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IN SILICO EVALUATION OF CAFFEIC ACID FROM RUTA GRAVEOLENS AGAINST MIGRAINE

Dhisha Vasudevan[1], Nikkila Devi Ravichandran[1], Priya Raman[2], Mahalakshmi Sundarapandian[1]*

[1]JSS Academy of Higher Education and Research, Ooty Campus.
[2]School of Agricultural Sciences, Amrita Vishwa Vidyapeetha, Coimbatore

***Corresponding author – mahasundar@jssuni.edu.in**

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ABSTRACT
The plants belonging to the Rutaceae family have many beneficial and harmful properties. *Ruta graveolens* is an herb, which has more medicinal qualities. Different varieties of essential oil and compounds have been already extracted from this plant. Many research works have been done using Rue. The compound selected for the study from Rue is **Caffeic acid**. It has activities like anti-inflammatory, anti-tumor, and antioxidant. This acid is present in many foods and vegetables which we consume regularly. Caffeic acid resembles aspirin in its molecular formula but with a different structural arrangement; hence caffeic acid was compared with aspirin for its activity. Aspirin is mainly used for migraine headaches. Migraine is a neurological disorder where headache occurs on one side of the head. Migraines trigger seizures which may cause epilepsy. So, *in silico* method (i.e.) molecular docking was done to study whether caffeic acid can replace aspirin and act as a lead to treat migraine. The ligand and protein were selected and docked using the software AutoDock Vina.
KEYWORDS: *Ruta graveolens*, Caffeic acid, Aspirin, Migraine, *In silico*.

INTRODUCTION

Migraine is a chronic neurological disorder where the main feature is a headache and other associated features like nausea, sound and light sensitivity, and a negative feeling. Migraine is considered a vascular disorder—the duration of a migraine headache last from 6 hours to 24 hours. In children, it can be less than 6 hours. Mainly migraine headaches occur on one side of the head; rarely, it is bilateral. A migraine headache can also affect the functions of other organs in the body [1,2]. Migraine triggers stress, hormone changes, foods, skipping meals, caffeine, changes in weather, physical activity, and changes to your sleep. There are different types of migraine-like, migraine during menses, silent migraine, migraine in the vestibules, migraine in the abdominal region, hemiplegic migraine, retinal and ocular migraine, classic migraine, status

migrainosus (migraine that lasts for more than 72 hours), and recurrent painful ophthalmoplegic neuropathy [3].

Epilepsy is a state that influences the brain and gives rise to periodic seizures, otherwise known as brain dysfunction. Seizures are bursts of uncontrolled (electrical) activity in the brain [4,5]. Epilepsy can start in all age groups, either in childhood or in people over 60 years [6]. Worldwide 50 million people are affected by epilepsy, of which 2-3 million are in the United States, 6 million in Europe, and in developing countries, it is about 40 million [7]. Seizures are classified into two types, namely generalized and focal. A person's seizure type determines what kind of epilepsy they have [8].

Many studies show that migraine has a connection with epilepsy, shortly known as migraine-induced epilepsy. People with migraines with epilepsy had an increased risk [9]. All plants synthesize a phenolic compound called caffeic acid. This acid is present in tea, coffee, wine, and propolis medicine. This compound has anti-inflammatory, antioxidant, and antitumor activity [10].

Caffeic acid (3,4-dihydroxy-cinnamic acid) is present in foods like turmeric, basil, thyme, oregano, radishes, mushrooms, kale, sage, cabbage, apples, strawberries, cauliflower, pears, and olive oil. Taking caffeic acid can prevent early aging, diabetes, neurodegenerative diseases, and so on. It protects against Alzheimer's disease, skin from the sun and used as a supplement for weight loss, also used to treat viruses like herpes and HIV, and to raise athletic performance [11].

