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Research Article

DOCKING STUDIES ON ANTIDIABETIC MOLECULAR TARGETS OF PHYTOCHEMICAL COMPOUNDS OF *SYZYGIUM CUMINI* (L.) SKEELS

SMRUTHI G, MAHADEVAN V, VADIVEL V*, BRINDHA P

Centre for Advanced Research in Indian System of Medicine, School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India. Email: vadivel@carism.sastra.edu

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ABSTRACT

Objectives: Different parts of jamun tree (*Syzygium cumini* L. skeels) which belongs to the family - Myrtaceae are well-known for their antidiabetic activity. Traditional practitioners in India are using the leaf, bark, and fruits of this medicinal plant over many centuries to manage the diabetic patients. Although several research works have been conducted to prove the efficacy of this plant extracts and also to explore the active principles of this plant drug, there is no information regarding the interaction of phytoconstituents of jamun tree with diabetic targets at the molecular level. Hence, this study focused to apply a computational approach to reveal the interaction of molecules of jamun tree with antidiabetic targets.

Methods: Lamarckian genetic algorithm methodology was used for docking of 22 phytoconstituents with α-amylase, a key enzyme that involved in carbohydrate metabolism using Autodock software.

Results: Analysis of binding energy of ligands with target receptors was remarkably lower especially for friedelin (−9.54 kcal/mol), epifriedelanol (−8.98 kcal/mol), betulinic acid (−8.60 kcal/mol), beta-sitosterol (−8.56 kcal/mol), petunidin-3-gentiobioside (−7.52 kcal/mol), kaempferol (−7.08 kcal/mol), petunidin (−6.21 kcal/mol), quercetin (−6.03 kcal/mol), myricetin (−5.80 kcal/mol), and bergenin (−5.27 kcal/mol) when compared to the synthetic drug acarbose (−2.43 kcal/mol).

Conclusion: Potential molecules identified from this study could be considered as a lead to design/synthesize anti-diabetic drug molecules in pharmaceutical industry.

Keywords: Jamun tree, *Syzygium cumini*, Phytochemicals, Diabetes, α-amylase, Molecular docking.

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INTRODUCTION

Pancreatic α-amylase (E.C. 3.2.1.1) is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated post-prandial hyperglycemia. Human pancreatic α-amylase in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in the treatment of diabetes [1]. Hence, retardation of starch digestion by inhibition of enzymes such as α-amylase would play a key role in the control of diabetes. However, the discovery of specific high-affinity inhibitors of pancreatic α-amylase for the development of therapeutics has remained elusive. Inhibitors currently in clinical use (e.g., acarbose, miglitol, and voglibose) are known to inhibit a wide range of glucosidases such as α-glucosidase and α-amylase. Because of their non-specificity in targeting different glucosidases, these hypoglycemic agents have their limitations and are known to produce serious side effects. Therefore, the search for safer, specific, and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional plant medicines offering great potential for discovery of new anti-diabetic drugs [2].

While plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems, very few of these traditional anti-diabetic plants have received proper scientific scrutiny despite recommendations by the World Health Organization. Ayurveda and other Indian traditional approaches have described more than 800 plants in the Indian subcontinent, known to possess anti-diabetic potential. In fact, only a few of them have been characterized for their mechanistic actions [3,4]. *Syzygium cumini* (L.) Skeels. (Syn: *Eugenia jambolana* Lam., Family: Myrtaceae) is one of the widely used plants for the treatment of diabetes by traditional practitioners over many centuries. It is commonly known as jambolan, black plum, java plum, Indian blackberry, Portuguese plum, Malabar plum, purple plum, Jamaica, and damson plum. It is a large evergreen and densely foliaceous tree with greyish-brown thick bark, exfoliating in woody scales. The wood is white; leaves are leathery, oblong-ovate to elliptic or obovate-elliptic with 6-12 cm long, the tip being broad and less acuminate. Flowers are scented, greenish-white, found in clusters and are round or oblong shaped in dichotomous paniculate cymes. The fruits are berries and are often obviously oblong, 1.5-3.5 cm long, dark-purple or nearly black, luscious, fleshy and edible, which contains a single large seed (Fig. 1).

