Journal of
Plant Science & Research

Open Science Publications

Volume 7, Issue 1 - 2020

www.opensciencepublications.com

Isolation of Flavonoids from *Ocimum sanctum* L. and its Docking Study as Angiotensin Converting Enzyme (ACE) Inhibitors

Research Article

Patel H* and Gamit D

Department of Biosciences, Veer Narmad South Gujarat University, India

***Corresponding author:** Patel H, Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India, Email: hinalbiotech@gmail.com

Article Information: Submission: 07/02/2020; Accepted: 16/03/2020; Published: 20/03/2020

Abstract

Hypertension is one of the most common health problems that carry a high risk of stroke, myocardial infection and renal disease. ACE is one of the important enzymes that responsible for hypertension. *Ocimum sanctum* plant is very important for their therapeutic potential. There are so many phytochemicals are present in *Ocimum sanctum* among which flavonoids are most important for their health benefits. In present study prepared flavonoid extract from *Ocimum sanctum* leaves using methanol and ethyl acetate solvent. Isolate flavonoids from prepared solvent extract using preparative HPTLC method and identified it with the help of HRLC-MS/Q-TOF technique. Molecular docking study was conducted using iGEMDOCK V.2 windows software to find out efficiency of isolated flavonoids with ACE along with standard drug. This information can be further utilized for design of potential therapeutic drug from plant flavonoid in regulation of hypertension.

Introduction

Hypertension is the most common serious persistent health problem that carries a high risk of arteriosclerosis, stroke, myocardial infarction and end stage renal disease [1]. Angiotensin converting enzyme inhibition is one of the Morden therapeutic approaches to treat hypertension [2]. ACE catalyses the degradation of bradykinin [3]. ACE also converts angiotensin I into potent vasoconstrictor angiotensin II [4]. Therefore to generate antihypertensive effect, inhibition of ACE is required. Captopril, enalapril like synthetic drugs are widely used to treat hypertension but they show some major side effects like renalin fatigue, angioneurotic edema etc. so there is a requirement of new Natural based ACE inhibitors that greatly beneficial to hypertensive patients.

Natural has many useful plants and herbs for human beings. The attention paid by health authorities to use of herbal medicine has increased considerably to meet its health needs. Medicinal plants are rich in secondary metabolites like alkaloids, flavonoids, glycosides, tannins and essential oil which are good source of drugs in pharmaceutical industries [5,6]. Among the plants having the medicinal value, the plants of genus *Ocimum* belongs to the family labiacea are very important for their therapeutic potential [7]. *Ocimum sanctum* has been used for thousands of the year in Ayurveda for its healing and other medicinal properties. In Hindu tradition, *Ocimum sanctum* get important symbol. *Ocimum sanctum* is a herbal remedy for many common ailments like healing power , fever, common cold, sore throat, anti-inflammatory action, antibacterial etc.

Secondary metabolite like flavonoids – A product of secondary metabolism produced by plant in higher amount and distributed widely in plant kingdom. Flavonoids represent a one of the important class of phytochemicals. They belong to group of polyphenols that are present in fruits, vegetables, medicinal plants and its products. Interest in isolation and structure elucidation of many natural flavonoids get increasing because of its ecology and health benefits

ISSN: 2349-2805

of bioactive flavonoids. They are renowned for their number of health [8]. Phenolic compounds such as ferulic acid and tannic acid and flavonoids such as quercetin, anthocyanins, flavones and flavonols have shown to exhibit a capacity to inhibit different zinc metalloproteinases, including ACE [9,10].

Molecular docking is a very important tool useful in the structure based rational drug designing. Using iGEMDOCK software of molecular docking, enzyme interact with ligands like smaller molecules such as inhibitors or drug candidate to form stable complex.

The main object of this study was to extract flavonoids from *Ocimum sanctum* leaves extract using solvent maceration method and get isolated using preparative HPTLC and identified by HRLCMS/Q-TOF technique. Then to perform molecular docking of that identified flavonoids (inhibitors) with ACE, to determined its binding efficacy.

Materials and Methods

Extraction of flavonoids

Dried leaves powdered of *Ocimum sanctum* plant (50 gm) extracted with direct methanol (95%) by maceration method for 24 h in shaking condition.

The extract was filtered and evaporated in vacuum condition. The filtrate obtained was resuspended in ethyl acetate and extract it successively to yield ethyl acetate fraction following the method [11] with some modification.

