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Identification of novel PPARγ agonist from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.): a computational molecular simulation studies

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ABSTRACT

Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a nuclear receptor family transcription factor that is expressed in several types of cancers. Antiproliferative and proapoptotic actions of PPARγ agonists suggesting that, it could be a promising therapeutic target for the treatment of variety of cancers. Therefore, the main aim of this study is attempt to identify the novel PPARγ agonist from presence of bioactive compounds in ethanolic extract of *Cayratia trifolia* using GC-MS analysis and Computational molecular simulation studies. The GC-MS analysis revealed that the ethanolic extract of *Cayratia trifolia* (L.) (whole plant) consist of 20 bioactive compounds which embrace many biological activities against variety of diseases. Molecular docking studies (Glide 5.5 from Schrödinger suite) exposed that, out of 20 bioactive compounds, Cyclopentadecane, 9-Borabicyclo [3.3.1]nonane, 9-(2-propen-1-yloxy)-.1, 4,8,12,16-Tetramethylheptadecan-4- olide, Oxirane and Vitamin E shows the better glide score. ADME properties (QikProp 2.3 from Schrödinger suite) of these bioactive compounds were under the acceptable range. Based on the result it can be concluded that, these bioactive compounds may act as a good agonist for PPARγ. In future it may focus on current discoveries in PPARγ activation and possible anticancer therapeutic option.

INTRODUCTION

The peroxisome proliferator-activated receptors (PPARs) belong to members of the nuclear hormone receptor superfamily, it consist of three members such as PPARα, PPARβ/δ, and PPARγ. They are involved in the pathophysiology of the different types of diseases (Park, 2012). The PPARγ plays a vital responsibility in the regulation of lipid homeostasis, adipogenesis and insulin resistance and in the development of diverse organs (Park, 2005). Aside from the recognized metabolic events, PPARγ has also been shown to be overexpressed in numerous human cancers, including breast, colon, bladder, and prostate cancer. It was also suggested to induce apoptosis in some malignant cell lineages (Sikka *et al.*, 2012). Furthermore, loss-of-function mutations of PPARγ have been found in several human colon and thyroid carcinomas. *In vitro* and *in vivo* studies have demonstrated antiproliferative and proapoptotic actions of PPARγ agonists signifying that, PPARγ could be a promising therapeutic target for the treatment of cancer (Sarraf *et al.*, 1999).

Numerous PPARγ agonist were developed and utilized clinically all over the world exert multiple effects with emerging potential benefits in other diseases (DeSouza and Fonseca, 2009). On the other hand, these medicines may also produce the toxicities, and some PPARγ agonist is no longer in use clinically because of serious complications arising in some patients (Kamijo *et al.*, 2012). Current research reports suggest that using medicinal compounds is always superior to the synthetic compounds. Due to this the recent research has been focused towards the plant compound isolation and compounds production at large scale against various diseases (Ma *et al.*, 2011).

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Comprehension of the chemical constituents of medicinal plant is helpful in the discovery of therapeutic agents as well as new sources of economic materials like oil and gums. Secondary metabolites of medicinal plants have proved to be an excellent reservoir of new medical compounds (Sarker and Nahar, 2012). In India, great number of plant species had been screened for their pharmacological properties but still vast wealth of rare species is unexplored (Velmurugan *et al.,* 2010). Medicinal plants are at interest to the field of novel drug development, as most of the drug industries depend on medicinal plants for the production of novel bioactive compounds (Peter *et al.,* 2012).

Cayratia trifolia (L.) is the medicinal plant belongs to the family of Vitaceae, It has been reported to contain huge amount of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins (Kumar, 2011; Gour, 2012; Gupta, 2012; Gupta, 2012; Kumar, 2012). Whole plant is used as diuretic in tumors, neuralgia and splenopathy (Pulliah, 2006). The bark extract has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities in animal models (Siriwatanametanon *et al.,* 2010). Therefore, the aim of the present work is to analyze the presence of bioactive compounds in ethanolic extract of *Cayratia trifolia* (L.) using GC-MS and to identify the novel PPARγ ligands using computational molecular simulation studies.

