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IN SILICO SCREENING OF ACTIVE COMPONENT IDENTIFIED IN ACETONE EXTRACT OF *ACACIA NILOTICA* LEAVES AGAINST CANCER TARGET PROTEINS

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ABSTRACT

Acacia nilotica is a widely distributed well known multipurpose medicinal tree used in ethno medical management of a wide range of disorders. In the present work, the phytochemical analysis of active acetone fraction of *A. nilotica* leaves extract was performed using GCMS and the major compound Butanedioic acid, hydroxyl also known as Malic acid was selected as ligand for *in silico* molecular docking study against cancer receptor proteins such as Gamma glutamyl transferase, Human estrogen receptor, BMX non receptor tyrosine kinase, cyclooxygenase 2 and neuron specific enolase. Malic acid showed higher docking score towards neuron specific enolase (63.026) and also shares higher number of hydrogen bonds (6), the least score was by cyclooxygenase 2, but it has highest PLP score that indicates the stronger receptor-ligand binding. So Malic acid proved good docking result towards all the five target proteins that bring conformational changes, hence, the virulence of cancer may be reduced.

KEYWORDS: *Acacia nilotica*, GCMS, Malic acid, Molecular docking.

INTRODUCTION

Cancer is one of the leading causes of adult deaths worldwide. Recent anticancer treatments, although successful for a limited number of tumor types, the efficacy of cancer therapies, especially for late-stage disease, remains poor overall. Due to the increasing rate of mortality associated with cancer and adverse or toxic side effects of cancer chemotherapy and radiation

therapy, discovery of new anticancer agents derived from nature, especially plants, is currently under investigation.

During the process of identifying preventive agents, dietary phytochemicals are thought to be safe for human use. It has emerged as modulators of key cellular signaling pathways. The task now is to understand how these chemicals perturb these pathways by modeling their interactions with their target proteins.[1] Structure-based drug design is perhaps the most elegant approach for discovering compounds exhibiting high specificity and efficacy. The cost of research and development in the pharmaceutical industry has been rising steeply and steadily to achieve any process in the last decade but the amount of time required in bringing new products to market take around ten to fifteen years. *In silico* technique is an inexpensive technique that shortens the length of time spending in testing the efficacy of drug.

Acacia nilotica (L.) Delile is well known multipurpose medicinal tree. It is commonly known as Gum Arabic or Babul tree. It is widely spread in Africa, Asia and Australia.[2] Almost all its parts are used in medication including root, bark, leaves, flower, gum, pods etc.,[3] It is a pioneer species, relatively high in bioactive secondary compound and is important for a variety of functions. Babul plant is therapeutic used as Anticancer, Anti tumors, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains and Diarrhea, Nerve stimulant, Cold, Congestion, Coughs, Dysentery, Fever, Hemorrhages, Leucorrhea, Ophthalmia and Sclerosis.[4]

Thus, with the previous knowledge about the anticancer activity of the plant *A.nilotica*, the phytochemicals are identified in the plant extract and docking study was performed against few important cancer receptor proteins so that a particular anticancer compound or a class of compounds can be identified.

MATERIALS AND METHODS

Sample collection and identification

The study plant *Acacia nilotica* was collected from T.M Palayam (Latitude 10.9º N and Longitude 76.9º E) of Coimbatore in Tamilnadu. The collected plant sample was identified at Botanical Survey of India, Southern regional Centre, Coimbatore.

Preparation of extract

The shadow dried *A.nilotica* sample was powdered with help of electronic blender. Twenty five gram of powdered plant material was taken in clean sterile Soxhlet apparatus and extraction was done with 250 ml of acetone. The crude extract was further purified by column chromatography and Thin Layer Chromatography. *A.nilotica* leaves acetone extract (0.1g) was then added above the column and eluted with different ratios of acetone and petroleum ether for preparation of different fractions. Each fraction was screened by TLC with the solvent system Toluene – ethyl formate – formic acid (5:4:1) for individual separation of active compounds in crude *A.nilotica* extracts. The active fraction was identified in antimicrobial assay. The fraction showing best result in antibacterial assay was concentrated.