Sagrawat *et al*. [5] reviewed the pharmacological actions and phytochemical constituents of jambolan. Various extracts of jambolan possess a range of pharmacological actions, viz., antibacterial, antifungal, antiviral, antigenotoxic, anti-inflammatory, antiulcerogenic, cardioprotective, antiallergic, anticancer, chemopreventive, radioprotective, free radical scavenging, antioxidant, hepatoprotective, antidiarrheal, hypoglycemic and antidiabetic effects [6]. Clinical and experimental studies of jambolan revealed that different parts of the plant especially fruits, seeds and stem bark possess promising antidiabetic activity. In the early 1960-1970s, some preliminary reports on the antidiabetic activity of different parts of jambolan in experimental animals have been reported [7-9].

Seeds were considered as more effective in experimental diabetes as they quickly reduced the sugar level in urine [10]. Administration of seed extracts caused hypoglycemia in mild and severe diabetic rabbits [11]. Seed methanolic extract showed inhibition in murine liver glucosidases activity [12]. Oral administration of seed extract to rats for 15 days lowered the blood glucose [13]. The ethanolic extract of seeds decreased blood sugar levels in alloxan induced diabetic rats [14].

Aqueous and ethanolic extract of the seeds administered orally to animals and to clinical patients at variable dose levels were found to be active [15]. Yadav *et al.* [16] reported that ethanol extract of seeds of jambolan in rats had a significant hypoglycemic and antihyperglycemic activity. *S. cumini* fruit seeds were reported to reduce blood glucose level, an increase in serum insulin level and exhibits insulinase activity [17]. The antidiabetic activity of hydroalcoholic extracts of *S. cumini* seeds was reported [18]. *In vitro* antidiabetic activity of the ethanol extract of *S. cumini* seeds was investigated using α-amylase inhibition assay [19].

The fruits are rich in citric acid, mallic acid, gallic acid, anthocyanins, delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyaniding diglycoside, petunidin, and malvidin [20-25]. The seeds of *S. cumini* were claimed to contain alkaloid, jambosine, glycoside jambolin, and ellagic acid [26]. The seeds have been reported to be rich in flavonoids [27]. Daulatabad *et al.* [28] reported the presence of epoxy and cyclopropenoid fatty acids in *S. cumini* seed oil. The leaves are rich in acylated flavonol glycosides, quercetin, myricetin, myricitin, myricetin-3-O-4-acetyl-L-rhamnopyranoside, triterpenoids, esterase, galloyl carboxylase, and tannin [29-32]. Kumar *et al.* [33] have evaluated the phytochemical composition of the leaf oil of *S. cumini*. The stem bark is rich in betulinic acid, friedelin, epifriedelanol, beta-sitosterol, eugenin, terpenoids and fatty acid ester of epifriedelanol [34],

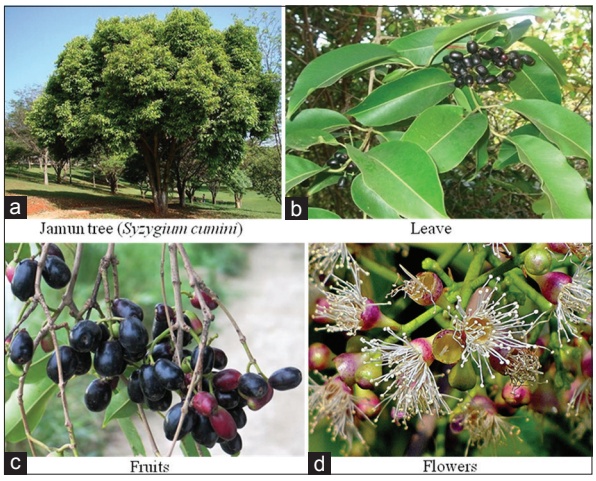

Fig. 1: (a-d) Morphology of leaf, flower, and fruits of jamun tree

quercetin kaempferol, myricetin, gallic acid and ellagic acid [35], bergenins [36], flavonoids and tannins [37]. The phytoconstituents of *S. cumini* flowers were analyzed by Nair and Subramanian [38]. The presence of flavonoid glucosides, such as isorhamnetin 3-O-rutinoside, was reported in the roots of *S. cumini* [39,40].