ABOUT THE PLANT

Rutaceae family, otherwise called as Citrus family and consists of aromatic plants. It comes under the order Sapindales. Plants under the family Rutaceae can be herbs, shrubs, or trees. *R. graveolens* is one of the essential shrubs under this family. The Mediterranean region is the native of *R. graveolens* and is used mainly in Europe [12,13,14]. *R. graveolens* has been used as traditional medicine since the ancient period [12,15]. *R. graveolens* is called 'Rue' or 'Garden Rue' or 'Herb of grace' [12,13,15]. Rue has more medicinal qualities and is a remedy for eye pain, gastric problems, headache, rheumatism, edema, inflammation, hypertension, skin problems, etc. [12,14,15].

Rue is an aromatic everlasting shrub with yellow flowers and blue-green leaves [13]. The plant is about one meter high (approximately), aromatic with an unpleasant odor and an ornamental evergreen shrub. *R. graveolens* leaves are small, oblong, pinnate, deeply divided, and glandular dotted. The small yellow flowers are arranged in cluster form in the branched stem. All flowers in this plant have four petals, whereas the flower in the centre has five petals in a particular season, such as spring and summer. The fruits of *R. graveolens* are round, small, 4 or 5 lobed, and greyish-brown. The fruits taste intensely bitter. The height of *R. graveolens* is about 2 to 2½ feet. *R. graveolens* seeds are ovoid, flattish in front, rounded on the back, angular, rough, and Testa blackish; the embryo is curved from base to apex, surrounded by fleshly endosperm [16,17]. The seeds will get ripen from August to October. The shrub can grow well in soil conditions such as well-drained soil and nutritionally poor soil. *R. graveolens* grows without shade or in half shade.

120 compounds are present in the plant. *R. graveolens* has been used in clinical conditions from ancient periods [18]. Common Rue consists of chemical constituents, alkaloid extract like acridone, and quinoline, where it has spasmolytic action [19]. *R. graveolens* has antioxidant activity, anti-inflammatory activity, and cytotoxic activity on the human cancer cell, anti-tumour activity, anti-arrhythmic activity, anti-oxidative activity, anti-microbial activity and cytotoxic

activities, anti-androgenic activity, anti-conceptive, anti-fertility activity and also anthelmintic, antiepileptic, antispasmodic, rubefacient, antidote, haemostatic, antidiarrheal, ophthalmic, and stomachic. This plant has antifungal properties and is used in infections like athlete's foot and dermatitis.

Ruta graveolens is used as traditional medicine against stimulants, emmenagogues, diuretics and resolvent [16]. This plant also treats stiff neck, gastric disorders, dizziness, and headache. The infusion can also treat infantile paralysis of the shrubs leaf. It is used as a nasal drop.

Figure 1: *Ruta graveolens* flower

Figure 2: *Ruta graveolens* fruit

Figure 3: *Ruta graveolens* leaves

Both migraine and epilepsy are different, but they cause paroxystic neurological cases. Welch and Lewis recorded migraine-epilepsy syndrome under "a classification of migraine-related epilepsy" [9]. Migraine affects people below the age of 50, and worldwide it is widespread. Symptoms of migraine include sorrow, pain in the neck, and stress [20]. Migraine is also associated with photophobia and phonophobia. The word migraine came from the Greek word hemicranias, meaning "half of the head"- migraine originates from factors like genetics and environment.

Different phases of migraine are,

- Prodromal phase
- Postdrome phase
- Aura phase
- Headache phase [21].

Some electrical disturbance occurs in the brain in these two diseases. The headache and aura phase of migraine may activate epileptic seizures [22]. Migraine causes cerebral damage to the brain and leads to epilepsy [23]. Both epilepsy and migraine can cause by some mutations in the genes. Epileptic seizures may be due to brain electrical impulses, and migraine is a chronic pain condition. Paroxysmal indication and episodic disease are identified in migraine and epilepsy [24]. Similar properties are present in the drugs which are used for the prevention of migraine and epilepsy. Primarily this is identified in paediatric neurology. Migraine raises the possibility of epilepsy and vice versa; a bidirectional mechanism proposed this. Most children are affected

by 'abdominal epilepsy'. "Migralepsy" is there in some patients in consecutive order of headache and seizure [25]. Severe migraine is seen in (PNEs) Psychogenic non-epileptic seizures rather than in epilepsy patients [26]. People with epilepsy have 52% migraines compared to those without epilepsy [27].

