Antidiabetic activity of Phytochemical isolated from *Lannea coromendelica* leaves – an *in silico* approach

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ABSTRACT

Diabetes mellitus is one of the major lives threatening metabolic disorder all over the world. To combat this disease, natural small molecules derived from plant plays a key role in diabetes therapy.Cyclin dependent Kinase 5 is a serine-threonine kinase expressed in pancreatic beta cells plays an important role in the pathogenesis of diabetes mellitus .Cdk5 prevents the secretion of insulin by phosphorylating voltage-dependent calcium channels leading tothe decreased concentration of cytosolic Ca^{2+}. The present study involves *in silico* docking of 2 Tropylpropanaltosylhydrazone a compound isolated from *Lannea coromendalica* (Houtt.) Merr as inhibitor of Cdk5. While comparing the results from the docking experiments and their interactions with Cdk5, 2 Tropylpropanaltosylhydrazone and Roscovitine the known Cdk5 inhibitor have the binding energy of −9.15 kcal and −6.27 kcal respectively. Moreover the compound satisfies the Lipinski rule and hence it maybe a potential lead compound in the treatment of diabetes.

KEY WORDS: Cyclin dependent Kinase 5, Diabetes mellitus, insulin, cdk5 inhibitor

1. INTRODUCTION

Diabetes Mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, negative nitrogen balance and ketonemia (Bahara, 2011) Diabetes mellitus has reached epidemic proportions and affects more than 170 million individuals worldwide (Stumvoll, 2005). The majority of diabetes (~90%) is type 2 diabetes (T2D) caused by a combination of impaired insulin secretion from pancreatic beta cells and insulin resistance of the peripheral target tissues, especially muscle and liver. The disease cannot be cured with allopathic medicine as the drugs used do not restore normal glucose homeostasis and moreover have side-effects. Traditional medicinal practitioners of various countries claim to cure diabetes through administration of medicinal plants. Compared with synthetic compounds, natural small molecules with special bioactivity have become the major resource of bioactive agents and play a key role in diabetes therapy (Liu, 2010).

One such plant is *Lannea coromandelica* (Houtt.) Merr belonging to family Anacardiaceae, is widely distributed in India and Africa. It had been used traditionally by various tribal people who inhabit the forests in India. Some of the traditional uses include Anti inflammatory, Analgesic, Anti ulcer, Aphrodisiac etc (Md. Manzur-ul-Kadir Miaet al., 2009) in 2012, Farhana Islam reported that *Lannea coromandelica* (Houtt.) Merr is used by tribals to treat diabetics. This plant contains flavonoids, alkaloids, glycosides, carbohydrate and phenolics (Bhakuni, 1971)

Cyclin-dependent kinases (Cdks) constitute a family of serine / threonine kinases whose activity is regulated by the interaction with proteins known as cyclins (Morgan, 1995). Cdk5 is a serine-threonine kinase that is ubiquitously expressed in mammalian tissues (Lew, 1994) predominantly present in neurons due to the presence of its activators, p35 and p39. (Tsai, 1994) Recent studies have shown that p35 and p39 are expressed in pancreatic beta cells (Lilja, 2004) suggesting the possible activation and potentialrole of Cdk5 in insulin secretion. Cdk5 plays an important role in the pathogenesis of diabetes mellitus (Ubeda et al., 2004) The overall effects of increased activity of Cdk5 are decreased rates of insulin release, reduced insulin production and diminished insulin gene expression (Ubeda, 2006) On the basis of these findings, it is obvious that Cdk5 might be a constructive target for the treatment of diabetes

The present study is designed to explore the pharmacological properties of *Lannea coromandelica* leaves, for its antidiabetic activity using in-silico molecular docking methods with Cdk5 as target protein.

