, octadecanoic acid methyl ester have showed highest interaction with processing α-Glucosidase I with high binding energy followed by, Bis-2ethylhexyl phthalate, Diisooctyl phthalate, Phenol 2,6-bis-2 hydroxy-5-methyl, Benzenedicarboxylic acid. The human pancreatic α-amylase have firmly interact with octadecanoic acid methyl ester with more binding energy followed Diisooctyl phthalate, Di methyl, 2,4-Bis (1-phenylethyl) phenol, Phenol 2,6-bis-2 hydroxy-5-methyl (Table 3, Fig. 3).

From *in silico* anti-diabetic activity, the octadecanoic acid methyl ester able to interact with all most all diabetic proteins/enzymes with high biding energy, whereas the diisobutyl isophthalate have conjugated with 1m1j, iso-octyl isophthalate on 1ogs and bis-2-ethylhexyl phthalate on 4acd with more binding energy. The phthalates have expressed as anti-diabetic potentials *in vitro* and *in silico* screening[43-45] (Table 3, Fig. 4).

The octadecanoic acid methyl ester is responsible for inducing antidiabetic activity reported by Iqbal *et al.*[46] and Hashim *et al.*[47] *in vitro* and *in vivo* conditions, respectively. Sasikala and Meenak[48], Rajkumar *et al.*[49] have reported that octadecanoic acid methyl ester was able to inhibit diabetic enzymes in *in silico* study. *In vitro* and *in silico* anti-diabetic activity of octadecanoic acid methyl ester was carried out by Raajshree and Chitra[50]. The present study clearly showed the presence of important phytochemicals which induced antidiabetic activity *in vitro* and *in vivo* conditions.

From online ADME test reveals that, octadecanoic acid methyl ester, dimethyl phthalate, Diisooctyl phthalate and bis-ethynyl phthalate are non-toxic AMETS test and non-carcinogens. The results confirm their degradable characters except for the octadecanoic acid methyl ester all are readily biodegradable. The online ADMET test for all the compounds was carried out but the data represented only for above mentioned four compounds are represented in Table 4.

Table 4: ADMET Predicted profile of the potent phytochemicals of *Penicillium* species

	Octodeconic acid methyl ester		Diisobutyl pthalate		Diisooctyl pthalate		Bis-2-ethylhexyl pthalate	
Property	Value	Probability	Value	Probability	Value	Probability	Value	Probability
Blood brain barrier	BBB-	0.7109	BBB+	0.9308	BBB+	0.9272	BBB+	0.9383
Human Intestinal absorption	HIA-	0.7586	HIA+	0.9745	HIA+	0.9547	HIA+	0.9797
Caco-2-permeable	CaCo2-	0.7737	CaCo2+	0.7151	CaCo2+	0.6810	CaCo2+	0.7003
P-glycoprotein-substrate	Substrate	0.7389	Non-substrate	0.6465	Non-substrate	0.5117	Substrate	0.5000
P-glycoprotein-inhibitor I	Non-inhibitor	0.6358	Non-inhibitor	0.7684	Non-inhibitor	0.7375	Non-inhibitor	0.7174
	Inhibitor	0.5434	Non-inhibitor	0.8162	Non-inhibitor	0.6907	Non-inhibitor	0.5466
Renal organic cation transporter	Non-inhibitor	0.7064	Non-inhibitor	0.8914	Non-inhibitor	0.8189	Non-inhibitor	0.8446
Distribution								
Subcellular localization	Mitochondria	0.8577	Mitochondria	0.8897	Mitochondria	0.9107	Mitochondria	0.8662
Metabolism								
CYP450 2C9 substrate	Non-substrate	0.7789	Non-substrate	0.8206	Non-substrate	0.8328	Non-substrate	0.8479
CYP450 2D6 substrate	Non-substrate	0.8547	Non-substrate	0.8820	Non-substrate	0.8727	Non-substrate	0.8655
CYP450 3A4 substrate	Substrate	0.5222	Non-substrate	0.6097	Non-substrate	0.5000	Non-substrate	0.5881
CYP450 1A2 substrate	Non-inhibitor	0.9191	Non-inhibitor	0.5666	Non-inhibitor	0.7392	Non-inhibitor	0.6539
CYP450 2C9 inhibitor	Non-inhibitor	0.9235	Non-inhibitor	0.6604	Non-inhibitor	0.7591	Non-inhibitor	0.7448
CYP450 2D6 inhibitor	Non-inhibitor	0.9270	Non-inhibitor	0.9218	Non-inhibitor	0.8986	Non-inhibitor	0.8432
CYP450 2C19 inhibitor	Non-inhibitor	0.8788	Non-inhibitor	0.7906	Non-inhibitor	0.7283	Non-inhibitor	0.6310
CYP450 3A4 inhibitor	Non-inhibitor	0.9659	Non-inhibitor	0.8983	Non-inhibitor	0.7913	Non-inhibitor	0.8309
CYP inhibitory promiscuity	Low CYP inhibitory	0.9402	Low CYP inhibitory	0.7003	Low CYP inhibitory	0.8125	Low CYP Inhibitory	0.6709
Excretion-toxicity								
Human ether-a-go-Related	Weak inhibitor	0.8836	Weak inhibitory	0.9738	Weak inhibitory	0.9078	Weak inhibitory	0.9063
gene inhibition	Inhibitor	0.5277	Non-inhibitor	0.9621	Non-inhibitor	0.8010	Non-inhibitor	0.7755
AMES test	Non- AMES toxic	0.8754	Non AMES toxic	0.9132	Non AMES toxic	0.9132	Non AMES toxic	0.9322
Carcinogens	Non-carcinogens	0.9735	Non-carcinogens	0.5391	Non-carcinogens	0.7411	Non-carcinogens	0.7116
Fish toxicity	High FHMT	0.8001	High FHMT	0.9862	High FHMT	0.9962	High FHMT	0.9959
Tetrahumena pyriformis toxicity	High TPT	0.9957	High TPT	0.9669	High TPT	0.9999	High TPT	0.9999
Honey bee toxicity	High HBT	0.6529	High HBT	0.6573	High HBT	0.5683	High HBT	0.5816
Biodegradation	Not ready biodegradable	0.8015	Ready biodegradable	0.6348	Ready biodegradable	0.5383	Ready biodegradable	0.6368
Acute oral toxicity	III	0.5355	IV	0.7836	IV	0.7863	IV	0.7176
Carcinogenicity (Three class)	Non-required	0.7385	Non-required	0.5420	Warning	0.5066	Warning	0.5434
Absorption								
Aqueous solubility	-1.7660	LogS	-4.5973	LogS	-6.5776	LogS	-6.2807	LogS
CaCo2 Permability	0.44624	LogPapp, cm sec^{-1}	1.2521	LogPapp, cm sec^{-1}	1.0364	LogPapp, cm sec^{-1}	1.0128	LogPapp, cm sec^{-1}
Distribution, metabolism, excretion, toxicity								
Rat acute toxicity	2.0969	LD50, mol kg^{-1}	1.2991	LD50, mol kg^{-1}	1.1979	LD50, mol kg^{-1}	1.0838	LD50, mol kg^{-1}
Fish toxicity	1.9240	pLC50, mg L^{-1}	0.3153	pLC50, mg L^{-1}	-0.0759	pLC50, mg L^{-1}	0.2941	pLC50, mg L^{-1}
Tetrahymena pyriformis toxicity	0.8498	pIGC50, µg L^{-1}	1.0247	pIGC50, µg L^{-1}	2.1100	pIGC50, µg L^{-1}	2.1076	pIGC50, µg L^{-1}

