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Molecular Docking Studies of *Limonia acidissima* Linn. Fruit Extract on Estrogen Receptor for Ovarian Cancer

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ABSTRACT: The development of computer aided drug design has become a challenging role in discovering drugs for the medication of various illnesses. Searching for the chemical compounds from natural sources is being safe without side effects than a synthetic one. Recently screening of medicinal plants using a computational method which establishes the natural product as a major source for drug design. The *Limonia acidissima* L. belongs to the Rutaceae family contains various bioactive components that have been used in traditional medicine. The present work aims to evaluate the phytoconstituents in *Limonia acidissima* L. fruit extract that could act as an anticancer drug for ovarian cancer through molecular docking. *In silico* docking analysis estrogen receptors alpha and beta complexes with genistein act as an inhibitor molecule for targeting the drug design. The bioactive components of *Limonia acidissima* L. fruit extract have been identified by the GC-MS technique. It shows the presence of various secondary metabolites contains viz. N-Isobutyl- (2E, 4Z, 8Z, 10E) - dodeca tetraenamide, Hexadecanoic Acid, 14- Hydroxy-16, 16- Dimethoxy-14-Methyl, Ethyl Ester, Histamine N-Trifluroacetyl-2-amino, etc. The drug design property of *Limonia acidissima* L. fruit extract has been determined by the Lipinski rule and the docking score of the compounds. The docking result shows that Hexadecanoic Acid,14- Hydroxy-16, 16- Dimethoxy-14- Methyl, Ethyl Ester and 19,19-Dimethyl-eicosa-8, 11-dienoic acid have a high docking score these lead molecules have interaction with the interacting residues are Arg 394, Glu 353, Glu 305 and Lys 401 which stabilizes the receptor structure for designing a therapeutic drug. In this paper we highlight the potential use of the medicinal plant *Limonia acidissima* plant as an anticancer source, first time we report the important bioactive compounds Hexadecanoic Acid, 14-Hydroxy-16, 16-Dimethoxy-14-Methyl, Ethyl Ester and 19, 19-Dimethyl-eicosa-8, 11-dienoic acid through molecular docking studies.

Keywords: Bioactive components, Drug design, Estrogen, Histamine N-Trifluroacetyl-2-amino, *Limonia acidissima*, Ovarian cancer.

I. INTRODUCTION

Progression of ailment presents a foremost warning in the modern period, it represents a difficult role to target and diminish the multiplication of communicable diseases. However, addressing the hazard factors of communicable and non-communicable diseases is due to the lifestyle of people worldwide [1]. In the modern world; most human beings are affected by cancer. Cancer differs from other diseases in that it can develop at any stage in life and any body organ. It is expected that 80% of cancers are due to definite environmental or behavioral triggers therefore cancers are potentially preventable [2]. As there are about 200 types of cancer which affects mainly breast, lungs, colorectal, and prostate in the world [3, 4]. Ovarian cancer which is the seventh most common cancer among women in the world is influenced by steroid hormones [5]. The three major types of ovarian cancer are epithelial (90%), germ cell (3%) and sex cord-stromal (2%) [6], about 10% to 15% of ovarian cancers are to be connected with genetic abnormalities [7, 8]. Genetic ovarian cancer appears to be heterogeneous diseases in comparison with sporadic cancer ovary which has been characterized by different molecular pathways [9]. A potentially helpful approach is to develop a drug for resistant tumor survival and growth. This can be achieved through the use of multi-targeted drugs [10]. Nowadays the uniqueness of drugs derived from herbal plants is an increase because herbal medicine is safer than costly synthetic drugs that possess side effects [11]. From ancient times, plants symbolize a superior and important source of natural products. The Indian ayurvedic system of medicine uses plant-based drugs or formulations to treat human disease [12]. The naturally occurring medicinal plants contain various bioactive components such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, etc. which have therapeutic values [13]. The phytochemical plays an important role in cancer prevention through a molecular mechanism that approaches chemo resistance and chemical modifications for greater importance [14]. Chemo preventive and anticancer activity of flavanoid with conventional chemotherapeutic agents has been studied by Kikuchi, *et al.*, (2019) [15]. Molecular docking is one of the important methods in modern drug design,