Caffeic acid has a vigorous antioxidant activity, more collagen (protein) production and stops premature ageing. It can also be used to treat dermal diseases because of its antimicrobial activity. The use of caffeic acid has become more in people [28]. The molecular formula of Caffeic acid is $C_9H_8O_4$.

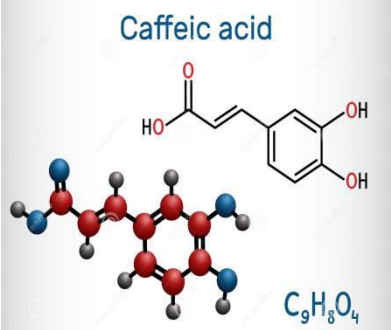

Figure 4: Caffeic acid [29]

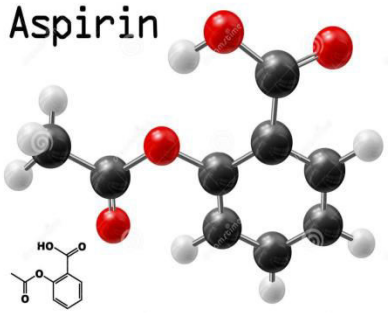

Figure 5: Aspirin [30]

There was an analysis of chlorogenic acid and caffeic acid in humans. They found that the small intestine absorbed all the caffeic acid and one-third chlorogenic acid. Finally, they found almost all the caffeic acid and only 11% chlorogenic acid was excreted in urine [31]. Propolis extract has a compound of a biological effect called CAPE (Caffeic acid Phenyl ester), i.e. 2-phenylethyl-3-(3,4-dihydroxyphenyl) acrylate [32]. It has more therapeutic qualities and is effective against stress, cancer, anxiety, and a few more. Caffeic acid inhibits microbes and insects, so it acts against pests, infections, and predators. It also protects the plant leaves from UV-B (Ultraviolet radiation B) [33].

Escherichia coli strain was constructed for the production of caffeic acid by a pathway [34]. Other common names for caffeic acid [35] are3,4-Dihydroxycinnamic acid, 2-Propenoic Acid, etc.

Aspirin has been used for treating headaches for many years, and now (NSAIDs – Nonsteroidal anti-inflammatory drugs) are mainly used, specifically in migraines [36]. The aspirin used in the early days was of low dose and was not considered under NSAID drug. NSAIDs include ibuprofen, diclofenac, naproxen, celecoxib, etoricoxib, mefenamic acid, indomethacin, and high-dose aspirin [37].

It also acts as an anti-inflammatory for migraine by blocking prostaglandin production [38]. Prostaglandins, a family of fat-derived molecules, which John Vane introduced in 1971. He is a British Pharmacologist. So, by taking aspirin, it can stop the production of prostaglandins [39]. Aspirin is available in the name of Acetylsalicylic acid (ASA). Felix Hoffmann was the man who synthesized Aspirin for the first time at Bayer. It is a synthetic drug. The molecular formula of aspirin is **$C_9H_8O_4$**. Aspirin is also known as 2-(acetyloxy) benzoic acid. Aspirin also has some side effects. Aspirin is called a COX inhibitor agent, where it inhibits the COX enzyme (Cyclooxygenase). It has two isoenzymes, COX 1 and COX 2, the prostaglandin-endoperoxide synthase [40].

By taking Aspirin, it stops the working of cyclooxygenase, and it will block the prostaglandins synthesis. Heart patients mostly take aspirin because it prevents diseases caused by blood clots [41]. The working of COX 1 will be stopped by taking Aspirin. Aspirin is mostly available in 2 doses,

- Baby dose (81 mg)
- Normal dose (325 mg) [42].

Thromboxane A2 (TXA2) was formed from Arachidonic acid produced by the prostaglandins with the help of COX 1, which causes platelet aggregation. So, taking aspirin stops the COX-1 enzyme, and further process will be stopped. COX-1is used in the prevention and treatment of heart attack and stroke. Whereas COX-2 in pain, inflammation, fever, and a few more. COX-2 has some inhibitors like etoricoxib, celecoxib, valdecoxib, rofecoxib, lumiracoxib [43]. But aspirin causes Reye's Syndrome when it is taken by youngsters and adolescents [44]. (Aggrerenox) an aspirin comb, where it has aspirin and dipyridamole. Aspirin stops COX-1 and then stops TXA2. Dipyridamole blocks the response to ADP, which is used only for secondary stroke prevention [45].

