Original Article

Molecular Docking Studies Of Canthin Alkaloids Present In Aerva Lanata With Matrix Metalloproteinases 2,9

J.V.Madhuri[1]*, LNS Prakash Goteti[2]

[1]*Geethanjali College of Engineering and Technology, Cheeryal, Hyderabad, E-mail:jvmadhuri.fe@gcet.edu.in
[2]Techmahindra Limited, Hyderabad, E-mail:- vmadhurijupudi@gmail.com

***Corresponding Author: -** J.V.Madhuri

*Geethanjali College of Engineering and Technology, Cheeryal, Hyderabad, E-mail:jvmadhuri.fe@gcet.edu.in
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Abstract

Rheumatoid arthritis (RA) is a systemic, chronic, autoimmune inflammatory disease affecting the joints of an individual hindering the quality of life. To treat RA, usually non-steroidal anti-inflammatory drugs, steroids, glucocorticoids are used. In the recent past, it has been observed that extracts and compounds derived from plants are effective in treating RA. Also, compounds that bind to MMP2, MMP9 can be potential drug targets for further study. Aerva lanata is a plant constituting diverse phytochemicals and a rich source for Canthin-6-one and ß-carboline alkaloids. This work focusses on binding of the compounds Canthin-6-one, 10-hydroxy-canthin-6-one, 9-Methoxycanthin-6-one and 7-methoxy-beta-carboline-1-propionic acid present in Aerva lanata with MMP2 and MMP9. From bioactivity spectrum, it is noticed that these molecules are biologically active suggesting further studies to be carried out for exploring potential drug activity. Bioactivity score, drug likeness character was assessed *in silico*. Binding affinity score of Canthin-6-one alkaloids with MMP2 and MMP9 obtained through molecular docking revealed that these molecules are potential drug targets for treating RA.

Keywords: Bioactivity, Aerva lanata, Matrix metalloproteinases, Rheumatoid arthritis, Canthin-6-one, ß-carboline alkaloids Molecular docking

1. INTRODUCTION

Globally usage of medicinal plants for the treatment of chronic diseases is becoming prominent because of their pharmacological activities and less side effects. Also, with increasing health consciousness among people, plant-based additives are finding their way in the development of novel products that alleviate health conditions [1-4]. Bioactive compounds present in these products exhibit therapeutic effects and do not have toxicological and carcinogenic effects. For treating Rheumatoid arthritis (RA), Nonsteroidal anti-inflammatory drugs (NSAIDs), Steroids, disease modifying anti rheumatic drugs **(**DMARDs) etc are used but as the disease is progressive in nature, prolonged use of these drugs has serious side-effects [5,6]. Another challenge in the treatment of RA is lack of strategies for its early detection. For this, biomarkers are helpful and starting the treatment with bioactive compounds could pave a path for effective treatment of RA [7-9]. In this work we present various aspects of Matrix metalloproteinases (MMP) that can be inhibited by bioactive compounds present in *Aerva lanata* and subsequent usage in the treatment of RA.

Matrix metalloproteinases (MMP) belongs to the class of zinc dependent metalloendopeptidases [10.11]. In humans, 23 different structural forms of MMP are observed and they are further divided into six groups namely, collagenases, gelatinases, stromelysins, matrilysins, membrane-type and non-classified MMP's based on their substrate specificity. MMP's play a role in cell growth, differentiation, survival and motility and are located in subcellular organelles, cytosol, nucleus. MMP's are secreted as inactive zymogens [12-13]. In the present study we are interested in the classification of gelatinases comprising of MMP-2,9. The name of the classification derives from the fact that MMP2,9 digest the denatured form of collagen, gelatin. These MMP's are associated with extracellular pathways and degradation of extracellular matrix (ECM) [14]. MMP's are associated with the pathogenesis of many diseases and in specific in Rheumatoid arthritis (RA), where matrix degrading enzymes are produced facilitating cartilage destruction.

