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Estimation of total flavonoids and molecular docking of important flavonoids of *Sacostigma kleinii* family-*Icacinaceae* with MAO-A

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Abstract

Sarcostigma kleinii Wight & Arn. (Erumathali,Odal, Vattodal, Velloda in Malayalam) is one of the potential medicinal plants, widely used as a source of drug in the treatment of several diseases including psychiatric illness.Depression, or major depressive disorder, is a mental health condition marked by a feeling of sadness, isolation and despair that affects how a person thinks, feels and functions. Objective of this study was to investigate total flavonoids in the ethyl alcohol extract of plant leaves (EAESk) by spectrophotometric method and molecular docking with MAO- A using software Autodock 4.2 using graphical interface pyrex. Total Flavanoid content of EAESk in terms of quercetin units.was found to be 28.06% by spectrophotometric method. Luteolin (a flavone) docked with docking score -8.5 which is having 5, 7, 3′ and 4′ substituted hydroxyls and a double bond presence at carbons 2 and 3, which are responsible for their multiple pharmacological effects. Quercetin (a flavonol) docked with docking score -8.3 that has an OH group attached at positions 3, 5, 7, 3′, and 4′. From these results we can conclude anti-depressant potential of Ethyl alcoholic extract of leaves of *Sarcostigma kleinii* in depression like symptoms due to presence of flavonoids which binds with MAO-A.

Keywords: ethyl alcohol extract of *Sarcostigma kleinii* (EAESK*)*, major depressive Disorder (MDD), total flavanoid content, molecular docking, monoamine oxidase a (MAO-A)

Introduction

Depression, or major depressive disorder, is a mental health condition marked by a feeling of sadness, isolation and despair that affects how a person thinks, feels and functions. The condition may significantly interfere with a person's daily life and may prompt thoughts of suicide. Depression isn't the same as sadness, loneliness or grief caused by a challenging life experience, such as the death of a loved one but a condition from which not able to come out by oneself. Depression can affect people of all ages, races and socioeconomic classes, and can strike at any time. The condition is found twice in women due to the hormonal changes than in men [1]. Antidepressant drugs are having many adverse effects which may lead to toxicity. Antidepressants help in altering mood by affecting naturally occurring neurotransmitters in brain. There are several categories of antidepressants [2], which includes MAO-A Reversible inhibitors like Moclobemide. To overcome adverse effects natural medicines can be used for treatment of depression which will have very less side effects. *Sarcostigma kleinii* Wight & Arn. (Erumathali, Odal, Vattodal, Velloda in Malayalam) [3] is one of the potential medicinal plants, widely used as a source of drug in the treatment of several diseases. This is seen in Evergreen and semi- evergreen forests, also in sacred groves and in all districts of Kerala. The plant's bark and leaves are bitter, acrid, thermogenic, anthelmintic, digestive, carminative, diuretic, anaphrodisiac, depurative, vulnerary and stomachic [4]. The entire plant was recognized as valuable drug and frequently used by many of the ancient traditional medical systems. The leaf extract showed the highest total phenolic content and total flavonoid content and the best antioxidant activity [5]. A wide number of biomolecules from plants of various families belonging to diverse chemical classes have been shown to inhibit human MAOs. Among them, flavonoids have attracted more interest as they possess a variety of biological activities such as anti-oxidation and their effects on the central nervous system. Only few docking studies are available on these compounds in order to clarify the interaction of the compounds with the active site of HMAO [6]. Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) dependent enzyme which is mainly localized on the outer mitochondrial membrane, responsible for the oxidative deamination of monoamines, including neurotransmitters. MAO-A preferentially deaminates noradrenaline and serotonin and regulates both the free intra neuronal concentration and the releasable stores of 5-HT and noradrenaline, which is inhibited by MAO A inhibitors [7].

Materials and Methods

Estimation of total flavanoids

The flavanoids estimation was performed by spectro photo metric method [5].

Stock Solution of Extracts:

10 mg of each extract was accurately weighed and made upto 1ml with DMSO.