Even though, several research studies have been carried out to evaluate the antidiabetic potential of *S. cumini* and also various phytochemical compounds were reported in different parts of jamun tree, no information available on molecular mechanism for their anti-diabetic action. Hence, in this study, an attempt has been made to decipher the interaction between phytochemical compounds of jamun tree with key carbohydrate digestive enzyme (α-amylase) using Autodock software.

METHODS

Preparation of protein receptor

The pancreatic α-amylase structure was downloaded from protein database (Ref. 4X9Y). It was a wild type human pancreatic α-amylase at true atomic resolution (1.07 A) as reported by Caner and Brayer in 2014 by X-ray diffraction study. All water molecules were removed, hydrogen atoms were added, non-polar hydrogen atoms were merged, and Gasteiger charge was assigned to the receptor molecule.

Preparation of ligands

The major phytochemical compounds of *S. cumini* such as citric acid, malic acid, gallic acid, delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyanidin diglucoside, petunidin, malvidin, ellagic acid, quercetin, myricetin, myricetin-3-O-4-acetyl-L-rhamnopyranoside, betulinic acid, friedelin, epifriedelanol, beta-sitosterol, eugenin, kaempferol, bergenins, and isorhamnetin 3-O-rutinoside were used as ligands (Table 1; Figs. 2 and 3). The 3D structure of each ligand was obtained from Pubchem and finally converted into PDF files using Open Babel.

Docking study

Automated molecular docking study was conducted with Autodock 4.2.6 (Scripps Research Institute, La Jolla, CA). The root of each ligand was detected, and torsion angles were identified for ten independent runs per ligand. A grid box of 126 × 100 × 96 points in x, y and z directions was built with a grid spacing of 0.575 A° for amylase macromolecule. The default settings were used for all other Autodocking parameters. At the end of docking, the best poses were analyzed for binding free energy (kcal/mol), docking predicted inhibition constant (Ki), intermolecular

Table 1: Molecular characteristics of different phytochemical compounds of jamun tree

S. No.	Name of the phytochemical	Number of non-polar hydrogen atoms	Number of aromatic carbons	Number of rotatable bonds
1	Berginin	11	6	7
2	Beta-sitosterol	49	0	7
3	Betulinic acid	46	0	4
4	Citric acid	4	0	9
5	Cyanidine diglycoside	20	15	18
6	Delphinidin-3-gentiobioside	19	15	19
7	Ellagic acid	2	12	4
8	Epifriedelanol	51	0	1
9	Eugenin	9	9	2
10	Friedelin	50	0	0
11	Gallic acid	2	6	5
12	Kaempferol	6	15	5
13	Malic acid	3	0	6
14	Malvidin	11	15	7
15	Malvidin-3-laminaribioside	25	15	19
16	Myricetin	4	15	7
17	Myricetin-3-O-4-acetyl-L-rhamnopyranoside	15	15	12
18	Petunidin	8	15	7
19	Petunidin-3-gentiobioside	22	25	19
20	Quercetin	5	15	6
21	Acarbose	29	0	32