which predicts the protein-ligand or protein-protein interaction forms a stable protein- ligand complex. The many biological processes have been proceeding through the Protein- ligand complexes [16]. The ligands bind with proteins through Vander Waals forces and the formation of hydrogen bonds [17]. Numerous molecular docking studies have been reported for bioactive components present in the natural products that could act as an anticancer drug. *In silico* docking analysis of phytoconstituents of *Crocus sativus, Curcuma longa, Cassia occidentalis* and *Moringa oleifera* on thymidylate synthase have been studied by Heble *et al*., (2016) [18]. They showed that all the phytoconstituents from *Crocus sativus* have better anticancer activity compared to that of the standard drugs. Raji *et al*., (2017) have been reported that crude extract and essential oil of citrus lemon leaf found to have anticancer activity against HeLa cell at the concentrations of 50µg/mL and 100 µg/mL respectively [19]. Daidzein compound acts as a selective estrogen receptor modulator for breast cancer has been investigated by Suryani *et al*., (2018) through molecular docking study [20]. Yugandhar *et al*., (2017) proposed that polyphenols from *Syzygium alternifolium* could be served as potential ER migrators for breast cancer therapy [21]. The review of literature shows that no such work has been carried out in docking analysis using *Limonia acidissima* L. fruit for the treatment of ovarian cancer.

II. MATERIALS AND METHODS

Protein selection and preparation: The crystal structure of the estrogen receptor was retrieved from the protein databank with PDB ID: 1X7R, 1X7J with a resolution of 2 Å and 2.3 Å. Docking analysis of the selected protein with a ligand molecule was analyzed using the Discovery studio software. The protein was preprocessed by removing the bounded ligands and the energy of the protein becomes minimized to form a stable structure for molecular docking. The protein consists of a single polypeptide chain with 245 amino acid residues. The initial docking was performed by selecting inhibitor binding site residues. The active sites residue are selected based on the binding of its inhibitor molecule genistein are Met 343, Thr 347, Glu 353, Arg 394 and His 524. Library docking is performed for identifying the binding affinity with the target molecule using the CHARMM force field.

Ligand selection and preparation: There are 10 ligands have been selected from the fruit extract of *Limonia acidissima,* Linn by GC-MS analysis. Using Chemdraw ultra, the selected ligand molecules are drawn and saved in PDB file format. The saved ligand molecules were later imported.SD format for the docking process. The CHARMM force is used in this study which helps optimize energy minimization and generate conformations, respectively.

Docking: The molecular docking studies of *Limonia acidissima* L. fruit extract was done through the discovery studio software 3.1 versions. In this study, we used the Libdock protocol for the docking scores findings. The docking score calculation was performed under the protein-ligand interaction. In this case, the ligand molecule would be structurally rearranged into the receptor molecules.

III. RESULTS AND DISCUSSION

Screening of bioactive components: The bioactive compounds led possess pharmacological properties which may help therapeutically to cure various diseases. The bioactive components of *Limonia acidissima* L. fruit extracts have been identified by GC-MS analysis is given in Table 1. Some of the phytocomponents of the fruit extract of the plant have been selected for the docking study basis on their biological activities. The screened bioactive components have given for docking analysis.

Table 1: Bioactive components of *Limonia acidissima* L. fruit by GC-MS.

