Strategies in the Design of Antidiabetic Drugs from *Terminalia Chebula Retz.* Using *in silico* and *in vitro* Approach

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Abstract: Diabetes mellitus is the fifth deadliest disease in the developing countries. Even with all the research and new drugs available, combating diabetes is still challenging. There are successes in finding new cost effective drugs without side effects, even if not perfect. In our investigation we studied binding mechanism of secondary metabolite of *T. chebula in silico.* It was observed that three compounds out of 16 have a higher binding affinity for the target proteins. Ellagic acid showed highest binding affinity with alpha amylase, beta glucosidase and alpha glucosidase with lesser binding energies -4.5kcal/mol, -5.36kcal/mol and -4.48kcal/mol respectively. Arjungenin has lesser binding energy of 4.77 kcal/mol with glucokinase while luteoline has binding energy of -7.25kcal/mol for enzyme glycogen synthase kinase. These entire compounds interacted with non-covalent interaction. Petroleum ether extract showed the significant alpha amylase inhibitory activity i.e. 51.22% as compared to standard drug (65.99%).TLC analysis revealed the presence of total 9 compounds in different plant extracts one of them might be a lead compound which could be further exploited for the development of novel safer and potent antidiabetic drug.

Keywords: diabetes mellitus, molecular docking, thin layer chromatography, alpha amylase, ellagic acid

1. Introduction

Diabetes mellitus is one of the major global health crises and is the fifth leading cause of the death [1].It is associated with severe pathological imbalances including long-term damage, dysfunction and failure in many organs [2, 3]. Prevalence of type II diabetes (T2D) is higher (90%) than that of the type I diabetes (T1D) mellitus [4].In spite of huge number of data available regarding the development and research of the antidiabetic drug, search of more safer natural antidiabetic drug still continued[5]. Herbal drug play a crucial role and are acceptable in the management of the type II diabetes mellitus (T2D) due to their less adverse effects and cost effectiveness but sill lot much efforts are needed to dig out an efficient drug from these natural sources [6,7]. 70-95% of the population of developing countries including India makes use of herbal drugs as an effective antidiabetic agent. The reason behind that these natural agents are easily available, it has very low costs and the most important unavailability of conventional antidiabetic drugs [8]. Herbal drugs are needed to be standardized, ensured for its quality, safety and reproducibility [9].

Terminalia chebula Retz. is popularly called the 'King of Medicine' as it has a remarkable property of healing every kind of disease including various asthma, candidiasis, diarrhea, vomiting, hiccough, bloody stools, dysentery, ulcer, gout, wound infection, urinary tract infection gastroenteritis, skin diseases, etc. [10, 11].It is a native plant of India and near about 250 species of *T. chebula* are spread all over a world specially in a tropical areas [12]. *T. chebula* is rich sources of tannins and other secondary metabolites like

tannic acid, gallic acid, chebulinc acid, chebulic acid, chebulagic acid, corrilagin, and triterpenoids [13, 14].The plant have demonstrated antioxidant and free radical scavenging activity [15], cytoprotective activity[16], anti-carcinogenic activity[17,18], anticonvulsant activity[19], anti-mutagenic activity[20] anti-ulcerogenic activity[21], anti-tussive activity[22] and anti-caries activity[23]. Herbal formulation known as a 'Triphala', recomonded by the Indian System of Medicine (ISM), contains the powder of fruit rind of *T. chebula* as one of the ingredient. Triphala is effective in the treatment of diseases of the mouth including dental caries, gingivitis, stomatitis. spongy and bleeding gums etc.[24].It possess hepatoprotective property[25] and also used in the treatment of cancer[26].

Based on the information regarding the usefulness of *T. chebula* in medicine without any harm, we hypothesized that one or more of its phytoconstituents may be useful in the diabetes to develop an effective antidiabetic drug. We have investigated the secondary metabolites present in the plant for their *in silico* and *in vitro* antidiabetic effect. For this purpose we have targeted some of the key enzymes those are used in the management of diabetes [27-31] and analyzed that up to what extent our secondary metabolites are effective in modulating the activity of the enzymes.