Materials required

- Methanol
- Aluminium Chloride
- Potassium Acetate solution
- Distilled water

Procedure

0.5mL of sample, 1.5 mL methanol, 0.1 mL aluminium chloride, 0.1 mL potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium

chloride with distilled water. The absorbance was measured at 415 nm using UV-VISIBLE spectrophotometer (Agilent, Cary 60) against the blank and the values obtained were interpreted using the standard graph of quercetin to get the milligram equivalents of quercetin.

Docking

Molecular docking-steps[8] using software autodock 4.2 using graphical interface pyrex.

Molecular Docking-steps [8] involve Ligand preparation-2D structures of compounds Chicoric acid, Proto catechuic acid, Luteolin and Quercetin were drawn using Chem Draw Ultra 8.0, converted to 3D structure using Chem 3D Ultra 8.0 of Chem office and saved in MDL Mol format, converted to SDF format making use of open babel system module of PYRX. Energy minimization was done using uff force field and saved in PDB format. Enzyme preparation-Downloaded 2Z5X (MAO A with native ligand Harmine) in PDB format which was opened in word pad and deleted native ligand part and saved file as 2Z5X without ligand. Ligand was prepared for docking by selecting the option "make ligand" and enzyme was prepared by selecting "make macromolecule". Grid generation was performed after selecting one aminoacid. Selected amino acid was Tyr 444. Then docking was performed by Vina Wizard module of PYRX selecting forward option, save docking result in CSV (Comma Separated Values) format. Final ligand pose after docking was saved in PDB format. Lig pose was opened in word pad, cut and pasted with 2Z5X (native ligand free) opened in pymolwin, and got photos. Labelled residues like Tyr 444, and saved image in PNG format. Ligand Interaction Diagram (LID) was obtained from Molegro Molecular viewer. Most negative docking score is most stable since after enzyme ligand interaction energy of system will reduce.

Results and Discussion

Total flavanoid content

Table 1: Standard table values of concentration Vs Absorbance - Flavanoids (quercetin)

Concentration (mg/mL)	Absorbance
Standard: Quercetin	
0.1	0.0364
0.2	0.0662
0.4	0.1047
0.6	0.1521
0.8	0.1997
1	0.2257

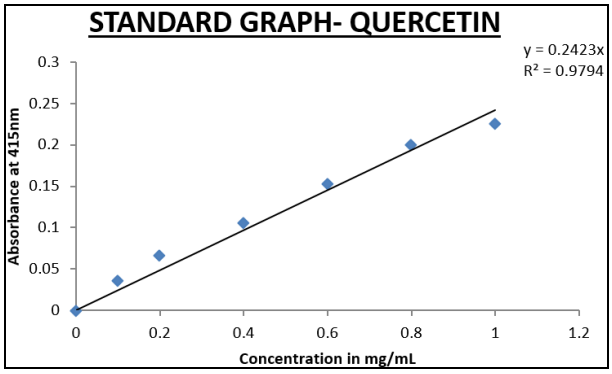


Fig 1: Standard graph of quercetin

From the equation y=0.2423 xx =0.3400/0.2423= 1.403219 ie, 0.5mL contains 1.403219 mg in 10mg So1mL contains 1.403219X2=2.806438 mg in 10 mg (stock solution of extract 10mg in 1mL)

So in 1 mg, 0.2806438 mg is present.

Table 2: Amount of flavanoid in terms of quercetin units

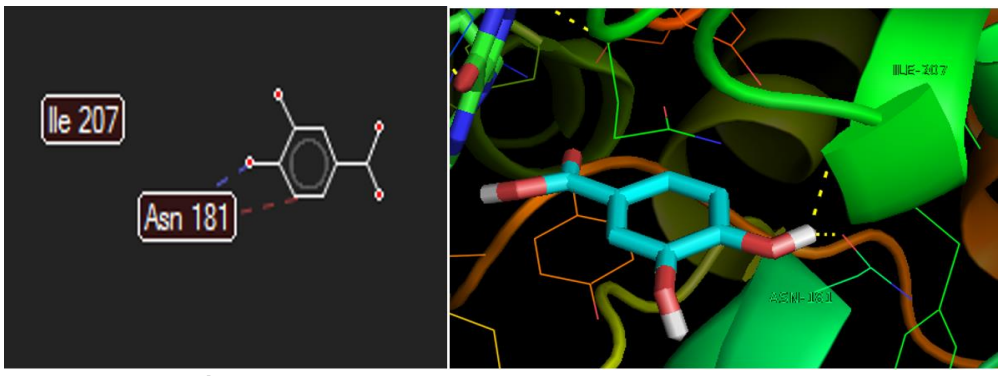
Sample code	Absorbance at 415nm	Amount of flavanoid in terms of quercetin units
EAESK	0.3400	0.2806