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder. Nearly 1% of world population is affected by RA and is predominantly more observed in women than men [15,16]. It is associated with pain, swelling and stiffness in the joints. RA progresses rapidly and results in irreversible dysfunction of the organs. It is crucial to diagnose and treat at the early stages of RA. Even though, its treatment started way back in 1897 through acetylsalicylic acid (asprin), it is in 1949 first breakthrough came by the administration of cortisone [17]. Later, glucocorticoids and anti-inflammatory drugs were used for the treatment of RA. However, progressing of the disease was irreversible. In 1998, first monoclonal antibody therapy was introduced based on the technique developed by Köhler and Milstein for producing monoclonal

antibodies. This revolutionised the treatment of RA. Immunosuppressive drugs used for the treatment of RA are referred to disease-modifying antirheumatic drugs (DMARDs) that can be synthesised as well as derived from biological agents [18]. With increasing demand for naturally derived compounds in treating RA, it is important to explore various parts of plants with biological importance [19, 20]. In the present work, we are suggesting phytoconstituents present in the plant *Aerva Lanata* as potential DMARDs.

Aerva lanata is a plant constituting diverse phytochemicals like alkaloids, flavonoids, tannins, aminoacids, cardiac glycosides, saponins and terpenoids. *Aerva lanata* belongs to the family *Amaranthaceae*, it is an erect plant found in the tropical regions like India, Africa, Philippines etc [21-22]. In Ayurveda, Indian traditional medicinal system, it is called as Pashanabheda possessing Antiurolithiatic and diuretic properties. Various studies were carried out for establishing the scientific data for the compounds present in *Aerva lananta* [23,24]. These studies revealed the presence of phenolic acids like vanillic acid, p-coumaric acid, ferulic acid, syringic acid, flavonols like kaempferol, 4'-methoxy kaempferol and 4', 7- dimethoxy kaempferol and quercetin. From this plant, Canthin-6-one and ß-carboline alkaloids were derived and are of specific interest in the present study due to their interaction with MMP2 and MMP9.

2. METHODS

Alkaloids identified in *Aerva lanata* chosen for the present study were, Canthin-6-one (CID 97176), 10-hydroxy-canthin-6-one (CID 158929), 9-Methoxycanthin-6-one (CID 9881423) and a ß-carboline alkaloid, 7-methoxy-beta-carboline-1-propionic acid (CID 12082492) with the structures as shown in Fig:1.