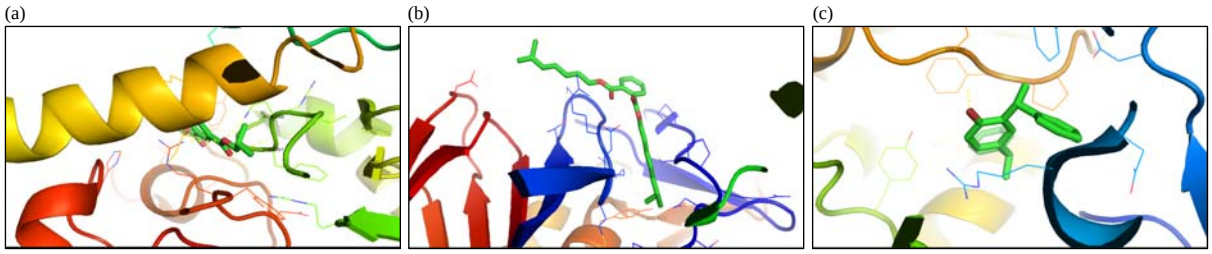

Fig. 4: Diisobutyl isophthalate, diisooctyl phthalate and bis-2-ethylhexyl phthalate and their conjugated with (a) 1m1j, (b) 1ogs and (c) 4acd of diabetic enzymes with high binding energy

CONCLUSION AND FUTURE RECOMMENDATIONS

Present day, the endophytes are being utilized as a source of novel drug compounds for the betterment of human health. The *Penicillium* species of *Tabebuia argentea* have shown medicinally important phytochemicals by GC-MS analysis. Anti-diabetic, endophytic fungal extract have inhibited the α-amylase, α-glucosidase, DPP IV activity strongly. The octadecanoic acid methyl ester and phthalates are responsible for inhibition of 21 diabetic proteins/ enzymes actively and they exhibited more binding energy. The present outcomes would provide alternate methods of natural product drug discovery which could be reliable, economical and environmentally safe. Using of these fungi, we can produce a high amount of bioactive compounds within short duration in laboratory conditions. In the study used almost all proteins or enzymes for *in silico* assay to know their activity on different enzymes and literature reveals that nobody has tried all these selected proteins for *in silico* anti-diabetic activity. Hence, further *in vivo* studies are suggested to investigate to isolate and identify pure compounds which are responsible for diabetic activity from *Penicillium* species.

SIGNIFICANCE STATEMENTS

The endophytic fungi, *Penicillium* species of *Tabebuia argentea* methanol extract yielded 18 different bioactive compounds. The same extract significantly reduced the activity of α-amylase, α-glucosidase and dipeptidyl peptidase IV enzymes in *in vitro* experiments. The molecular docking studies help to know inhibitory activity and binding mode of endophytic fungal phytochemicals with anti-diabetic target proteins. The octadecanoic acid methyl ester, dimethyl phthalate, di-iso-octyl phthalate and bis-ethylhexyl phthalate have showed the highest binding affinity and good hydrogen bond interactions with active site residues.

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