2.Materials and Methods

2.1 *In-silico* Approach

2.1.1 Molecular Docking

The 3-D crystal structure of the all five enzymes (Alpha amylase -PDB ID: 1HNY, beta glucosidase-PDB ID: 1JFE, glycogen synthase kinase-3β-PDB ID: 4ACD, glucokinase-PDB ID: 1V4T and alpha-glucosidase -PDB ID: 3W37) which can be regulated to cure diabetes retrieved from the protein data bank (http://www.rcsb.org/pdb/). Total sixteen phytochemicals were screened against these target proteins.

With the help of literature study of *T. chebula*, 16 phytochemicals (shown in Table 1) were found out for docking analysis [23, 32-35]. Structures of 16 compounds were obtained in .sdf file format from Pubchem database and converted to .pdbqt file which is required ligand file format with the aid of Open Babel software. Energy minimization was done and carried forward for further docking analysis with Auto dock module available in PyRx Version 0.8 software (http://pyrx.sourceforge.net/) and the results were analyzed with the help of LigPlot (https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/)[36].

2.2 *In vitro* approach

2.2.1 Plant Material and Extract Preparation

Seeds of *T. chebula* were purchased from local market of India and authenticated by the, Department of Botany, Padmashri Vikhe Patil College, Pravaranagar (Loni), Tal. Rahata, District: Ahmednagar, (MS), India. Then the seeds were crushed manually to a powder with a mortar and pestle at room temperature in the absence of sunlight. Powered material was subjected to successive extraction by maceration in petroleum ether, chloroform, ethanol and water (increasing order of their polarity).Extract was then filtered and evaporated to obtain concentrate [37].

2.2.2 *In-vitro* Alpha amylase inhibitory activity assay

The assay was carried out according to published protocols [38, 39]

The total reaction mixture containing 250 µl of 0.02M sodium phosphate buffer, 250 µl of enzyme (α-amylase) and the plant extracts at a different concentration of 100-1000 µg/ml were incubated at room temperature for 10 min followed by addition of 250 µl of 1% starch. The reaction was then terminated with addition of 500 µl of di-nitro salicylic acid color reagent and all tubes were then placed in boiling water bath for 5 min, cooled to room temperature and diluted upto 5 ml with distilled water and the absorbance

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was measured at 540 nm). The control samples were without any plant extracts showing 100% enzyme activity.

The % inhibition of the assay was calculated using the formula:

$$\% \text{ Inhibition} = [(\text{Abscontrol} - \text{Absextracts})/\text{Abscontrol}] \times 100$$

2.2.3 Chromatographic separation

Chromatoplates were prepared by silica gel G coating in 1:2 ratios with distilled water. Thickness of the coat was ≈0.5mm.Plates were allowed to dry at room temperature and activated in an oven at 110^{0}C for 1hr. 10 µl of extract was spotted and kept in a developing chamber containing appropriate solvent (Table 3). After development plate were brought out, allowed to dry and analyzed by exposure to iodine vapour [40]. Rf value was calculated by using following formula:

Rf = Distance travelled by solute (cm) /Distance travelled by solvent (cm)