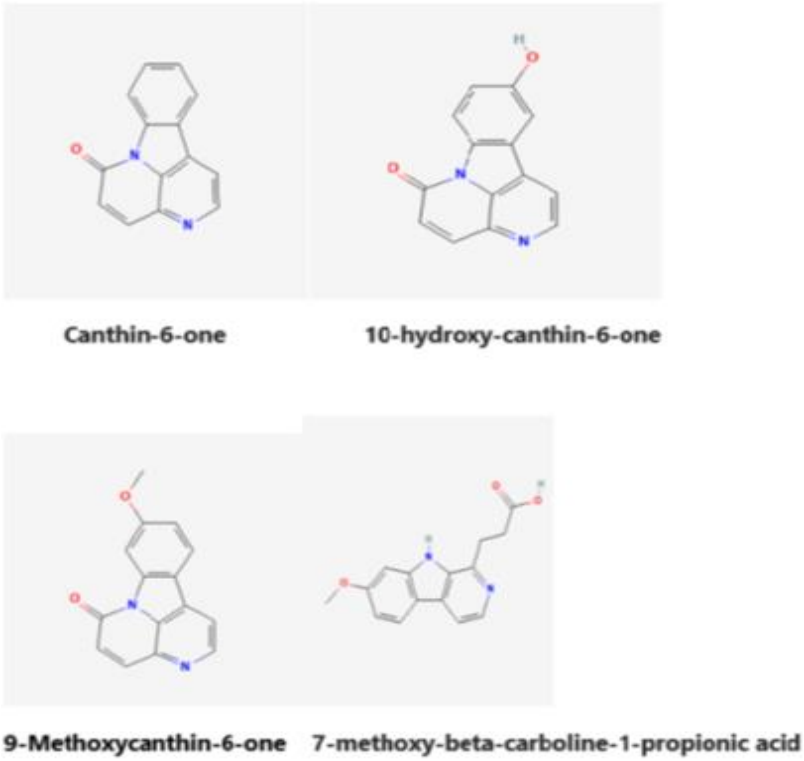

Fig:1 Structures of Canthin Alkaloids present in *Aerva lananta*

MMP2 (1ck7) and MMP9 (1l6j) were taken from protein data bank (www.rcsb.org) and these structures were used for docking and *insilico* analysis. Bioactivity score of the molecules was calculated using Monolinspiration (/www.molinspiration.com), by interacting with GPCR ligand, kinase, protease and enzyme inhibitors then comparing it with a standard drug. The molecule is considered to be active if its bioactivity score is greater than 0, moderately active if the bioactivity score lies between -5.0 and 0.0 and inactive if less than -5.0. Using Lipinski's rule of 5 drug likeness of the molecule is calculated [25].

PASS tool predicts the biological activity spectrum of the compounds based on its chemical structure and is considered as an intrinsic property. Pa (probability of the compound to be active) and Pi (probability of the compound to be inactive) were generated for each biological activity by using Multilevel Neighbourhoods of Atoms (MNA) descriptors generated in the program based on the training set [26]. Values of Pa and Pi range between 0.000 to 1.000 and Pa+Pi is always less than 1.

Dockthor program is a docking method using multiple solution genetic algorithm developed by GMMSB/LNCC group and it uses MMFF94S force field as scoring function [27]. The program generates topology and cofactor files for the ligand using MMFF94S force field and OpenBabel tools. X, Y, Z coordinates of a reference atom in the 3D space taken

as the centre. Grid size corresponds to the grid box. The docked poses are clustered by using DTStatistic program. Binding affinity, total energy was calculated to predict the compound's affinity in binding with MMP2 and MMP9.

3. RESULTS AND DISCUSSION

For the treatment of RA, it is important to search and innovate for plant-based drugs so that side effects arising due to prolonged use may be minimised. In the entire plant of *Aerva lanata Canthin*-6-one and ß-carboline alkaloids are observed. In Ayurveda, this plant is used to treat many conditions and the plant extract is possessed to have diuretic, nephroprotective, hepatoprotective, immunomodulatory, anti-hyperglycaemic, anti-inflammatory etc properties but there is very limited scientific evidence [28]. Hence, this plant rich in alkaloids is chosen to carryout insilico analysis of the chosen compounds present in *Aerva lanata* with MMP2 and MMP9.

The binding affinity of an alkaloid towards a biological target determines its pharmacological activity, the most common targets being enzymes, receptors, ion channel modulators etc. In the present study, the bioactivity score of the Canthin 6-one alkaloids is calculated by binding to G protein-coupled receptor (GPCR), ion channel modulator, nuclear receptor and kinase, protease, enzyme inhibitors and is presented in Table1.

S.No	Compound	Bioactivity score parameters					
		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1.	10-hydroxy-canthin-6-one	-0.28	0.31	0.24	-0.43	-0.56	0.06
2.	9-methoxy-canthin6-one	-0.33	0.15	0.21	-0.54	-0.57	-0.05
3.	7-methoxy beta carboline 1-propionic acid	0.26	0.34	0.43	0.18	-0.11	0.36
4.	Canthin-6-one	-0.42	0.25	0.10	-0.76	-0.66	-0.07

Table 1: Bioactivity score of Canthin 6-one alkaloids

It is observed that for Canthin 6-one alkaloids the bioactivity scores are within the range 0.0 and 5.0 suggesting moderate activity and from tables 1,2 it is also observed that for ion channel modulator, the bio activity is high. Hence, it is relevant to explore potential drug properties of Canthin 6-one alkaloids.