MATERIALS AND METHODS

Plant collection and authentication

The whole plant of *Cayratia trifolia* (L.) was collected from in and around the area of Kumbakonam, Tamil Nadu, India and it was authenticated by Dr. P. Sathyanarayanan, Botanical survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2010-2011/Tech.1527. The fresh whole plant material was washed under the running tap water, dipped on saline overnight, air dried and finely powdered for further use.

Extraction preparation

Based on the previous studies (Perumal et al. 2012), the 300g of dried plant powder was extracted in 1500ml of ethanol in an sporadic shaker for 72 hrs at room temperature. The extract was collected and concentrated at 40° C under reduced pressure using rotary evaporator. The dried extract was store at 4° C until further compound isolation process.

GC-MS analysis

GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.) was performed using the equipment Agilent technologies 7890 A. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm×0.25 mm ID×0.25 μm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results obtained have been tabulated (Gomathi *et al.,* 2013).

In silico analysis

Preparation of protein structure

The 3D structure of PPARγ was retrieved from the Protein Data Bank (PDB ID: 4HEE) and it was prepared by protein preparation wizards (standard methods) that are available in grid-based ligand docking with energetics (Protein Preparation Wizard, Schrödinger, 2012). Protein was optimized using sample water orientation and minimized by using RMSD 0.30 Å and OPLS (2005) force field.

Active site prediction

The active site (binding pockets) and functional residues of PPARγ was identified and characterized by Site- Map module from Schrodinger package (SiteMap 5.5, Schrodinger, 2012). SiteMap calculation begins with an initial search step that identifies or characterizes- through the use of grid points- one or more regions on the protein surface that may be suitable for binding ligands to the receptor. Contour maps were then generated, produced hydrophobic, hydrophilic maps hydrogen binding possibilities which may guide the protein- ligand docking analysis.

Ligand preparation

The bioactive compounds (from GC-MS analysis) were used in molecular docking studies. These ligands were prepared using the LigPrep 2.4 (LigPrep 2.4, Schrodinger, 2012). The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting.

Molecular docking analysis

All docking analysis were performed by using the standard precision (SP) which is Standard mode of Glide (Grid-based Ligand Docking with Energetic) module from Schrodinger2012 (Glide 5.6, Schrodinger, 2012). All bioactive compounds were docked in to the binding site PPARγ using GLIDE. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20Aº. The co-ordinates of this enclosing box with the help of the active site residues to be set default. The force field is using for the docking protocol is OPLS_2005. The lowest-energy docked complexes were found in the majority of similar docking conformations.

ADME properties prediction

The PPARγ ligands were checked for their ADME properties using QikProp 2.3 module. QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Predicted significant ADME

properties such as Molecular weight (MW), H-Bond donor, H-Bond acceptor and log *P* (O/W) (QikProp 2.3, Schrodinger, 2012).

RESULTS AND DISCUSSION

PPARs are involved in the pathophysiology of the various types of diseases. Variety of PPAR-related medicines has been developed and employed clinically all over the world. It is used in multiple effects, including regulation of hypolipidemic, antidiabetic, antiinflammatory, antifibrotic, and antiproliferative pathways, with emerging potential benefits in other diseases (Pazienza, 2012). Alternatively, these medicines may also exert diverse toxicities, and some PPAR drugs are no longer in use clinically because of serious problem arising in some patients (Li et al. 2012). Therefore, this study was focused to identify the novel and better PPARγ agonist from the bioactive compounds because it shows lesser side effects compared with synthetic ligands.