2. MATERIALS AND METHODS

2.1. Plant sample extraction: *Lannea coromandelica* leaves were collected in Chennai, Tamilnadu. The leaves were shade dried and powdered and stored in an air tight container. 50gm powdered plant material was soaked in 200ml of absolute alcohol overnight and then filtered through Whatmann filter paper No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1ml.The extract contains both polar and non-polar phytocomponents. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. The filtrate was analyzed for metabolites by using gas chromatography/mass spectrometry (GC-MS) analysis. After analysis, the compounds were identified by matching with known compound library

The target protein Cdk5 (PDB ID: 1UNG), was retrieved from the protein data bank (PDB) (http://www.rcsb.org/pdb/). The compound 2 Tropylpropanaltosylhydrazone identified by GC-MS analysis was screened against the target protein. The compound details were retrieved from the Pubchem database, and the chemical structure was generated from SMILES notation byusing the Chemsketch software (http://www.acdlabs.com). Autodock4.2 version, the most common and freely available software, (http://autodock.scripps.edu) was used for the docking analysis. Lig Plot software (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) was used to study the interaction between ligand and protein.

3. RESULT AND DISCUSSION

The metabolite from *L. coromendalica* was analyzed by GC-MS .Figure.1. From the list of metabolites (Table - 1) obtained those which possess antidiabetic and significant biological activity is thoroughly studied and identified as 2 Tropylpropanaltosylhydrazone. Auto dock software was used to dock the isolated compound 2Tropylpropanaltosylhydrazone and Roscovitine against the target protein Cdk5. The docking interaction of the protein , ligand and their docking energy interaction is shown in Table -2 .

From the interaction studies shown in Fig-2 it has been found that 2 Tropylpropanaltosylhydrazone have docking energy of − 9.15 kcal/mol and forms two hydrogen bond with lys 268 and leu 267 and non interaction bonds with Gln264, Ala244, Leu218, Thr 246, Leu248, Leu219, Thr221, Gly220, Pro222, Gln 226, Asn 270, Cys 269, Gln273 . Lesser is the binding energy more is the binding capacity of the ligand. This compound satisfies the Lipinski rule and is depicted in Fig -3

Whereas, roscovitine interaction studies in Fig -2 shows three hydrogen bond with Ala 244 and Leu 219 and non-bonding interactions with, Gln264, Lys268, Cys 269, Gly 226, Gly220, Pro 222, Thr 221, The245, Ser 247, Thr246 and have the binding energy of −6.27 kcal/mol. To study the feasibility of the compound as a probable drug candidate the ADMET properties were also studied and are depicted in Fig 3 & 4. 2 Tropylpropanaltosylhydrazone satisfies the Lipinski rule .

Cyclin dependent kinase 5 (Cdk5) prevents the secretion of insulin by suppression of gene expression by induction of transcription factors such as CCAAT enhancer binding protein β, which acts as negative regulator of insulin gene transcription and by phosphorylating the loop II and loop III of α1c subunit of L-typevoltage-dependent calcium channels (L-VDCCs) so that calcium could not find the entry into the pancreatic β cell as the L-VDCCchannel activity is deterred due to the phosphorylation leading to the decreased concentration of cytosolic Ca^{2+}.

Studies reported that insulin secretion is enhanced by inhibition of Cdk5 (Kitani et al., 2007) From comparing the interaction studies it has been found that 2 Tropylpropanaltosylhydrazone inhibits the activity of cdk5 similar to that of roscovitine , ie by competing with ATP for binding to Cdk5. One possible explanation for the mechanisms is that these compounds prevent phosphorylation thereby allowing the entry of calcium in pancreatic β cell .and preventing the suppression of insulin gene expression.