Preparative High Performance Thin Layer Chromatography

High performance thin layer chromatography was carried out after successful development of TLC plate by using toluene: ethyl acetate solvent system. HPTLC was carried out using CAMAG HPTLC system for prepared flavonoid extract. Prior to sample application, activate HPTLC plate (silica gel 60 F254, Merck) at 100 °C for 30 min.

Impregnated 200 l of prepared extract on HPTLC plate (20×20) as a single band of 180 mm length using CAMAG automatic TLC sampler III (CAMAG, Switzerland).The plate was then air dried and kept for development in the twin trough chromatographic chamber containing 200 ml standardized solvent system, toluene: ethyl acetate (9.3:0.7).

As the above procedure, 5 HPTLC plates were impregnated with 200 l of same sample same as performed for the above plate. Examine under UV chamber at 366 nm wavelength, after the successful development of plates.

Isolation of flavonoid constituent

The plate which was developed was further proceeded for isolation of flavonoid constitution. The developed plate was then marked with graphite tip from 1 to 10 cm with mm marking scale along the solvent system movement. Put the plate under UV chamber to mark flavonoid band on the scale. By using sharp scalpel, scratched the selected area along with silica and collected in Eppendorf tube and eluted flavonoid constituent from silica gel with ethyl acetate and pooled the content.

The pooled content was evaporated at room temperature to form concentrated and store in small glass bottle for further analysis.

HR-LCMS/Q-TOF

Identification of extracted flavonoid from desired plant solvent extract was analysed by LC/Q-TOF Mass Spectrometer, Agilent technologies, Bombay India. The HR-LCMS analysis of plant material was analysed by using UHPLC-PDA detector Mass spectrometer. Sample was separated on SB-C18 column(2.1×50 mm, 1.8 particle size). The solvents used were Water: Methanol. MS detection was performed in MS /Q-TOF Mass spectrometer.

Molecular Docking

Identified flavonoids through HR-LCMS get derived from PubChem in 3D structure along with standard drug, captopril. Convert their structure in mol. Format using software OPENBABEL. Three dimensional experimentally determined X-ray crystal structure of Angiotensin converting Enzyme (ACE) were taken from protein data bank(PDB)(www.rcsb.org/) having PDB code108A. Once the ligand and protein have been prepared, download the file in iGEMDOCK software. Docking was performed using iGEMDOCK software. At the end of each run, compare the binding of ligand and protein by binding energy i.e. the lowest energy shows highest binding affinity.

Result and Discussion

Cardioprotective potential of flavonoids gained more interest in research for other potential properties [12]. Flavonoids or their derivatives are widely used as pharmaceutical agents for their Vasoprotective properties [13]. Flavonoids have been extracted from the leaves of *Ocimum sanctum* using methanol and ethyl acetate as a solvent and prepared concentrated extract in ethyl acetate. Thin layer chromatography process confirmed the presence of flavonoids by observing the florescent band. Extraction method of phytochemicals is one of the most important procedure in pharmaceuticals use of plant species having medicinal importance [14]. Now a day in botany, HPTLC become very useful technique for pattern generation, separation and further isolation. The crude flavonoid extracts revealed brown, one bright florescent band along with light blue band. The clear bright blue florescent band was considered to be possible flavonoids and was selected for isolation (Figure 1).

HRLC-MS analysis lead to the identification of flavonoids from leaves extract of *Ocimum sanctum* which showed the presence of four flavonoids are tubulated (Table 1). Identified flavonoids were Daidzin, Apigenin, and Rutin and Quercitrin. Retention time,

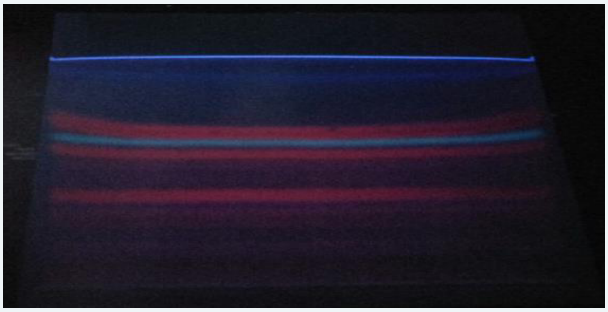

Figure 1: Preparative HPTLC with florescent band shows presence of flavonoids.