Total Flavanoid content of EAESk was found to be 0.280644 mg in terms of quercetin units.

Quercetin was used as a standard.Various concentrations of quercetin solution were used to make a standard caliberation curve.Total Flavanoid content percentage of EAESk in terms of quercetin units.=28.06% This indicates that the flavonoids are the major phenolic compounds present in the *S. kleinii* plant.

Docking

Proto catechuic acid



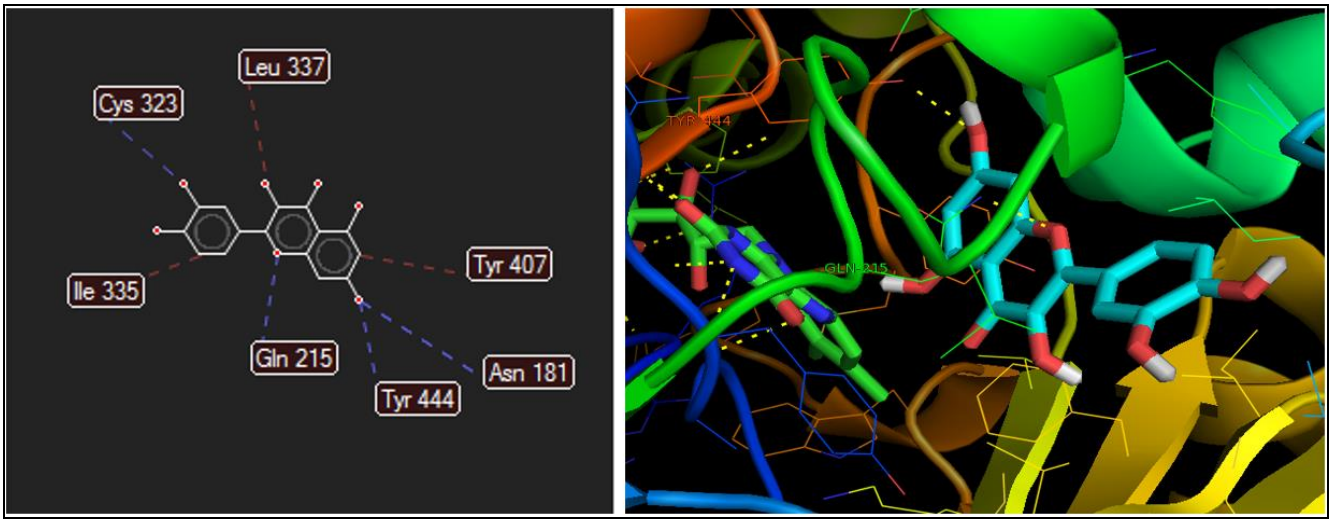
A **B**

Fig 2

A. Electrostatic and van der Waals interactions of proto catechuic acid
B. two dimensional orientations of proto catechuic acid with active site of MAO-A

The analysis of structures shows that proto catechuic acid shows many Electrostatic and van der Waals interactions with MAO-A. of the two hydroxyl groups attached to benzene ring one –OH attached to benzene ring will be showing hydrogen bonding with ASN-181.