GC-MS analysis shows that, 20 bioactive compounds were present in ethanolic extract of *Cayratia trifolia* (L.). A peak level in the chromatogram graph indicates the maximum amount of phytol (40%) present in the extract was showed in figure 1 and table1. These bioactive compounds posses many biological activities such as anti-cancer, anti-inflammatory, anti-microbial, anti-diabetic etc., (Nakashima, 2013; Yang, 2011; Mahendran, 2012; Ajila, 2010; Sasidharan, 2011). The PPARγ protein was retrieved (from PDB (PDB ID: 4HEE)) and prepared for further studies. On the other hand, the bioactive compounds (from *Cayratia trifolia* (L.)) were prepared. The best active site (binding pocket/site) was preferred based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity. The binding site residues of PPARγ were predicted and it may involve in the binding of substrate and small molecule. Thus, all these residues were confirmed as PPARγ active site residues and picked to generate grid in the centroid of these residues for molecular docking approach.

The molecular docking is frequently used to predict the binding orientation of small molecule drug candidate to their protein targets in order to predict the affinity and activity of the small molecule. The docking results 20 bioactive compounds were complexes with PPARγ protein shown in table 2. Among these bioactive compounds Cyclopentadecane, 9-Borabicyclo [3.3.1]nonane, 9-(2-propen-1-yloxy)-.1, 4,8,12,16-Tetramethylheptadecan-4- olide, Oxirane and Vitamin E shows the better Glide score of -6.532, -5.163, -7.181, -5.062, -7.221 respectively and the glide energy was -25.769, -20.777, -35.952, -16.249, -42.524 kcal/mol respectively. These bioactive compounds complexes with PPARγ were shown in figure 2 to 6. The highest negative value of glide score and glide energy indicated that, these complexes may have good affinity (Walker and Eldowney, 2013). The limitations of ADME properties are:

- Not more than 5 hydrogen bond donors.
- Not more than 10 hydrogen bond acceptor.
- A molecular mass less than 500 daltons.
- An octanol- water partition coefficient log P not greater than 5

The ADME properties prediction results (shown in table 3) of these bioactive compounds were under acceptable range.

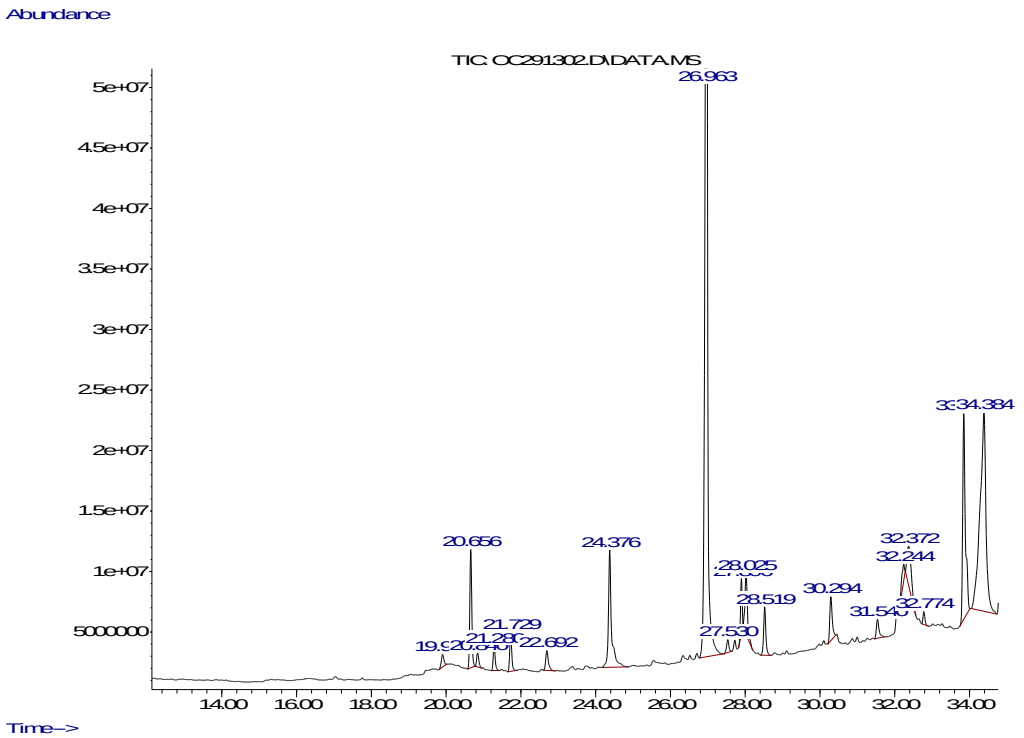

Fig. 1: GC-MS peak level in the chromatogram graph of ethanolic extract of *Cayratia trifolia* (L.)