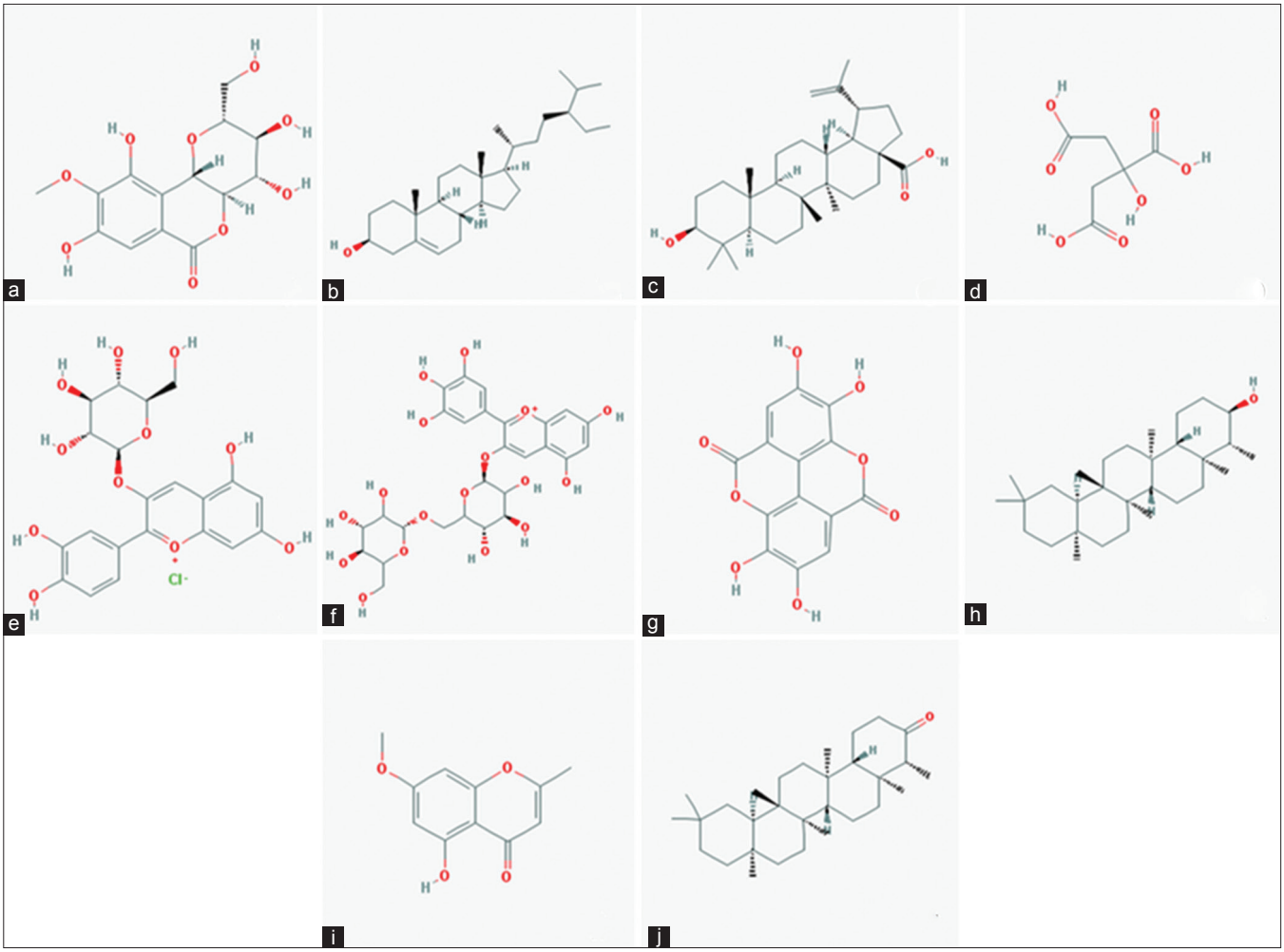

Fig. 2: Structure of phytochemical constituents of jamun tree: Berginin (a), beta-sitosterol (b), betulinic acid (c), citric acid (d), cyanidin diglucoside (e), delphinidin-3-gentiobioside (f), ellagic acid (g), epifriedelanol (h), eugenin (i), and friedelin (j)

energy, internal energy, torsional energy, root mean square and number of H bonds formed were recorded, and the molecular interaction images were visualized using Chimera software.

RESULTS AND DISCUSSION

Antidiabetic effect of jamun tree parts was deeply studied in previous research projects. Jamun seed kernel aqueous extract significantly prevented hyperglycemia and hyperinsulinemia-induced by high fructose diet induced experimental animals [41]. Type 2 diabetic individuals consuming seed kernel extract showed a decrease in serum glucose level [42]. Chaturvedi *et al*. [43] reported that blood glucose level remained stable in mild diabetic rats after STZ administration and showed antihyperglycemic effect on the 10th day. Aqueous and alcoholic extracts and lyophilized powder of kernels in diabetic animals showed significant antihyperglycemic activity in mild to moderate degree of hyperglycemia [44]. Fruit aqueous extract caused a marginal reduction in plasma glucose levels in diabetic mice [45]. The fruit pulp ethanolic extract produced a good effect in both mild and severe diabetic animals and 100 mg/kg body weight of water extract showed maximum effect [46]. Reduction of glycemia was observed after 7 days treatment with the ethanolic, aqueous and butanolic fractions of leaf [47]. In another study, administration of 20 and 40 mg/kg of ethanolic leaf extract exhibited significant results in normal and hyperglycemic adult rabbits [48]. The antidiabetic activity of hydroalcoholic extracts of *S. cumini* leaves was noticed by Shankar and Suthakaran [49]. Aqueous extract of stem bark showed a significant decrease in serum glucose levels in alloxan-induced diabetic rats [50]. Methanolic extract of stem bark decreased the blood glucose levels in mice [51].