Quercetin



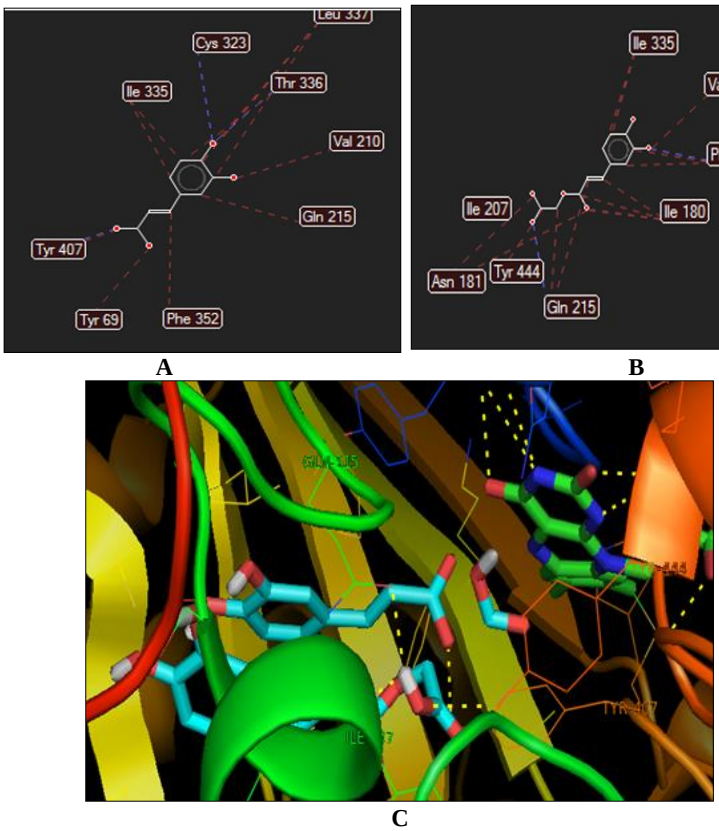
A **B**

Fig 3

A. Electrostatic and van der Waals interactions of Quercetin.
B. Two dimensional orientations of Quercetin with active site of MAO-A.

The analysis of structures shows that quercetin shows many Electrostatic and van der Waals interactions with MAO-A. The –OH groups present in the structure will be forming hydrogen bonds with Cys-323, Gln-215, Tyr -444, and Asn-181.

Chicoric acid



A **B**

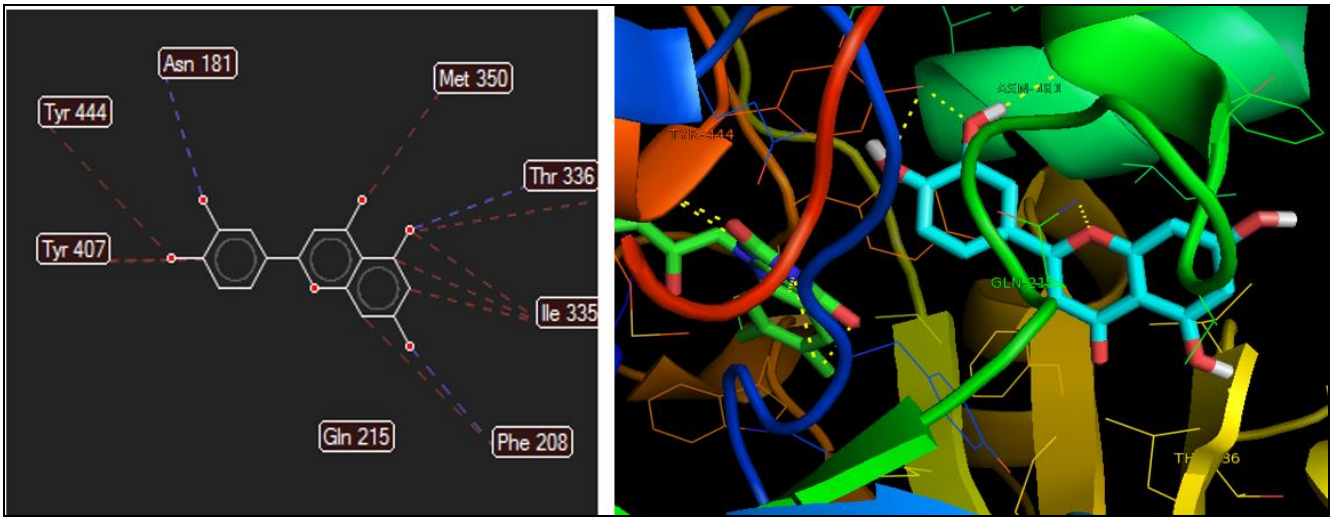
C

Fig 4: A and B. Electrostatic and van der Waals interactions of Chicoric acid C. Two dimensional orientations of chicoric acid with active site of MAO-A

The analysis of structures shows that Chicoric acid shows many Electrostatic and van der Waals interactions with MAO-A.