GCMS analysis

The purified fraction of acetone extract was subjected to GC-MS to identify the bio active constituents present in it. The GC-MS analysis is done in the SITRA (South Indian Textile Research and association), Coimbatore, Tamilnadu, India. The analysis was performed using the equipment Thermo GC - Trace Ultra Ver: 5.0, Thermo MS DSQ II with the capillary column of DB 5 - MS capillary standard non - polar column which is 30m long, 0.25mm internal diameter with film thickness of 0.25µm. 1µl of sample was taken for analysis. Helium was employed as carrier gas with flow of 1ml per minute. The oven temperature program was initially set as 70ºC and raised up to 260ºC at 6ºC per minute. The run time of the sample was 37 minutes. The mass spectra of compounds in samples were obtained by electron ionization at 70 eV and the detector operated in scan mode from 50 to 650 atomic mass units. Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST.

***In silico* screening**

Ligand Preparation: The single major peak in the GCMS was identified as Malic acid and chosen as a ligand for *in silico* studies. The three dimensional structure of Malic acid was downloaded from Pubchem database. The selected compound from Pubchem were drawn by using Chemsketch and saved in. mol format. The structure is loaded in discovery studio 2.1.

Protein preparation: The five target proteins were retrieved from protein Data Bank (www.rcsb.org) and the 3D structure of receptors was saved as 1VQQ. Crystallographic

water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. Following the above steps of preparations, the protein was subjected to energy minimization using the CHARM force field.

Docking process: Before beginning the docking, it was necessary to specify a binding site of the receptor. Ligand fit uses a method based on protein shape searching for cavities. Often the largest cavity was part of the ligand binding site. The 3D structure of ligand and protein was loaded in Accelrys Discovery Studio Software and interaction was studied. The determination of the ligand binding affinity was calculated using LigScore and PLP1, JAIN and Dock score were used to estimate the ligand binding energies.

RESULT AND DISCUSSION

GCMS: GC-MS analysis was performed to identify different compounds in the *A.nilotica* active acetone fraction. Thirty compounds were identified in GC-MS by library search tools and it is screened for its bioactive compounds among which four compounds (Orphenadrine, Pheniramine, Chlorophenamine and Butanedioic acid, hydroxyl or Malic acid) were identified as bioactive compound and were listed in Table 1. Among them a single major peak was observed as Butanedioic acid, hydroxyl, also commonly known as Malic acid with RT value of 26.96 with peak area of 97.54%. The molecular formula is C4H6O5 with molecular weight 134 Da. The chromatogram of GC-MS is shown in Fig. 2 and the library search result for structural prediction and identification is shown in Fig. 3. When analyzing the bioactivities of the four compounds, Malic acid was found to have various activities,[5] which include antitumor property. So, it is selected as a ligand for the further docking studies against various cancer targeted proteins to prove its antitumor property.

Further, the Lipinski rule stated that in general, an orally active drug has no more than one violation of the following criteria: (1) No more than five hydrogen bond donors, (2) No more than 10 hydrogen bond acceptors, (3) A molecular mass less than 500 Daltons (4) log P not greater than 5.[6] Hence Malic acid with 3 hydrogen bond donors, 5 Hydrogen bond acceptors, 134 Daltons molecular weight and -1.3 Log P value confirmed Malic acid to act as a drug.

Table 1: Bioactive compounds identified from the active acetone fraction of *A.nilotica* leaves extract by GCMS method.

S.No	RT	Compound	Area	Bio activity
1	4.61	Orphenadrine	0.17	Pain reliever
2	22.62	Pheniramine	0.56	H1 antagonist; Antipuritics; Antiallergic
3	25.54	Chlorophenamine	0.03	H1 antagonist; Antiallergic; Antihistamine
4	26.98	Butanedioic acid, hydroxyl OR Malic acid	97.54	Antiatherosclerotic; Antibacterial; Antifibromyalgic; Antioxidant Synergist; Antiseborrheic; Antiseptic; Antitubercular; Antitumor; Bacteristat; Hemopoietic; Laxative; Mycobactericide.