Citation: Patel H, Gamit D. Isolation of Flavonoids from *Ocimum sanctum* L. and its Docking Study as Angiotensin Converting Enzyme (ACE) Inhibitors. J Plant Sci Res. 2020;7(1): 189

molecular formula, m/z value were evaluated in (Table 1). Nature, molecular formula and structure of flavonoids compounds were analyzed by using mass spectrometer at different time. In these, appearances of peaks are due to large compounds splits into small ones at various m/z ratios and these HRLC-MS spectra are fingerprint of that compounds and it identified with data library [15]. The cleaned and optimized structure of identified flavonoids was derived from PubChem. 3D structure of ACE was derived from PDB. All the procedure has been performed using iGEMDOCK V.2 windows software. Flexible docking has been done between ACE and identified flavonoids. And also between ACE and standard drug captopril. Low energy with high binding affinity of flavonoids with ACE is given in (Table 2). Best conformation with highest binding affinity with low energy have been chosen for the protein-ligand interaction study and shown in Figure 2-6.

Table 1: Identified flavonoids from *Ocimum sanctum* leaves.

Compound Name	Retention Time	Molecular Formula	m/z Ratio
Rutin	5.328	$C_{27}H_{30}O_{16}$	611.162
Daidzin	8.396	$C_{21}H_{20}O_{9}$	417.117
Quercitrin	14.367	$C_{21}H_{20}O_{11}$	449.107
Apigenin-7-o-glycoside	17.373	$C_{21}H_{20}O_{9}$	455.094

Table 2: Results of iGEMDOCK analysis.

Ligand	Binding energy (Kcal/mol)	VDW	H-Bond	Electrostatic energy
Apigenin-7-o-glycosides	-88.9	-73.04	-15.86	0
Daidzin	-89.71	-74.34	-15.37	0
Rutin	-114.62	-81.52	-33.1	0
Quercitrin	-89.1	-53.0	-36.11	0
Captopril	59.19	-38.84	-18.33	0

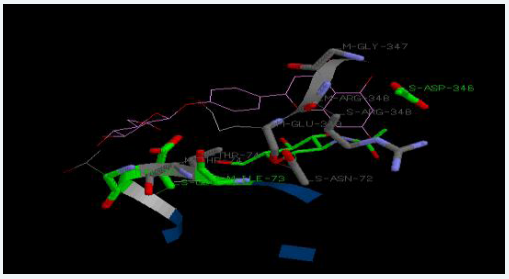

Figure 2: Docking of apigenin-7-o- ACE.

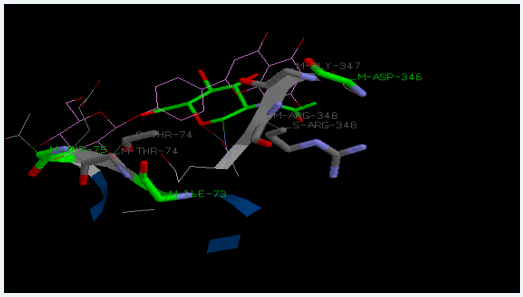

Figure 3: Docking of Daidzin with ACE glycoside with ACE.

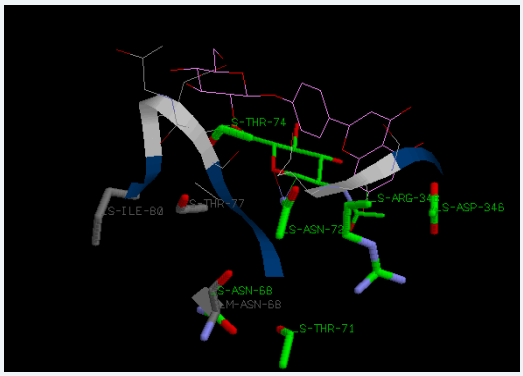

Figure 4: Docking of Rutin with ACE.

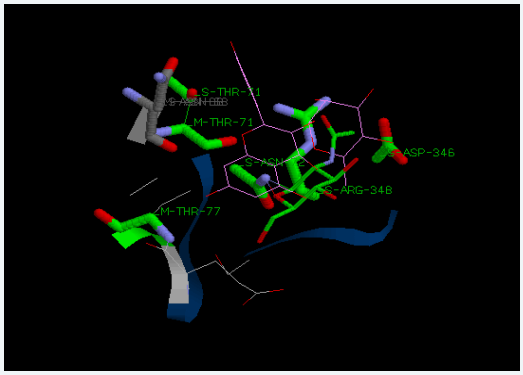

Figure 5: Docking of quercitrin with ACE.