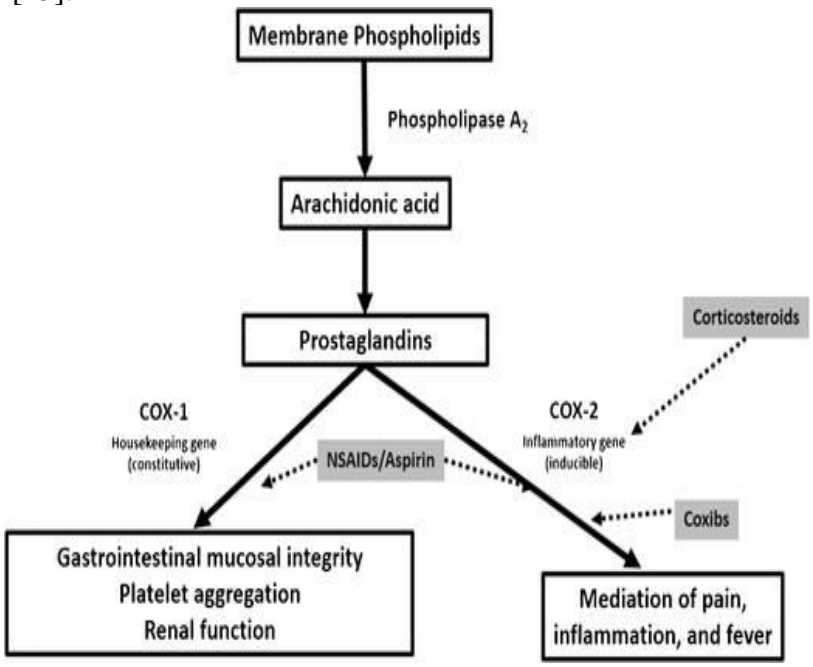

Figure 6: Aspirin mechanism of action in Migraine [46]

Molecular docking comes under the *insilico* methodology. Molecular docking is a tool used to design a drug. There are different kinds of software used for docking. Docking mainly requires Protein and Ligand [47].

Recently molecular docking is also used in food science, food safety and nutrition [48]. Molecular docking can be a protein-protein binding or protein-ligand binding. The ligand binds to the target site of protein to receive specific activity. Molecular docking gives information for which protein and ligand to bind, which is called binding affinity [49]. The protein-ligand binding has two parts: rigid and flexible docking