Molecular interaction of phytochemical constituents of jamun tree with α-amylase enzyme has been investigated in this study using Autodock software, and the results are given in Table 2 and Figs. 4 and 5. When compared to the synthetic drug acarbose (−2.43 kcal/mol), phytochemicals like friedelin (−9.54 kcal/mol), epifriedelanol (−8.98 kcal/mol), betulinic acid (−8.60 kcal/mol), beta-sitosterol (−8.56 kcal/mol), petunidin-3-gentiobioside (−7.52 kcal/mol), kaempferol (−7.08 kcal/mol), petunidin (−6.21 kcal/mol), quercetin (−6.03 kcal/mol), myricetin (−5.80 kcal/mol) and berginin (−5.27 kcal/mol) were exhibited remarkable interaction with α-amylase at lower energy level. This indicates, the phytoconstituents of jamun tree could inhibit α-amylase in a more efficient way even with low binding energy than the synthetic compound acarbose. The results obtained from the present investigation given significant information that the phytochemicals of jamun tree could work better with the diabetic targets by binding with α-amylase and slows down the starch digestion and leads to slow release of glucose in the blood stream. Due to this mechanism, the jamun fruit seeds could control the blood sugar level more effectively. Hence, we could take some leads from phytochemicals of jamun tree towards the development/synthesis of α-amylase inhibitors for the management of diabetic patients.

The results of this study were supported by earlier research regarding the anti-diabetic activity of selected phytochemicals of jamun tree. Bergenin was isolated from the roots of *Caesalpinia digyna* and evaluated for antidiabetic (type 2) activity in streptozotocin (STZ)-nicotinamide-induced diabetic rats [52]. Antidiabetic and antioxidant potential of β-sitosterol isolated from *Solanum surattense* was evaluated