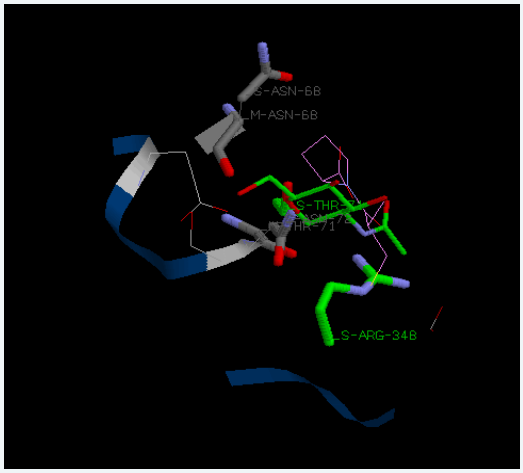

Figure 6: Docking of captopril with ACE.

Conclusion

In most of the studies, it have been shown that plant extracts rich in phytochemicals found to be very effective in angiotensin converting enzyme (ACE) inhibition. It can be conclude that, *Ocimum sanctum* contain flavonoids, that may important for the ACE inhibition. The above stated procedure is say to be an efficient and simple workout for the isolation of compounds from plant extract. Present molecular modeling studies demonstrated that isolated flavonoids have as same

Citation: Patel H, Gamit D. Isolation of Flavonoids from *Ocimum sanctum* L. and its Docking Study as Angiotensin Converting Enzyme (ACE) Inhibitors. J Plant Sci Res. 2020;7(1): 189

binding efficiency as standard drug. There for these compounds may be an effective therapeutic candidate for control of ACE. Further studies with structural modification are required to develop potential therapeutic entities for the treatment of hypertension and related disorder.

References

1. Jung WK, Mendis E, JeJY, Park PJ, Son BW, et al. (2006) Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (Limanda aspera) frame protein and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 94: 26-32.
2. Adeneye AA, Ajagbonna OP, Mojiminiyi FBO, Odigie IP, Etarrh RR, et al. (2006) The hypotensive mechanisms for the aqueous stem bark extract of Musanga cecropioides in Sprague-Dawley rats, J Ethnopharmacol 106: 203-207.
3. Fillardi P (2015) ACEi and ARBS in Hypertension and Heart Failure. 5: 10-13.
4. Patten GS, Abeywardena MY, Bennett LE (2016) Inhibition of Angiotensin Converting Enzyme, Angiotensin II Receptor Blocking, and Blood Pressure Lowering Bioactivity across Plant Families. Crit Rev Food Sci Nutr 56: 181-214.
5. Krishnaiah D, Sukla AR, Dhawan V (2009) Effect of herbal polyphenols on atherogenic transcriptome. Mol cell biochem, 278: 177-184.
6. Sobli SN, Pushphpangadan P (1982) Studies in the genus Ocimum: cytogenetics, breeding and production of new strains of economic importance. Regional laboratory council of scientific and industrial research, Jammu-Tawi India.
7. Yang SS, Cragg GM, Newman DJ, Bader JP (2001) Natural Product-Based Anti-HIV Drug Discovery and Development Facilitated by the NCI Developmental Therapeutics Program. J Nat Prod 64: 265.
8. Wanchai DE, Kaoru U, Jan FS (2015) Flavonoids: separation and quantitation. The Scientific World Journal 2.
9. Alam MA, Sernia C, Brown L (2013) Ferulic acid improves cardiovascular and kidney structure and function in hypertensive rats. J Cardiovasc Pharmacol 61: 240-249.
10. Ende C, Gebhardt R (2004) Inhibition of matrix metalloproteinase-2 and -9 activities by selected flavonoids. Planta Med 70: 1006-1008.
11. S. Subramanian and Nagarajan (1969) Flavonoids of seeds of Crotalaria retusa and Crotalaria striat. Current Science 38: 365.
12. Liu Z, Ma LP, Zhou B, Yang L, Liu ZL (2000) Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. Chem Phys Lipids 106: 53-63.
13. Gohel MS, Davies AH (2009) Pharmacological agents in the treatment of venous disease: an update of the available evidence. CurrVascPharmacol 3: 303-308.
14. Mishra GJ, Reddy MN, Rana JS (2012) Isolation of flavonoid constituent from Launaea procumbens Roxb. by preparative HPTLC method. IOSR J Pharm *2*: 5-11.
15. Jain DJ, Rajurkar SK (2019) Chemical composition of methanolic extract of Tulsi leaves (Ocimum sanctum L.). International journal of Research and Scientific innovation 5: 113:114.

Citation: Patel H, Gamit D. Isolation of Flavonoids from *Ocimum sanctum* L. and its Docking Study as Angiotensin Converting Enzyme (ACE) Inhibitors. J Plant Sci Res. 2020;7(1): 189