Table. 1: Gas chromatogram graph peak level of ethanolic extract of *Cayratia trifolia* (L.).

Peak	RT	Compound Name	%
1	4.187	Cyclopentadecane	1.13
2	19.904	9-Borabicyclo[3.3.1]nonane, 9-(2- propen-1-yloxy)-	0.55
3	20.656	3-Octadecyne	3.87
4	20.84	Ethanol, 2-(octadecyloxy)-	0.5
5	21.28	3-Octadecyne	0.84
6	21.729	9-Octadecyne	1.33
7	22.692	6-Octen-1ol, 3,7-dimethyl-, formate	0.9
8	24.376	Hexxadecanoic acid, ethyl ester	6.1
9	**26.963**	**Phytol**	**42.55**
10	27.53	Trans-13-Octadecenoic acid	0.51
11	27.896	9,12-Octadecadienoic acid, ethyl ester	1.6
12	28.025	Ethyl Oleate	2.46
13	28.519	Octadecanoic acid, ethyl ester	1.78
14	30.294	3-Eicosene	1.82
15	31.54	4,8,12,16-Tetramethylheptadecan-4-olide	0.84
16	32.244	Hentriacontane	1
17	32.372	Heptadecane	2.71
18	32.774	Oxirane, hexadecyl-	0.41
19	33.844	1-Heptacosanol	9.24
20	34.384	Vitamin E	19.86

Table. 2: Docking results of PPARγ protein with 20 bioactive compounds from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.)

S. No	Compounds	Glide Score	Glide Energy
1	**Cyclopentadecane**	**-6.532**	**-25.769**
2	**9-Borabicyclo 3.3.1]nonane, 9-(2-propen-1-yloxy)-**	**-5.163**	**-20.777**
3	3-Octadecyne	-1.213	-31.063
4	2-(Octadecyloxy)ethanol	-3.740	-35.707
5	9-Octadecyne	-1.769	-32.005
6	citronellyl formate	-1.410	-26.014
7	Hexadecanoic acid	-1.611	-31.521
8	Phytol	-4.813	-40.634
9	Trans-13-Octadecenoic acid	-3.173	-38.192
10	Linoleic Acid	-3.226	-35.414
11	Ethyl Oleate	-4.754	-40.260
12	Stearic acid	-1.998	-34.948
13	3-Eicosene	-0.835	-33.805
14	**4,8,12,16-Tetramethyl heptadecan-4- olide**	**-7.181**	**-35.952**
15	Hentriacontane	-4.601	-39.473
16	Heptadecane	-0.361	-29.927
17	**Oxirane**	**-5.062**	**-16.249**
18	Heptacosanol	-4.659	-39.938
19	**Vitamin E**	**-7.221**	**-42.524**

Table. 3: ADME properties of selected PPARγ agonist from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.)

S. No	Ligands	Molecular Weight (g/mol)	H-Bond Donor	H-Bond Acceptor	Log *P* O/W
1	Cyclopentadecane	210.402	0	0	5.7
2	9-Borabicyclo[3.3.1]nonane, 9-(2-propen-1-yloxy)-	178.078	0	0	2.8
3	4,8,12,16-Tetramethylheptadecan-4- olide	324.546	0	3	6.0
4	Oxirane	100.16	0	2	0.8
5	Vitamin E	430.713	1	1.5	8.9

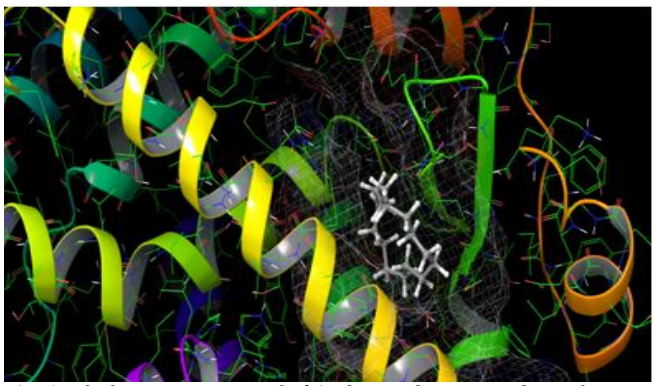

Fig. 2: The bioactive compound of Cyclopentadecane complex with PPARγ protein.