Physiochemical parameters like molecular weight, lipophilicity, polar surface area, H-bonding etc affect the bioactivity by influencing the interaction between bioactive compound and the lipophilic cell membrane. Lipinski's rule of 5 suggests that molecular weight to be less than 500, partition coefficient (log P) around 5, 'H' bond donors less than 5 and allows 10 'H' bond acceptors [25,29]. Typically, topological polar surface area (TPSA) is within the range 0-140˚A^2. Applying Lipinski's rule of 5 for the Canthin 6-one alkaloids, it may be observed from the table2 that, almost all the compounds fit into the rule, and hence may be concluded that these compounds have drug like properties.

S. No	Compound	Mol weight g/mol	LogP	TPSA (Å^2) Topological polar surface area	H-bond acceptor	H-bond donor	Number of rotatable bonds	Vol.
1.	10-hydroxy-canthin-6-one	236.22	2.19	54.61	4	1	0	196.81
2.	9-methoxy-canthin6-one	250.25	2.72	43.61	4	0	1	214.34
3.	7-methoxy beta carboline 1-propionic acid	270.28	2.34	75.22	5	2	4	239.01
4.	Canthin-6-one	220.23	2.69	34.38	3	0	0	188.80

Table 2: Physiochemical parameters of Canthin 6-one alkaloids

In the studies carried out using 'PASS' tool, biological activity spectrum of these compounds is studied. The Pa and Pi values are as shown in the Table 3. Canthin 6-one alkaloids exhibit various biological activities like antiviral, antibacterial, antitumor, and antifungal. There are very few studies relating to the anti-inflammatory activity of these compounds and from the biological activity spectrum, it is observed that these alkaloids are potential drug targets for various pathological

events that are triggered due to cigarette smoking, pollution, inflammation etc before the clinical onset of the disease. Understanding the Pathogenesis of RA is heterologic and provides potential insights to the treatment.

	Biological activity							
	Glutathione thiolesterase inhibitor		Nicotinic alpha2beta2 receptor antagonist		2-Hydroxyquinoline 8-monooxygenase inhibitor		Gluconate 2dehydrogenase (acceptor) inhibitor	
Compound	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
10-hydroxy-canthin-6-one	0.883	0.003	0.782	0.012	0.843	0.004	0.726	0.043
9-Methoxycanthin-6-one	0.713	0.016	0.706	0.025	0.785	0.006	0.774	0.023
7-methoxy-beta-carboline-1-propionic acid	-	-	-	-	-	-	0.794	0.018
Canthin-6-one	0.871	0.004	0.867	0.004	0.802	0.005	-	-

Table 3: Bioactivity spectrum of Canthin 6-one alkaloids

Molecular docking studies with MMP2 and MMP9 with Canthin 6-one alkaloids using dockthor gave information about the binding affinity and binding mode. In RA patients, Zinc present in the catalytic domain of MMP interacts with cysteine residue, and becomes inactive and is active when this interaction is disrupted by pro-protein converting enzymes like furin. [30]. Catalytic domain of MMP-2 and MMP-9 comprises of fibronectin type II motif that enables them to bind and degrade gelatin. cytokine-stimulated synoviocytes produce matrix metalloproteinases (MMP), which are released into the synovial fluid. Cartilage is degraded by these enzymes and absorbed. Molecular docking score of the compounds is depicted in Table 4.

Compound	**Binding affinity kcal/mol**		**Total Energy kcal/mol**		**Vander Waals energy kcal/mol**		**Elec. Energy kcal/mol**	
	MMP-2	**MMP-9**	**MMP-2**	**MMP- 9**	**MMP-2**	**MMP-9**	**MMP-2**	**MMP- 9**
10-hydroxy-canthin-6-one	-9.427	-8.080	5.157	4.541	-27.10	-14.395	-2.394	-15.812
9-Methoxycanthin-6-one	-7.541	-8.501	19.487	15.607	-13.703	-21.281	-10.009	-6.251
7-methoxy-beta-carboline-1-propionic acid	-7.002	-8.006	-6.047	-2.474	-5.805	-17.484	-32.325	-20.594
Canthin-6-one	-8.183	-8.291	13.040	10.551	-17.642	-21.143	-5.273	-4.261