3. Result and Discussion

3.1 Molecular docking analysis

The workflow was directing towards the screening of bioactive compound from 16 secondary metabolites obtained from the *T. chebula*, which would prove a potent antidiabetic agent by getting docked with the said target enzymes and interacting favorably with them. Molecular docking is the computational method for structure-based drug designing which gives an idea about the proper and stable conformation of ligand and target protein and also tells about suitable protein ligand interactions [41].Binding energy is nothing but the binding strength of the ligand which not only help predicting the stable conformation of ligand-protein complex but also optimize the newly formed bonds [42].Docking result based on their binding energies is shown in the Table 1. Analysis of docking results shows binding energies in a range of -4.48 Kcal/mol to -7.25kcal/mol. Three secondary metabolites found to have best docking interaction with the target proteins. Ellagic acid showed highest binding affinity with alpha amylase, beta glucosidase and alpha glucosidase with binding energies -4.5kcal/mol, -5.36kcal/mol and -4.48kcal/mol respectively. Arjungenin has lesser binding energy of -4.77 kcal/mol with glucokinase. Among all these metabolites luteoline has highest binding affinity with much lesser binding energy of -7.25kcal/mol for enzyme glycogen synthase kinase-3β (GSK). Promising binding poses of all theses enzymes are shown in Figure1b and Figure 3. The non-covalent interactions are conventional methods and are proven to be effective in prediction of different binding modes of protein ligand complexes [41]. Non-covalent interactions include hydrophobic interactions, hydrogen bonding, van der waals interaction and the electrostatics interaction [43]. The residues take part in non-covalent interactions of luteoline with GSK via hydrogen bonding and hydrophobic interactions are Ser215, Tyr216, Arg220, Ile228, Arg223, Asp260, Ser261, Gly262 and Gln 265.Out of these only two residues of B chain Ser215 and Ile228 are involved in hydrogen bonding with the bond distance 2.94 A^0 and Ile225 A^0 respectively while others interact with hydrophobic interactions (Figure1a). Ellagic acid forms two hydrogen bonds with a single amino acid residue, Lys227 of A chain of alpha amylase (Figure 2A). The bond length of these two bonds is 2.66A^0 and 2.97 A^0 . Ellagic acid also formed a hydrogen bond with beta glucosidase at the distance of 3.01 A^0 with amino acid residue Glu424 (Figure 2B).

Table 1: Binding energies of *T chebula* revealed during docking analysis of the compounds with their target enzymes

Sr. No.	Compounds	Alpha-amylase	Beta-glucosidase	Glycogen synthase kinase-3β	Glucokinase	Alpha - Glucosidaes
1	Ethylgallate	-2.9	-3.09	-5.42	-2.28	-2.43

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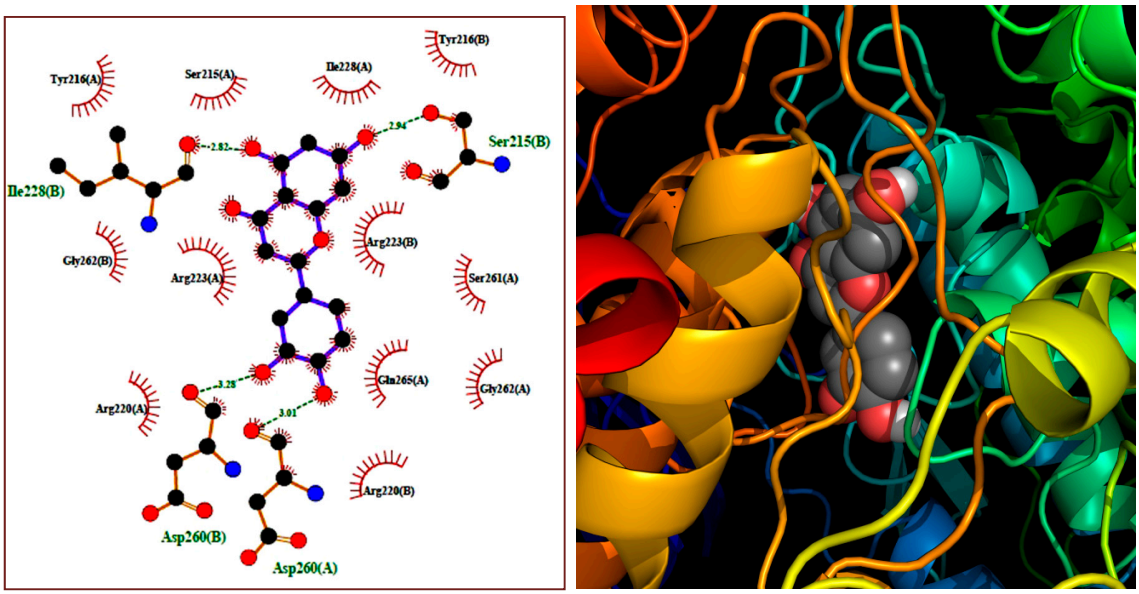
2	Arjungenin	-4.38	-4.42	-0.15	**-4.77**	-4.45
3	Castalagin	-2.85	-3.28	7000	-2.29	-2.43
4	Terflavin A	1.4	0.36	3500	1.19	-0.12
5	Chebullagic acid	-1.2	-2.21	1630	-0.92	-1.14
6	Gallic acid	-2.57	-2.43	-5.31	-3.49	-2.76
7	ChebulosideII	-4.19	-2.58	99.63	-1.54	-2.09
8	Terchebin	1.3	0.29	1430	0.62	0.04
9	Punicalagin	-2.81	-3.14	7680	-2.19	-2.38
10	Arjunglucoside1	-3.15	-3.37	449.91	-1.87	-3.06
11	Luteoline	-4.31	-4.53	**-7.25**	-3.91	-3.44
12	Chebulinic acid	1.63	2.4	1730	1.3	2.27
13	Ellagic acid	**-4.5**	**-5.36**	-6.98	-3.91	**-4.48**
14	Quercetin	-0.94	-0.12	3.56	-0.55	-0.8
15	Chebulic acid	-2.38	-2.54	-5.56	-1.26	-1.95
16	Flavogallonic Acid	-1.42	-1.91	-2.53	-1.03	-2.2
17	Acarbose	-0.46	2.72	2.66	1.87	2.05