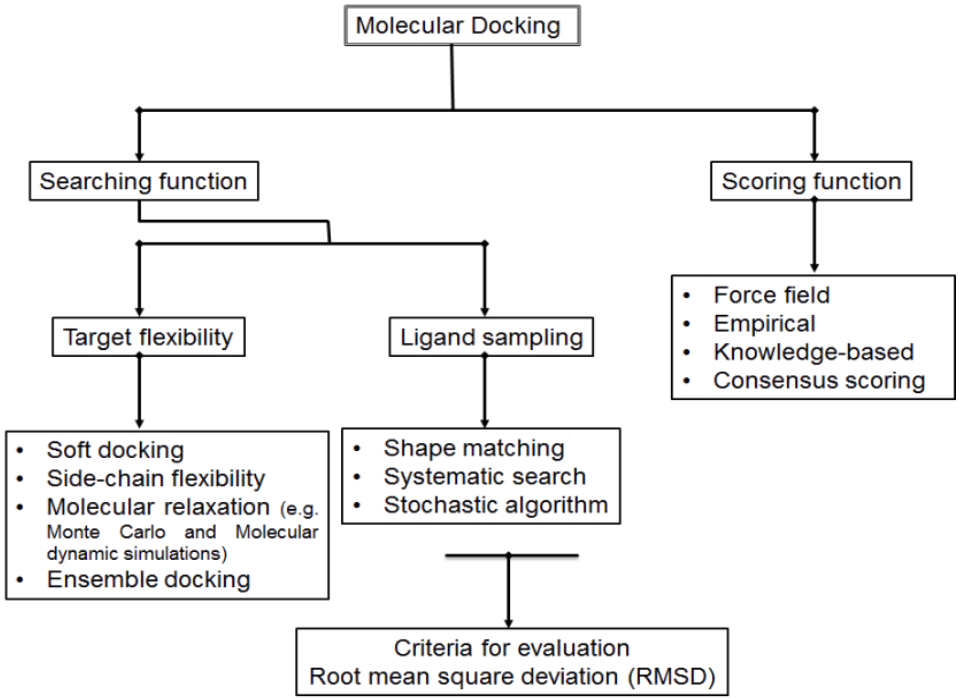

Figure 7: Steps involved in Molecular docking

MATERIALS AND METHODS

- Files Required:
  - ✓ PDB File of Receptor
  - ✓ PDB File of the Ligand Molecule
  - ✓ PDBQT File of the Ligand and the Receptor
- Software Required:
  - ✓ Auto Dock Vina
  - ✓ FireDock
- Online Server Requirements:
  - ✓ hz.rcsb.org

METHODOLOGY:

In this *in silico* based screening, the receptor prostaglandins COX 2 was selected, and the ligand Aspirin and Caffeic acid were selected.

Preparation of Protein Molecule:

Prostaglandins COX 2 was downloaded from the Protein Data Bank (PDB), and refinement was done using FireDock. The numbers of lipids made at the sites of tissue damage are known as Prostaglandins. Prostaglandins can control blood flow and the blood clot

Selection of Ligand Molecule:

The ligand Aspirin and Caffeic acid was selected from PubChem.

MOLECULES

1. **Aspirin:** Carboxylic acid is the principal functional group of Aspirin

2. **Caffeic acid:** The compounds belonging to hydroxycinnamic acid (i.e.) sodium caffeate and trans-caffeate is also known as Caffeic acid.

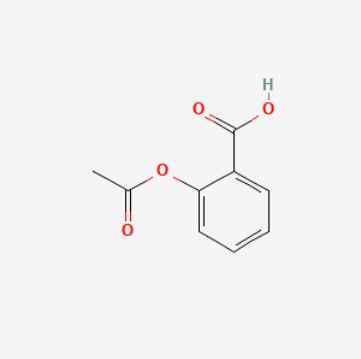

Figure 8: Aspirin

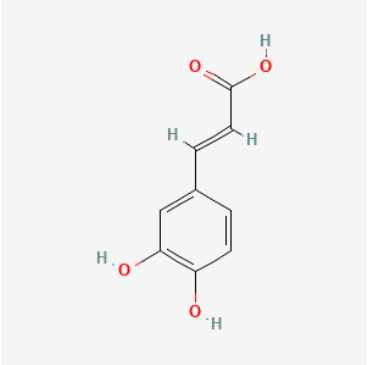

Figure 9: Caffeic acid

DOCKING:

AutoDock Vina was the software used in the present docking study.The most used tool for the *in silico* method is AutoDock Vina. Through this tool, the ligand and protein molecules are docked.

Inputs:

- In AutoDock, there are some steps to examine docking. The form has the sample file that was directly uploaded.

Target Protein Selection:

- With the help of PDB ID or files, the proteins were loaded after processing.
- The protein Prostaglandins COX 2 was loaded.
- The active sites of the receptor were taken care of before uploading the target protein.