Table 4: Bioactivity spectrum of Canthin 6-one alkaloids

From the table 4, it is evident from the binding affinity score that these compounds effectively bind with MMP2, 9

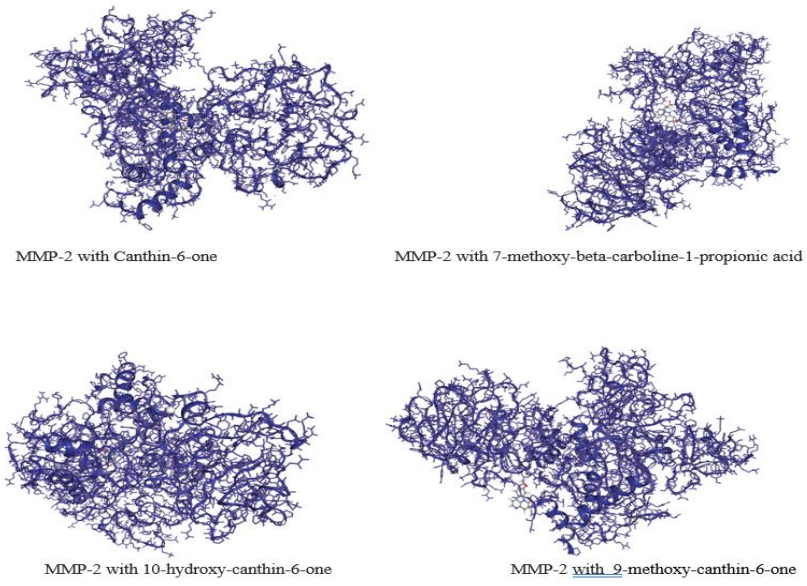

Fig: 2 Molecular docking of the compounds with MMP-2

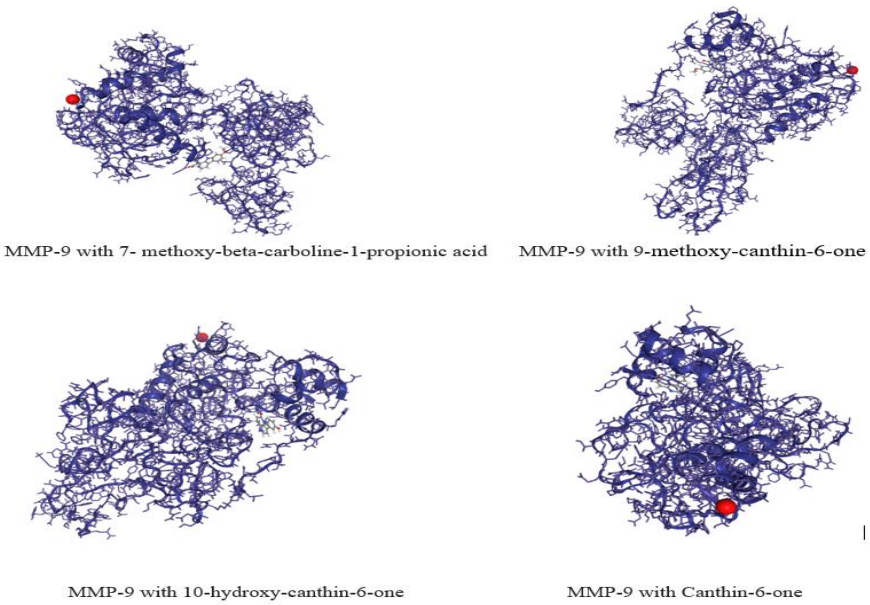

Fig: 3 Molecular docking of the compounds with MMP-9

CONCLUSIONS

Aerva Lanata is an interesting example from traditional Ayurvedic medicine that is used for treating various medical conditions. From the present study, it is clearly evident that the plant extract may be further explored for treating Rheumatoid arthritis. Canthin-6-one alkaloids are structurally unique and are potential candidates for commercial purposes from which nutraceuticals and functional foods may be designed to meet the nutritional as well as therapeutic needs of the patient suffering from RA.

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