(a) **(b)**

Figure 1: (a) Hydrophobic and hydrophilic interactions of luteoline with Glycogen synthase kinase-3β residues (GSK). Brown colored half circle indicates the hydrophobic reactions of luteoline with the target enzymes GSK. Green dotted lines indicate the hydrogen bond while green colored value indicates their bond length. (b) Promising binding pose of luteoline with GSK.

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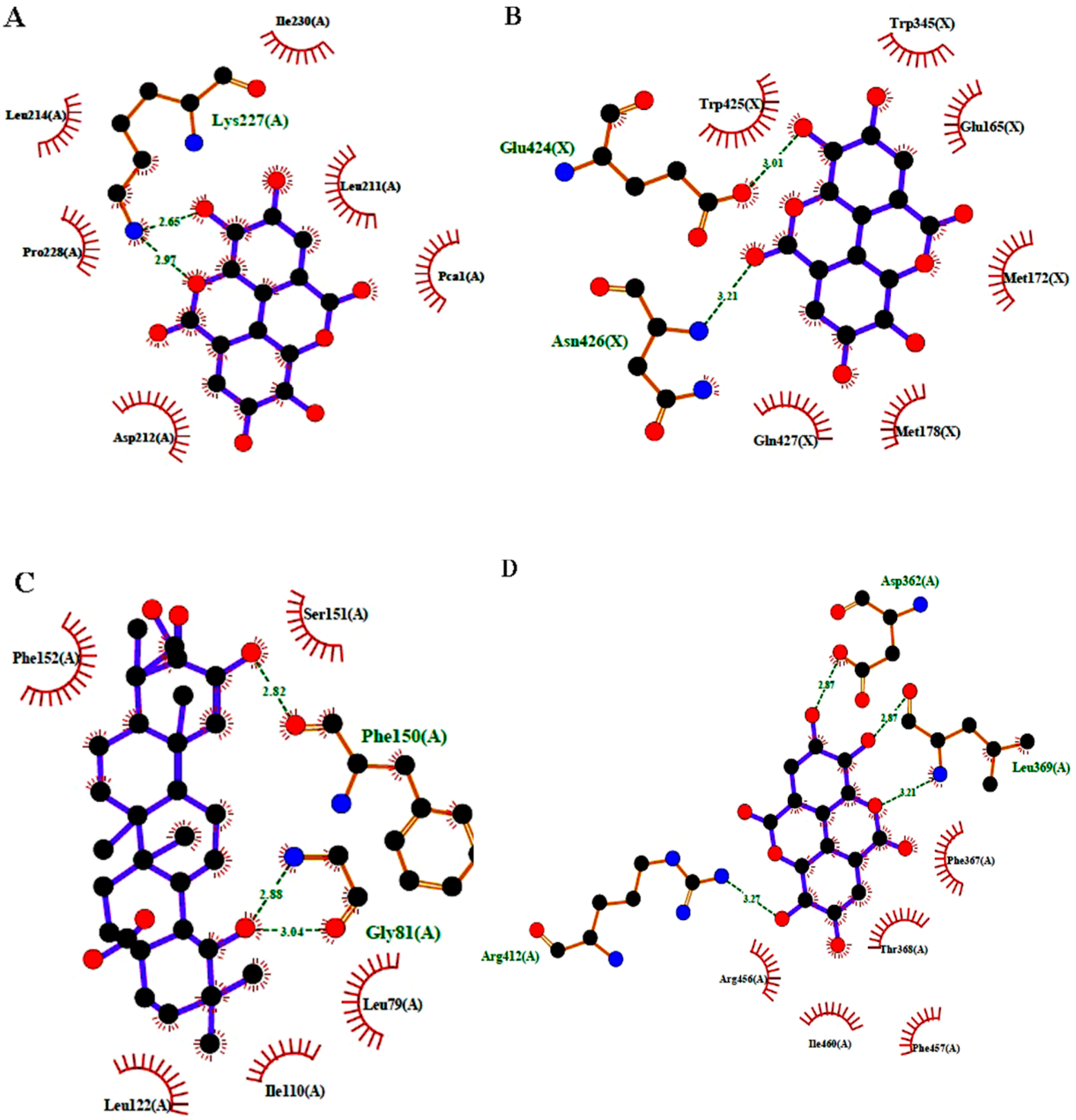