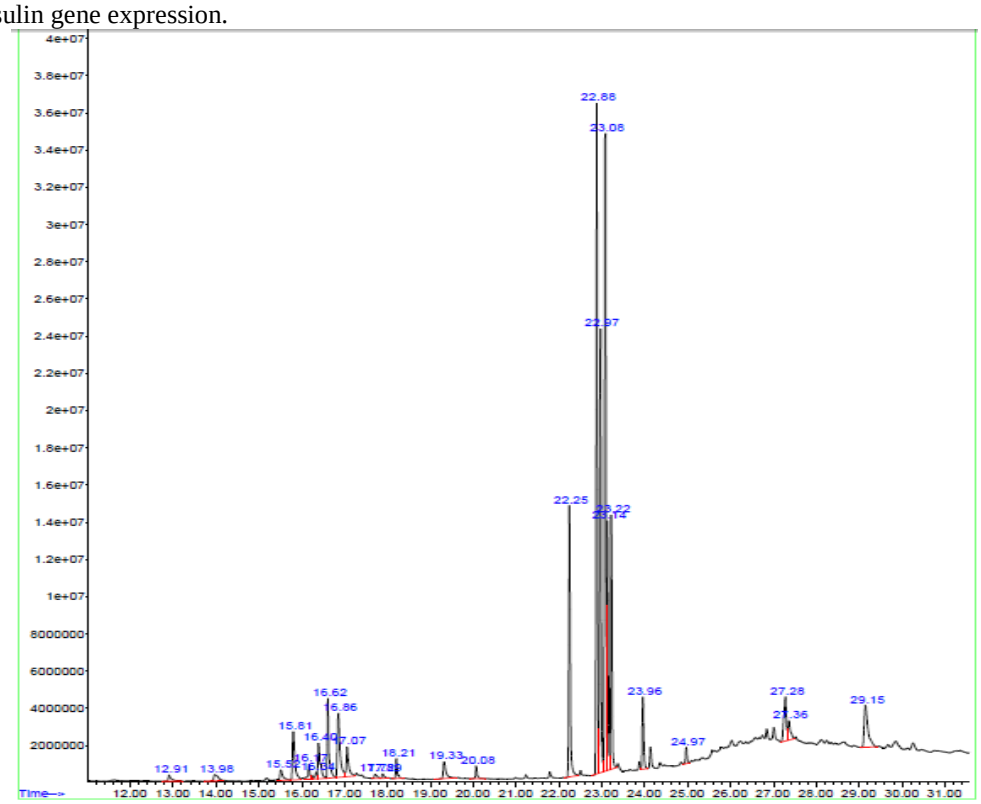


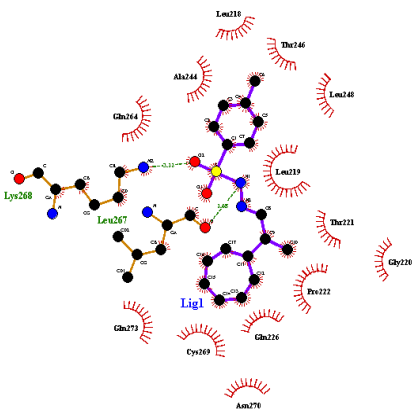
Figure 1, GC MS spectrum of crude extract from *Lannea coromendalica* leaves

Table.1.List of compounds present in the crude extract of *Lannea coromendalica* leaves