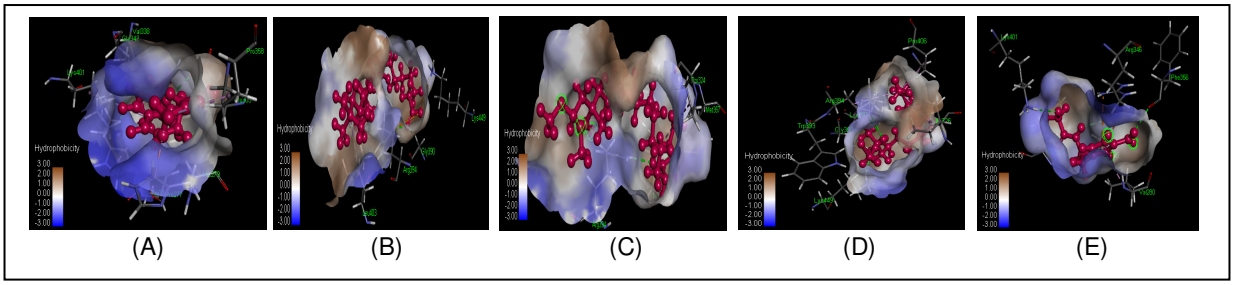
Name of the ligand	Molecular formula	Molecular Weight
19, 19-Dimethyl-eicosa-8,11-dienoic acid	$C_{22}H_{40}O_2$	336
N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	$C_{16}H_{25}ON$	247
7-Oct-2-one	$C_8H_{14}O$	126
Bromoacetic acid, 2-tetradecyl ester	$C_{16}H_{31}O_2Br$	334
1-Pentene-5-Methoxy	$C_6H_{12}O$	100
1,3 Propanediol,2,2-dimethyl-diacetate	$C_9H_{16}O_4$	188
N-Aetylcycloserine	$C_5H_8O_3N_2$	144
Hexadecanoic Acid, 14-Hydroxy-16, 16-Dimethoxy-14- Methyl, Ethyl Ester	$C_{21}H_{42}O_5$	374
Histamine, N-Trifluroacetyl-2-amino	$C_7H_9ON_4F_3$	222
1, 2-Epoxynonane	$C_9H_{18}O$	142

Table 2: *In-silico* docking analysis of *Limonia acidissima* L. fruit on Estrogen receptor.

Name of the ligand	Interacting Residues	Interaction	Dock Score	Rank score
19,19-Dimethyl-eicosa-8,11-dienoic acid	ARG 394	H-bond	126.5	-7870
N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	LYS 401 GLU 305	H-bond	114.6	-7495
Bromoacetic acid, 2-tetradecyl ester	LYS 449	H-bond	117.3	-8024
Hexadecanoic Acid, 14-Hydroxy-16, 16-Dimethoxy-14-Methyl, Ethyl Ester	ARG 394 LEU 387 GLU 353	H-bond	126.3	-7516
Histamine, N-Trifluro acetyl-2-amino	PHE 406 LYS 401	H-bond	99.40	-8648

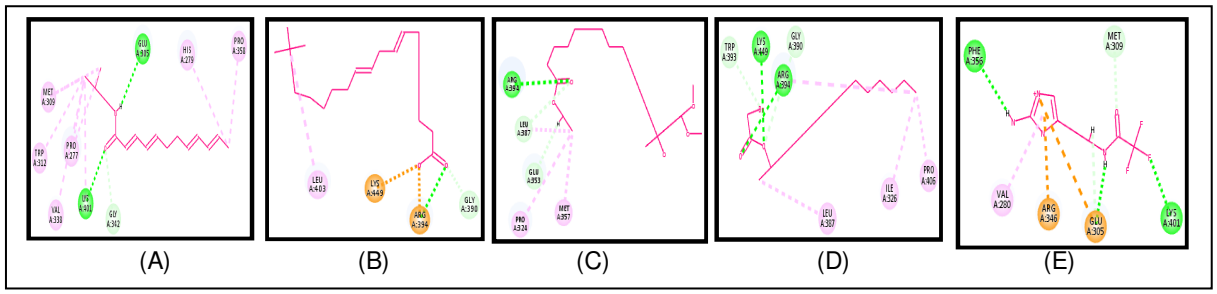
Table 2 shows that 19, 19-Dimethyl-eicosa-8, 11-dienoic acid, Hexadecanoic Acid-14-Hydroxy-16, 16-Dimethoxy-14-Methyl- Ethyl Ester molecules possess higher docking score and re-ranking score which corresponds to the higher affinity. Furthermore, the results indicate that Histamine, N-Trifluroacetyl-2-amino compounds possess poor binding interaction with the target molecule.