Figure 2: Hydrophobic interactions and hydrogen bonds of ellagic acid with amino acid residues of A. Alpha amylase, B. Betaglucosidase and D. Alpha glucosidase while that of arjungenin with C. Glucokinase. Brown colored half circle indicates the hydrophobic reactions of compounds with the target enzymes. Green dotted lines indicate the hydrogen bond while green colored value indicates their bond length.

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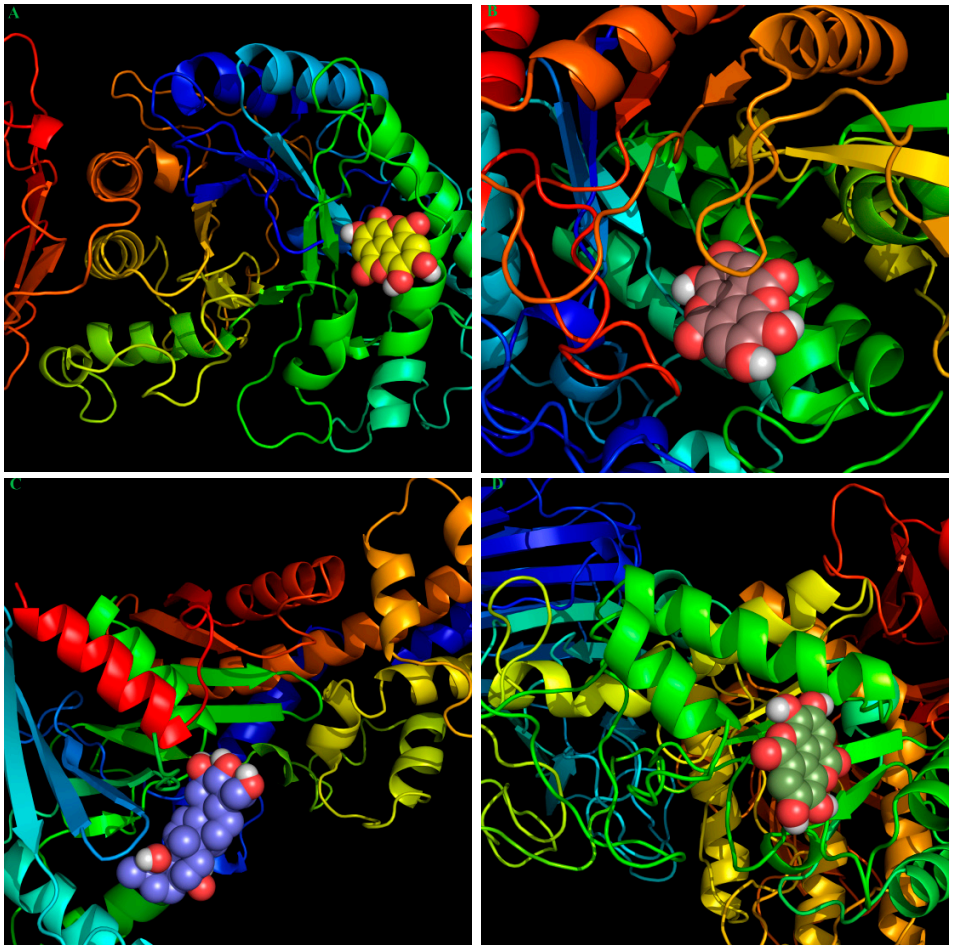