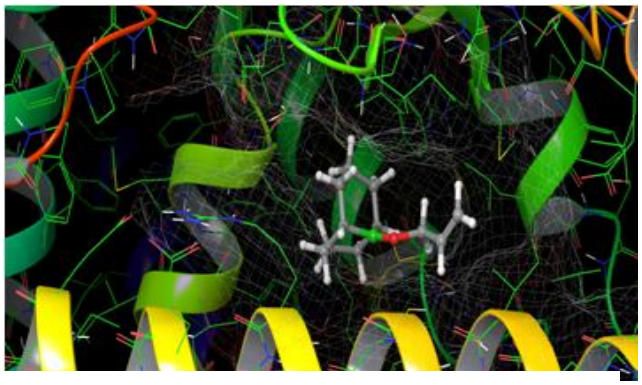

Fig. 3: The bioactive compound of 9-Borabicyclo [3.3.1]nonane, 9-(2-propen-1-yloxy)-.1 complex with PPARγ protein.

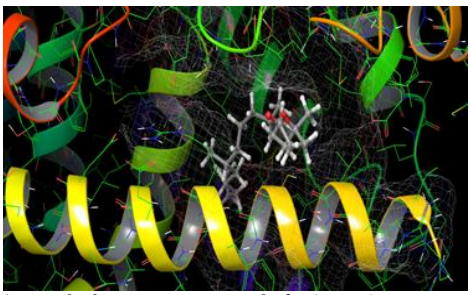

Fig. 4: The bioactive compound of 4,8,12,16 -Tetramethylheptadecan -4- olide complex with PPARγ protein.

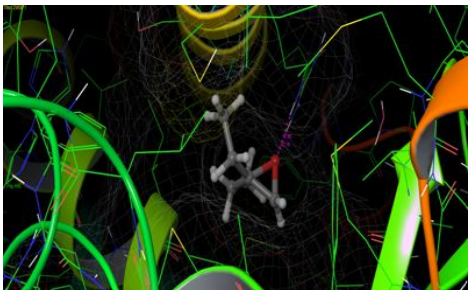

Fig. 5: The bioactive compound of Oxirane complex with PPARγ protein.

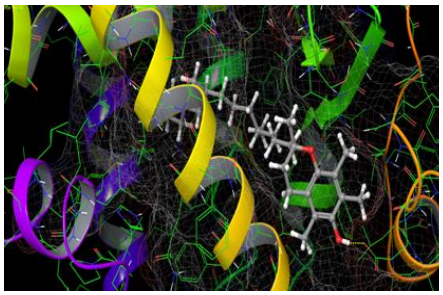

Fig. 6: The bioactive compound of Vitamin E complex with PPARγ protein.

CONCLUSION

In the present study, the GC-MS analysis identified that, 20 bioactive compounds were present in ethanolic extract of *Cayratia trifolia* (L.) which hold many biological activities against variety of diseases. The molecular docking studies exposed that, out of these bioactive compounds, Cyclopentadecane, 9-Borabicyclo [3.3.1]nonane, 9-(2-propen-1-yloxy)-.1, 4,8,12,16-Tetramethylheptadecan-4- olide, Oxirane and Vitamin E shows the better interaction with PPARγ protein. The ADME properties prediction of these compounds was under acceptable range. Thus, based on the results this study concluded that, these bioactive compounds of may act as a good agonist for PPARγ. In future, it may lead to develop a novel PPARγ agonist and therapeutic agent for the cancers.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

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