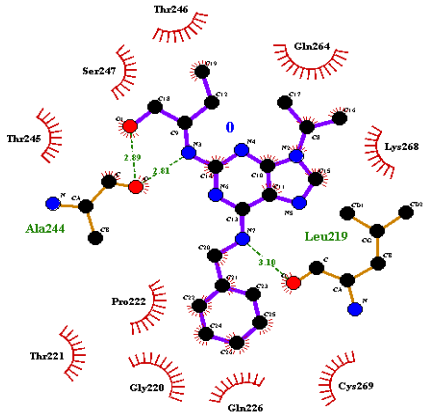
Peak no	Retention time(min))	Area %	Chemical name
1	3.332	1.95	Styrene
2	12.918	0.43	Phenol, 2,4-bis(1,1-dimethylethyl)-
3	13.978	0.54	Diethyl Phthalate
4	15.517	0.69	N-Benzyl-1H-benzimidazole
5	15.808	2.54	Benzene, 1,1'-(1,2-cyclobutanedi
6	16.171	0.58	E-15-Heptadecenal
7	16.345	0.22	13-Octadecenal
8	16.403	1.81	cis-9-Hexadecenal
9	16.621	2.85	Bicyclo[3.1.1]heptane
10	16.853	3.71	3-Tetradecyne
11	17.071	1.45	3,7,11,15-Tetramethyl-2-hexadece
12	17.725	0.24	3-Octyne, 5-methyl-
13	17.899	0.22	Phthalic acid,
14	18.218	0.62	E-15-Heptadecenal
15	19.322	0.96	Phytol
16	20.077	0.46	1-Heneicosyl formate
17	22.241	8.10	2-Tropylpropanal tosylhydrazone
18	22.880	16.57	1 Bis(2-ethylhexyl) phthalate
19	22.967	13.23	1H-Indole, 5-methyl-2-phenyl
20	23.084	19.54	1H-Indole, 5-methyl-2-phenyl-
21	23.142	6.43	Methadone N-oxide
22	23.229	7.29	(2,3-Diphenylcyclopropyl)methyl
23	23.955	2.28	(2,3-Diphenylcyclopropyl)methyl
24	24.972	0.53	Squalene
25	27.281	2.27	Vitamin E
26	27.368	1.06	1H-Isoindole-1,3(2H)-dione, 2-bu
27	29.154	3.39	1 .gamma.-Sitosterol

Table.2.Docking studies of ligand against Cdk5

Target protein	Ligand name	Docking energy level (Kcal/mol)	Hydrogen donar/acceptor
Cyclin dependent kinase5 (1UNG)	2 Tropylpropanaltosylhydrazone	−9.15	Two hydrogen bond with Lys 268(3.22A) and Leu(3.65 A)
Cyclin dependent kinase5 (1UNG)	Roscovitine	−6.27	Three H bond with Ala244(2.89A and 2.81A) and leu267 (3.65 A)



1UNG 9602285



1UNG Roscovitine

Figure.2.The docking interaction of Cdk5 with 2 Tropylpropanaltosylhydrazone and roscovitine with their predicted ligand binding site residues.

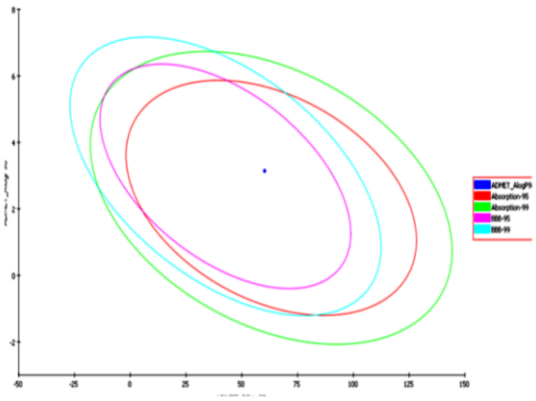


Figure.3.ADMET property of 2-Tropylpropanaltosylhydrazone

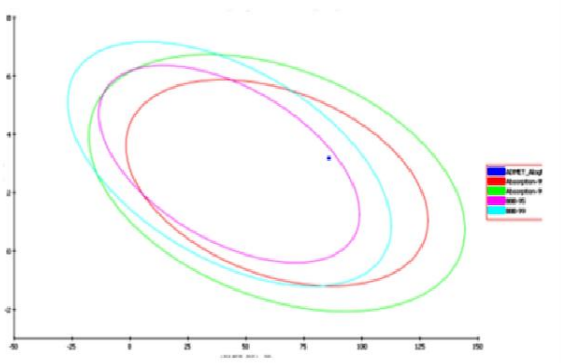


Figure.4.ADMET property of Roscovitine

4. CONCLUSION

The present study showed that 2 Tropylpropanaltosylhydrazone could be used as a potential lead compound in the treatment of diabetes. Further exploration of the function of the compound will facilitate a better understanding towards developing 2 Tropylpropanaltosylhydrazone as an antidiabetic agent.

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