Figure 3: Promising binding poses of ellagic acid with A. Alpha amylase, B.Beta glucosidase and D. Alpha glucosidase and arjungenin with C. Glucokinase

Two hydrogen bonds are formed in between ellagic acid and alpha glucosidase through Leu369 and Arg412 amino acid residues and the bond length is 3.21 A^0 and 3.27 A^0 respectively(Figure 2D). Arjungenin also involved in hydrogen bond formation via gly81 residue of glucokinase and forms a bond from the distance 2.88 A^0 away (Figure 2C).It is cleared from the above result that three phytochemicals ellagic acid, luteoline and arjungenin interacted well with the key regulatory enzymes and hence can prove to have an ability to be a future drug.

3.2 *In vitro* alpha amylase inhibitory activity assay analysis

Inhibition of digestion of dietary carbohydrates in small intestine is a widely used method in the management of diabetes mellitus [44]. Inhibition of these carbohydrates by alpha amylase is one of the therapeutic approaches taken to reduce postprandial glucose level [45]. Keeping the same approach in mind we proceeded to find out the potential alpha amylase inhibitory activity from different solvent extract of *T chebula*. All plant extract have shown a great potential antidiabetic activity in comparison with standard drug acarbose (Table 2). TCPE showed the 51.22% inhibition for alpha amylase at the concentration of 1mg/ml while at the same concentration standard drug showed 65.99% inhibition. TCCE showed the 39.71% percent inhibition value while TCEE showed 38.03% inhibition of alpha amylase activity. TCWE inhibited alpha amylase activity 35.59%. Figure 4 shows the comparison of all this plant extracts with the

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standard drug acarbose. Comparing the extracts among them showed that TCPE showed a significant capacity to inhibit the enzyme and thus may contain one of three compounds (ellagic acid, luteoline, arjungenin) as a bioactive compound.

Table 2: Alpha amylase inhibition by *T. chebula* using different solvent extracts. Tests were carried out in triplicate manner and values are expressed as the mean ± SD. The IC50 value is the concentration of inhibitor which inhibits 50% of its activity under the assayed conditions. (TCPE- *T. chebula* petroleum ether extract, TCCE- *T. chebula* chloroform extract, TCEE- *T. chebula* ethanol extract, TCWE- *T. chebula* water extract)