Hydrogen bonding is seen between -OH group in chicoric acid and cys 323 and the second-OH group attached to benzene ring also shows hydrogen bonding with phe 208 and one–OH group in the chain shows hydrogen bonding with Gln-215.

Luteolin



A **B**

Fig 5: A. Electrostatic and van der Waals interactions of Luteolin. B. 2 dimensional orientations of Luteolin with active site of MAO-A.

The analysis of structures shows that luteolin shows many Electrostatic and van der Waals interactions with MAO-A. The –OH groups present in the structure will be forming hydrogen bonds with Asn-181, Thr-336 and Phe-208.

The analysis of structures showed that luteolin with docking score -8.5 shows many Electrostatic and van der Waals interactions with MAO-A. The–OH groups present in the structure will be forming hydrogen bonds. Asn-181with -OH group of 3' position of ring B, Thr-336 with -OH group of 5th position and Phe-208 with -OH group of 7th position of ring A, formed hydrogen bonds. The analysis of structures also showed that quercetin with docking score -8.3 shows many Electrostatic and van der Waals interactions with MAO-A. The –OH groups present in the structure will be forming hydrogen bonds. Cys-323 with -OH group of 3' position of ring B, Gln-215 with_hetero atom (O) of 1st position of ring C, Tyr -444 with -OH group of 7th position and also Asn-181 with -OH group of 7th position of ring A, formed hydrogen bonds.

Conclusion

From Amount of flavanoid in terms of quercetin units there is a possibility of having great potential as biomedicine for depression.

Luteolin (a flavone) with docking score -8.5 was found to have 5, 7, 3′ and 4′ substituted hydroxyls and a double bond presence at carbons 2 and 3, which are responsible for their multiple pharmacological effects. Quercetin is a flavonol with docking score -8.3 that has an OH group attached at positions 3, 5, 7, 3′, and 4′. The results showed that all of the flavonoids tested possessed a degree of selectivity for MAO-A, which suggests that these compounds are highly selective towards MAO-A. Among them, Luteolin appears as remarkably selective, since binding was better than that of other tested flavonoids. From these results we can conclude anti-depressant potential of Ethyl alcoholic extract of leaves of *Sarcostigma kleinii* in depression like symptoms due to presence of flavonoids (which are the major phenolic compounds present in the *S. kleinii* plant.) which bind with MAO-A.

Discussion

Computational docking analysis confirmed the selective binding of some flavanoids to MAO-A showing a potential therapeutic application for the treatment of depression.The main purposeof docking analysis in this study was to determine favorable binding conformations between flavonoid members and MAO-A. The results suggest two compounds, luteolin and quercetinas potential natural starting molecules for developing novel selective MAO-A inhibitors, for prevention and treatment of psychiatric disorders such as depression, anxiety etc. *In silico* studies revealed that all the selected compounds have relatively lesser binding energies but luteolin and quercetin showed lowest. Hence this study has widened the scope of developing these compounds or their derivatives as promising antidepressant agents.The present study provides an evidence for the isolated flavonoids from *Sarcostigma kleinii* as new potent and selective MAO-A inhibitor. The results suggest two compounds, luteolin and quercetin as potential natural starting molecules for developing novel selective MAO -A inhibitors, for prevention and treatment of psychiatric disorders such as depression, anxiety etc. They may serve as new candidates for selective MAO-A inhibitor. The MAO inhibiting activity of *Sarcostigma kleinii* can be primarily due to the presence of flavonoids such as luteolin and quercetin supported by computational docking analysis of MAO-A complexed with luteolin and quercetin.

Conflicts of Interest

No conflicts of interest

Acknowledgement

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