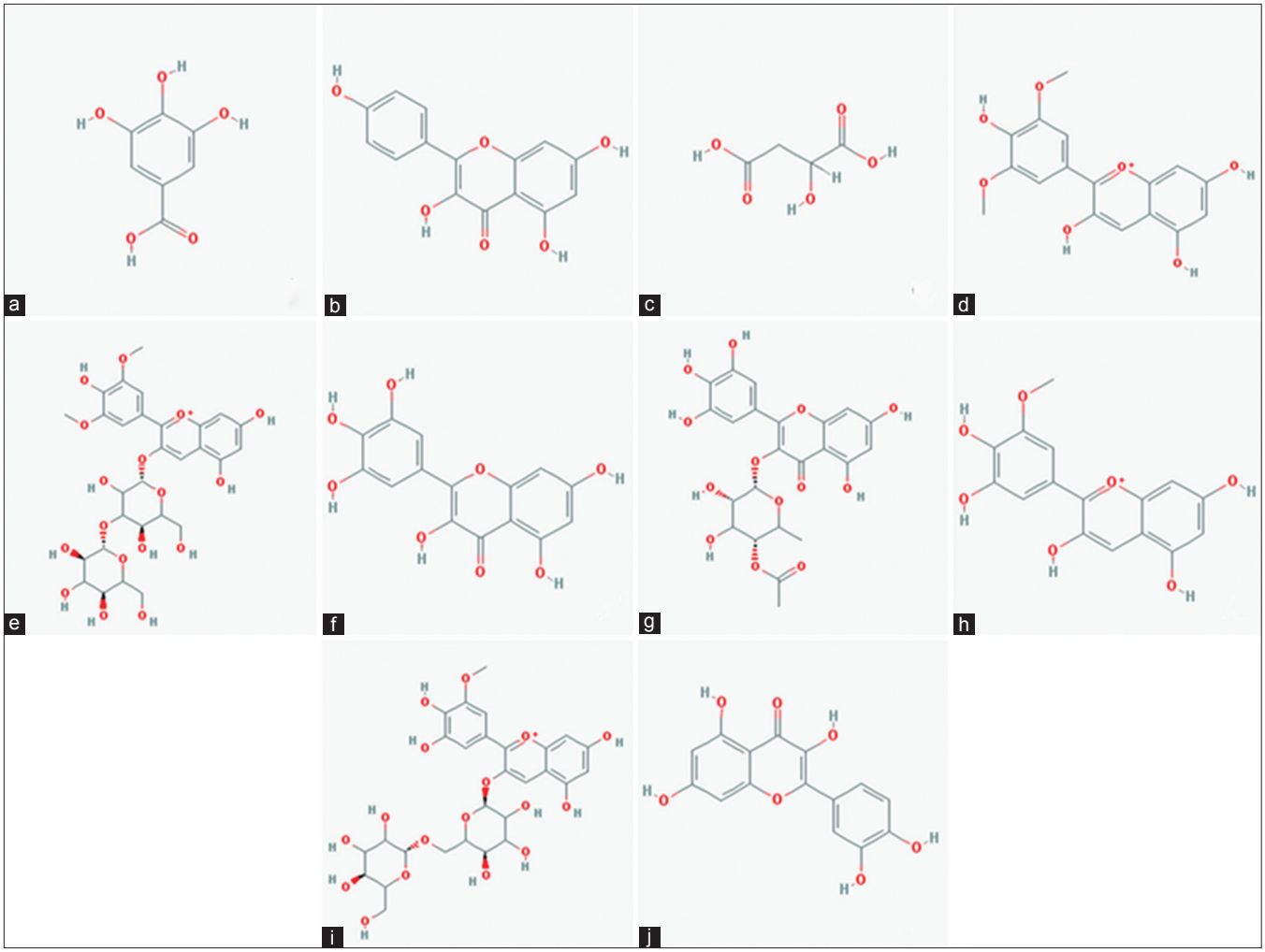

Fig. 3: Structure of phytochemical constituents of jamun tree: Gallic acid (a), kaempferol (b), malic acid (c), malvidin (d), malvidin-3-laminaribioside (e), myricetin (f), myricetin-3-o-4-acetyl-l-rhamnopyranoside (g), petunidin (h), petunidin-3-gentiobioside (i) and quercetin (j)

Table 2: Molecular docking results of α-amylase with different phytochemical compounds of jamun tree

S. No.	Name of the ligand	Binding free energy (kcal/mol)	Docking predicted inhibition constant (Ki)	Intermolecular energy	Internal energy	Torsional energy	RMS	Number of H bonds formed
1	Berginin	−5.27	136.98 uM	−7.36	−3.59	2.09	42.99	3
2	Beta-sitosterol	−8.56	527.25 nM	−10.65	−0.88	2.09	43.16	2
3	Betulinic acid	−8.60	500.92 nM	−9.79	−0.44	1.19	54.83	3
4	Citric acid	−3.20	4.55 mM	−5.88	−3.01	2.68	51.25	3
5	Cyanidine diglycoside	−3.92	1.33 mM	−9.29	−8.34	5.37	33.32	7
6	Delphinidin-3-gentiobioside	−2.30	20.63 mM	−7.97	−10.61	5.67	67.61	2
7	Ellagic acid	−6.69	12.52 uM	−7.88	−2.07	1.19	55.44	4
8	Epifriedelanol	−8.98	260.67 nM	−9.28	0.03	0.3	44.61	2
9	Eugenin	−5.48	96.88 uM	−6.07	−0.75	0.6	53.51	1
10	Friedelin	−9.54	102.15 nM	−9.54	0	0	45.66	1
11	Gallic acid	−4.60	421.71 uM	−6.1	−1.75	1/49	66.71	3
12	Kaempferol	−7.08	6.47 uM	−8.57	−1.51	1.49	51.74	2
13	Malic acid	−3.14	4.98 mM	−4.93	−3.06	1.79	65.26	1
14	Malvidin	−5.98	41.23 uM	−8.07	−1.19	2.09	45.4	2
15	Malvidin-3-laminaribioside	−2.94	6.96 mM	−8.61	−8.27	5.67	64.27	2
16	Myricetin	−5.80	56.48 uM	−7.88	−3.56	2.09	57.90	7
17	Myricetin-3-O-4-acetyl-L-rhamnopyranoside	−3.69	1.98 mM	−7.27	−7.36	3.58	33.78	2
18	Petunidin	−6.21	27.88 uM	−8.3	−1.1	2.09	49.87	3
19	Petunidin-3-gentiobioside	−7.52	3.06 uM	−13.19	32.56	5.67	65.73	1
20	Quercetin	−6.03	37.74 uM	−7.82	−2.87	1.79	45.61	1
21	Acarbose	−2.43	16.58 mM	−8.99	−10.52	6.56	39.08	4