Insilico docking analysis of bioactive constituents from *Limonia acidissima* L. fruit on estrogen receptor (PDB ID: 1X7R, 1X7J) which possess 539 amino acid residues in alpha and 540 residues in the beta position. The residues in the receptors which are involved in the binding to the ligand molecules are ARG 394, LYS 401, GLU 305, LYS 449, LEU 387, GLU 353, PHE 404, PRO 325 and LEU 327. The interaction of residue with the ligand molecule is represented in Fig. 1. The binding affinity of the ligand molecule is explained by the formation of hydrogen bonds and hydrophobic interaction. In Fig. 2 green dotted lines shows the hydrogen bond and the pink line corresponds to hydrophobic interaction. The drug design property of *Limonia acidissima* L. fruit was explained by the Lipinski rule of five, it states that the compound must possess hydrogen bond donor is <5, hydrogen bond acceptor is <10, molecular weight <500, partition coefficient log p-value is between 0.4 to 5.6 r range. The Table 3 shows that N-Isobutyl-(2E, 4Z, 8Z, 10E)-dodecatetraenamide and Histamine, N-TFA-2-amino compounds possess drug design property.



(A) N-Isobutyl-(2E, 4Z, 8Z,10E) dodecatetraenamide (B) 19, 19-Dimethyl-eicosa-8, 11-dienoicacid (C) Hexadecanoic Acid, 14-Hydroxy-16, 16-Dimethoxy-14-Methyl, Ethyl Ester (D) Bromoacetic acid, 2-tetradecyl ester (E) Histamine, N-Trifluroacetyl-2-amino.

Fig. 1. Schematic representation of docked ligands molecules on estrogen receptor.



(A) N-Isobutyl-(2E, 4Z, 8Z,10E) dodecatetraenamide (B) 19, 19-Dimethyl-eicosa-8, 11-dienoicacid (C) Hexadecanoic Acid, 14-Hydroxy-16, 16-Dimethoxy-14-Methyl-, Ethyl Ester (D) Bromoacetic acid, 2-tetradecyl ester (E) Histamine, N-Trifluroacetyl-2-amino.

Fig. 2. Interactions of ligands molecules with amino acid residues.

Table 3: Drug likeness properties of bioactive components by Lipinski rule of five.

S. No.	Name of the ligand	LOG P	HBA	HBD
1	19, 19-Dimethyl-eicosa-8, 11-dienoic acid	6.235	21	3
2.	N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	-2.546	3	2
3.	Bromoacetic acid, 2-tetradecyl ester	2.657	2	0
4.	Hexadecanoic Acid,14-Hydroxy-16,16,Dimethoxy-14-Methyl, Ethyl Ester	2.657	7	2
5.	Histamine, N-Trifluro acetyl-2-amino	-5.302	4	2

IV. CONCLUSION

In this present study, the potential drugs for curing ovarian cancer have been identified by docking analysis. The drug design property of phytocomponents of *Limonia acidissima Linn.* has been developed by protein- ligand interaction. It could explain with the help of a docking score and re-ranking score which stabilizes the receptor structure. Docking results show that 19, 19-Dimethyl-eicosa-8, 11-dienoic acid and Hexadecanoic acid- 14-Hydroxy-16, 16-Dimethoxy 14-Methyl-, Ethyl Ester has high docking and re-ranking score which indicates that the molecule possesses a good binding affinity with the target molecule. The lead molecule has interaction with amino acid residues at the site of Arg 394, Leu 387, and Glu 353; Estrogen receptors are helpful to select the active inhibitor for ovarian cancer. Lipinski rule of five concludes that Isobutyl-(2E, 4Z, 8Z, 10E)-dodeca tetraenamide, and Histamine, N-TFA-2-amino compounds satisfied the

drug design property than the other bioactive components present in of *Limonia acidissima Linn.* fruit extract. The phytoconstituents present in *Limonia acidissima* fruit extract could create a new path for the discovery of the novel anticancer drug.

Conflict of Interest. No conflict of interest.

V. FUTURE SCOPE

Further investigation has to be done for the isolation of bioactive components from *Limonia acidissima* plant, since the fruit is used for antitumor treatment in traditional medicine.

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