Sr. No.	Concentration (µg/ml)	% Inhibition				
		Acarbose	TCPE	TCCE	TCEE	TCWE
1	100	42.15 ± 0.68	29.55±1.15	29.45±1.46	12.5±3.09	21.17±1.71
2	200	57.88 ± 2.42	31.83±1.37	30.36±0.71	15.07±4.20	22.305±1.22
3	400	62.24 ± 0.53	32.88±0.05	31.83±1.45	19.79±1.50	23.535±1.54
4	600	63.76 ± 0.96	34.31±1.40	33.92±1.48	23.22±1.53	26.465±2.02
5	800	64.66 ± 1.26	41.88±5.37	38.49±6.26	26.62±1.23	28.09±2.02
6	1000	65.99 ± 1.76	51.22±4.29	39.71±6.21	38.03±4.71	35.59±7.76
IC50 values (µg /ml)		52	1115	1712	1589	2168

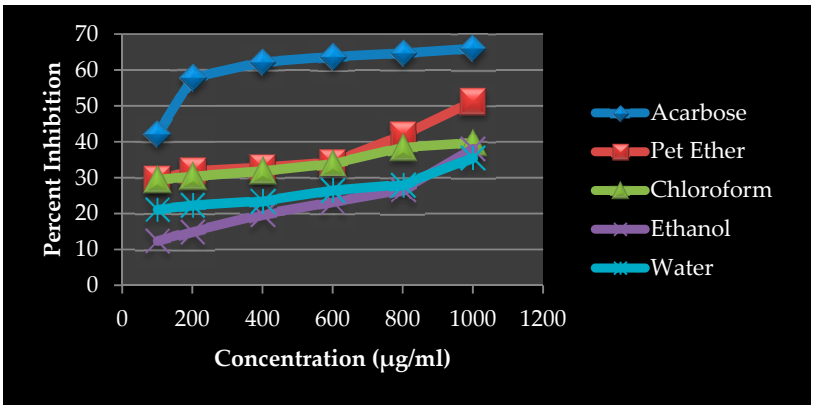

Figure 4: The enzyme inhibitory activity of different extracts of *T. chebula* seed extract on α-amylase

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3.3 Thin Layer Chromatography analysis

Thin layer chromatography is one of the analytical techniques which is been developed to standardize the natural products isolated from the medicinal plants [46]. To analyze the bioactive compound(s) in the present in the mixture of different solvent extracts and to document the phytoconstituents in this extracts we performed TLC. Different solvent system used for analysis showed better resolution for different plant extracts (Table 3). In solvent system chloroform: ethyl acetate (3:1), petroleum ether extract of *T. chebula* showed two spots /compounds with Rf value 0.56, 0.41 while in solvent system chloroform: ethyl acetate (4:6) of chloroform extract showed three spots showing Rf values, 0.94, 0.54, 0.90. Ethanol extract in the solvent system ethyl acetate: methanol: water (5:1.1:1) showed two spots with Rf values 0.8, 0.63. Finally aqueous extract gave better results in toluene: ethyl acetate (4:1) mobile phase and the Rf values calculated were 0.17, 0.10.Totally 9 compounds were isolated by TLC method one or all them containing potential for antidiabetic potentially.

Table 3: TLC result of different extracts of *T chebula* visualized by iodine chamber

Sr No	Extract	Solvent system used	Rf value
1	Petroleum ether	Chloroform: ethyl acetate (3:1)	0.56, 0.41
2	Chloroform	Chloroform: ethyl acetate (4:6)	0.94, 0.54, 0.90
3	Ethanol	Ethyl acetate :Methanol: Water (5:1.1:1)	0.8, 0.63
4	Aqueous	Toluene: Ethyl acetate (4:1)	0.17, 0.10

4. Conclusion

Our study confirmed that the secondary metabolites of *T chebula* extract specially ellagic acid, luteoline and arjungenin possess a great potential of antidiabetic activity. One of the probable mechanisms of theses phytoconstituents for the antidiabetic action is the inhibition of carbohydrate metabolizing enzyme, alpha amylase. Alpha amylase inhibitory activity is higher in petroleum extract which contain two compounds (TLC analysis).Further analysis is necessary to purify the bioactive compound and study its potential for antidiabetic activity.

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Authors' contributions

All authors have contributed significantly. They have performed the laboratory works and prepared the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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