RMS: Root mean square

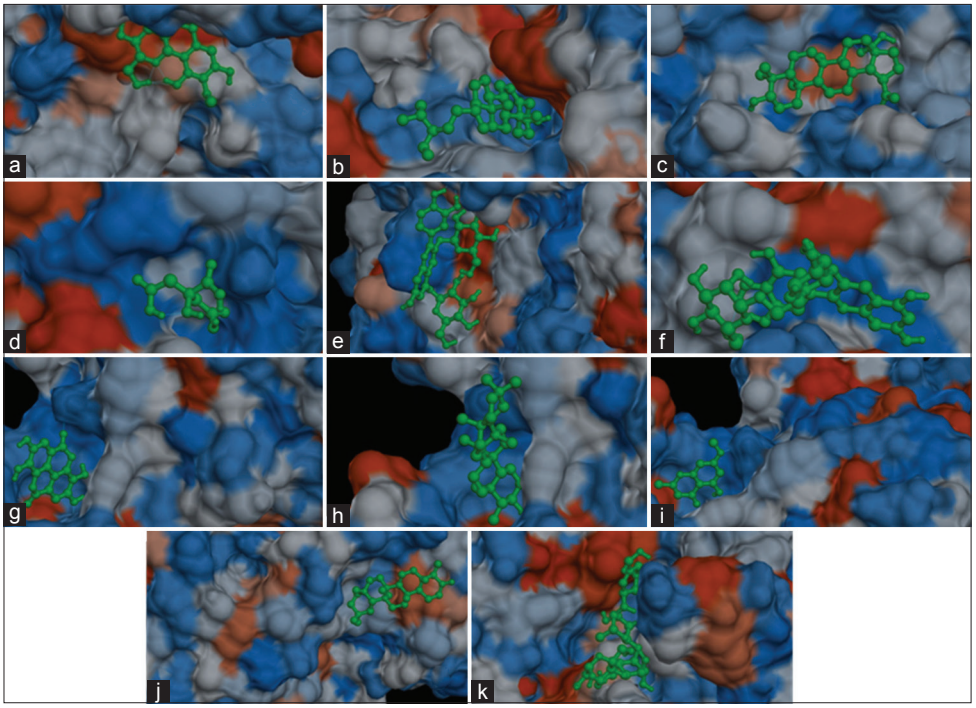

Fig. 4: Docking images showing the interaction of berginin (a), beta-sitosterol (b), betulinic acid (c), citric acid (d), cyanidine diglycoside (e), delphinidin-3-gentiobioside (f), ellagic acid (g), epifriedelanol (h), eugenin (i), friedelin (j) and acarbose (k) with α-amylase