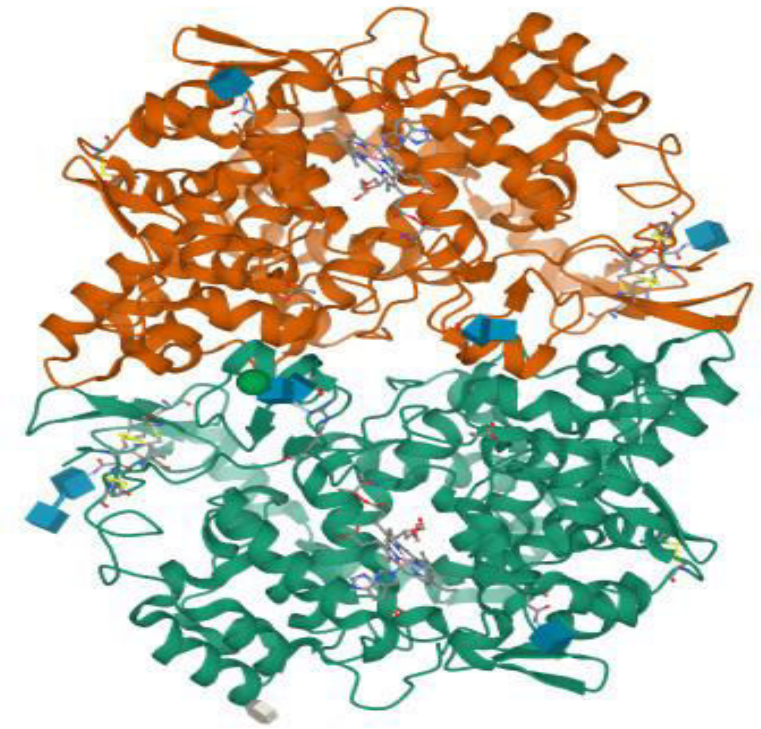

Figure 10: Murine COX-2 S530T mutant

Ligand molecule selection:

- By uploading the PDB File of the ligand molecule the ligand was selected.
- The ligand Aspirin and Caffeic acid was selected and moved on to the next step.
- Both ligands undergo all the possibilities of the active site. And then docked onto the molecule.

Outcome:

To understand whether the ligand chosen can be an effective inhibitor, a text file with the value of the docking energy is obtained after docking is completed. Based on the values ligands can be selected.

Analysis and Active Site Production:

The active site of Prostaglandins is COX 1 and 2. For the present study COX 2 binding site was selected.

Docked Molecule - Structural Analysis:

- The binding energy values obtained after docking were compared with the reference molecule Aspirin.
- The Caffeic acid-binding energy is checked from the nine confirmations.
- And the comparison of the molecule is made.

RESULTS & DISCUSSION

Caffeic acid compounds from *R. graveolens* which belongs to the Rutaceae family, were assessed for the activity of migraine and migraine-induced seizures by using an AutoDock software program.

Different parameters, which include the interactions and binding energy, are acquired from the software AutoDock Vina. Readings which obtained was compared with the reference molecule Aspirin.

Through number of modes the binding affinity of the compound has been found. If the binding energy is low, it indicates the best affinity.

Aspirin:

In the 9 confirmations, the ligand aspirin has binding affinity of **-5.8 (kcal/mol)**.

Caffeic acid:

The ligand Caffeic acid has a best binding affinity of **-6.2 (kcal/mol)**.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-5.8	0.000	0.000
2	-5.8	2.562	2.999
3	-5.6	70.782	71.717
4	-5.5	73.771	74.730
5	-5.5	3.472	5.904
6	-5.3	40.767	41.210
7	-5.2	80.056	81.432
8	-5.1	40.873	42.189
9	-5.1	73.656	74.562

Figure 11: Aspirin

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000
2	-6.0	22.061	23.632
3	-6.0	1.731	2.418
4	-5.7	1.872	2.484
5	-5.7	22.649	23.970
6	-5.6	68.656	70.566
7	-5.6	2.313	3.036
8	-5.6	20.219	21.710
9	-5.6	15.486	17.097

Figure 12: Caffeic acid

When Aspirin and Caffeic acid was compared, it was found that a phenolic compound Caffeic acid was having more affinity power than a reference compound Aspirin. Where the binding energy of Aspirin is **-5.8 (kcal/mol)** and binding power of Caffeic acid is **-6.2 (kcal/mol).**

In the table rmsd is known as **(**Root Mean Square Derivation) and (l.b, u.b) means lower and upper bond. In which the different ligands (Aspirin and Caffeic acid) are docked in the same protein (COX- 2) lacking the presumption of known atomic sequence between two files.

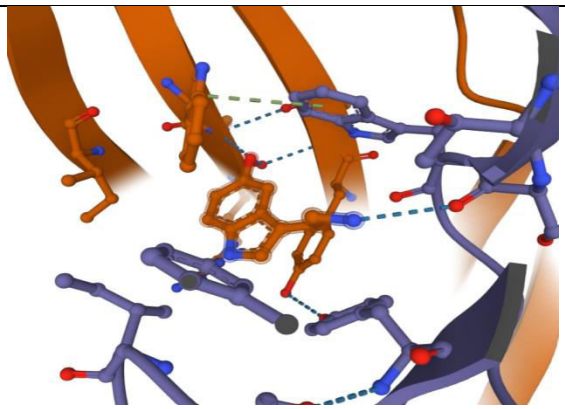

Figure 13: Docking of Caffeic acid
Blue color represents caffeic acid and brown color indicates COX 2.Caffeic acid has best affinity power.

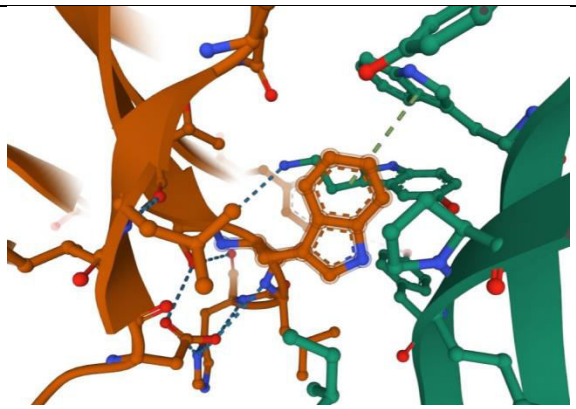

Figure 14: Docking of Aspirin
COX 2 is seen in the color brown and aspirin in green color. Affinity binding is -5.8 (kcal/mol).

SUMMARY AND CONCLUSION

Ruta graveolens an evergreen herb is known worldwide. Due to its bitter taste, the usage of this plant has been reduced. This plant is used as a flavoring agent and is approved by FDA. This plant has many activities. Actions like antiseptic, stimulant, emmenagogue irritant and abortifacient are present. For children, the juice of this plant is given because of its expectorant and antispasmodic action. In the case of worms, the leaves of rue are hung around the children's neck. It is used in the first stage of paralysis and as a fumigant. Vermicidal activity is seen in the oil extract of Sudab. *R. graveolens* should not be used during pregnancy.

R. graveolens consists of many compounds, where caffeic acid was selected because of its beneficial actions. Caffeic acid is a phenolic compound. It is present in various fruits, many vegetables, and seasonings. In the human diet, coffee is the primary source of caffeic acid. Wine contains a maximum amount of caffeic acid. Aspirin and caffeic acid had more or less similar structures and functions. Aspirin is a drug that is commercially available in the market for treating pain, fever, and another disease. Aspirin is commonly used to treat migraine headaches. The aspirin mechanism of action in migraine was studied, and found that COX-2 prevents fever, inflammation, and pain. Migraine is prevalent in children, adults, and older adults. In the early stage, it is difficult to identify and cure. When it is not treated properly, it may also lead to migraine-induced seizures.

So, to know the action of caffeic acid molecular docking was done. Here the binding affinity of caffeic acid was compared with aspirin, and docking was done with the protein COX-2. After docking, the binding affinity of aspirin was **-5.8 (kcal/mol),** and caffeic acid was **-6.2 (kcal/mol).**

Finally, caffeic acid has good affinity power when compared to aspirin. So, taking caffeic acid regularly in our diet may relieve many diseases. Caffeic acid usually is present in coffee, tea, wine, turmeric, basil, thyme, oregano, radishes, mushrooms, kale, sage, cabbage, apples, strawberries, cauliflower, pears, and olive oil. Intake of caffeic acid may cure migraine headaches and also migraine-induced seizures. Drinking a cup of coffee can cure migraine headaches and other neurological diseases. This study proves that caffeic acid may replace aspirin in the near future.

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