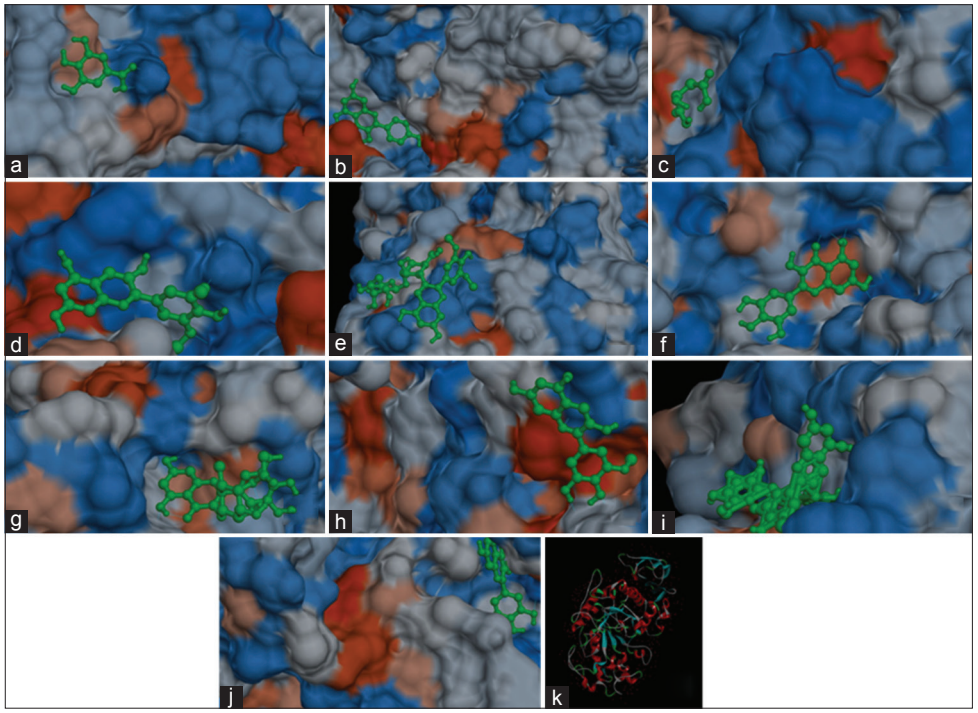

Fig. 5: Docking images showing the interaction of gallic acid (a), kaempferol (b), malic acid (c), malvidin (d), malvidin-3-laminaribioside (e), myricetin (f), myricetin-3-o-4-acetyl-l-rhamnopyranoside (g), petunidin (h), petunidin-3-gentiobioside (i) and quercetin (j) with α-amylase (k)

in STZ-induced diabetic rats [53]. Betulinic acid from *Dillenia indica* was reported to exhibit 47.4% α-amylase inhibition and significant anti-diabetic activity in STZ-nicotinamide induced diabetic mice at the dose of 10 mg/kg [54]. Uddin *et al*. [55] showed α-amylase inhibitory activity and hypoglycemic activity of *Citrus macroptera* fruit containing citric acid in hyperglycemic rats. Structure-activity relationship of cyanidin and its glycosides to inhibit pancreatic α-amylase was investigated under *in vitro* conditions [56]. Delphinidin-3-rutinoside was reported to stimulates glucagon-like peptide-1 secretion in Murine GLUTag cell line via the Ca2+/calmodulin-dependent kinase II pathway, and this mechanism is effective for controlling blood glucose levels in type 2 diabetic patients [57].

Fatima *et al*. [58] reported that the ellagic acid in *Emblica officinalis* exerts antidiabetic activity through the action on β-cells of pancreas. Gallic acid can increase GLUT4 translocation and glucose uptake

activity in an Akt-independent but wortmannin-sensitive manner [59]. Antidiabetic activity of kaempferol and its glycoside-rich fraction isolated from soybean leaf was evaluated in genetically type 2 diabetic mice [60]. Possible hypoglycemic effect in type 2 diabetic patients by *Aloe vera* high molecular weight fractions with malic acid was explained by Yagi *et al.* [61]. Antidiabetic and pancreatic regeneration potential of aerial parts of *Clitoria ternatea* containing Malvidin were reported [62]. Therapeutic potential of myricetin in diabetes mellitus was reviewed by Li and Ding [63]. Inhibitory effects of muscadine anthocyanins including petunidin on α-glucosidase were investigated by You *et al.* [64]. Intra-peritoneal injection of quercetin in STZ-induced diabetic rats significantly and dose-dependently decreased the plasma glucose level [65].

CONCLUSION

Interaction of phytochemical compounds of jamun tree with a well-known diabetic target α-amylase was revealed by the present study. Some of the phytochemical constituents such as friedelin, epifriedelanol, betulinic acid, beta-sitosterol, petunidin, kaempferol, quercetin, myricetin, and berginin can bind more effectively than the synthetic drug acarbose and inhibit the activity of α-amylase enzyme and thus slows down the glucose release in blood stream. Hence, the results of the present investigation offer scientific evidence for the antidiabetic potential of jamun tree parts by elucidating the mechanism of action shown by its bioactive principles. The chemical constituents of jamun tree with high potential to inhibit α-amylase could be chosen as a lead for further study and chemical synthesis as anti-diabetic agents for the management of type 2 diabetic patients.

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