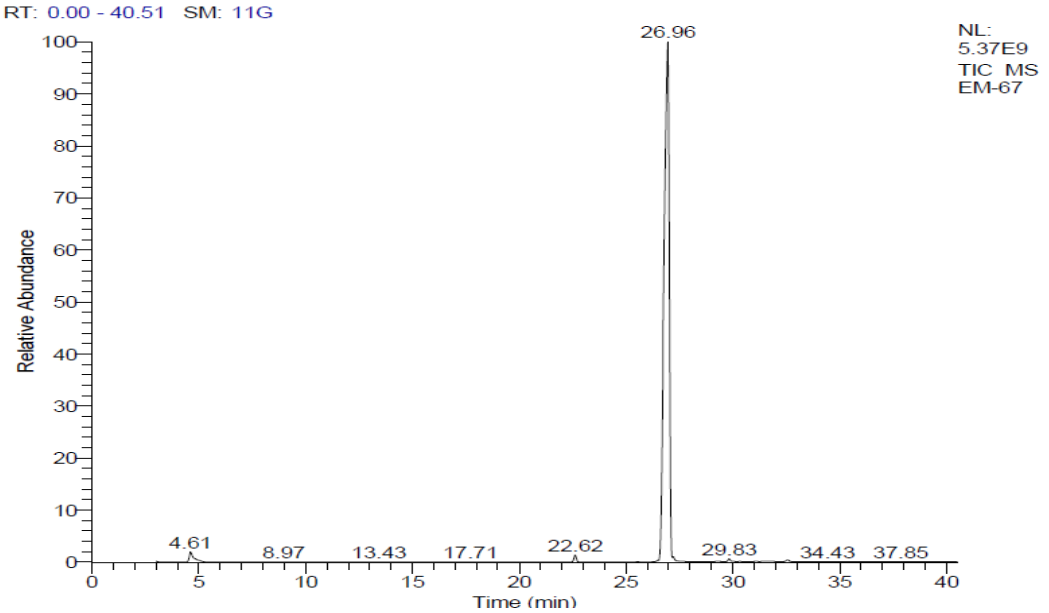

Fig. 2: GC-MS chromatogram of active acetone fraction of *Acacia nilotica* leaves extract.

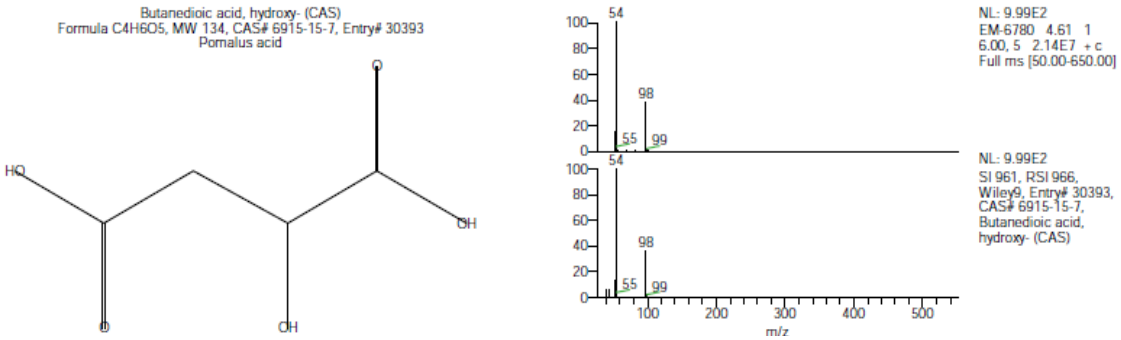

Fig. 3: Library search graphics table for Malic acid identification.

Validation of docking results

The molecular study was performed with the ligand, Malic acid against five selected cancer cell receptor proteins namely Gamma glutamyl transferase, Human estrogen receptor, BMX

non receptor tyrosine kinase, Cyclooxygenase 2 and neuron specific enolase. The results were analyzed on the basis of docking score (Table 2) and hydrogen bonds between the ligand and proteins (Table 3). Our study showed that among five receptor proteins, Malic acid showed higher docking score towards neuron specific enolase (63.026) and also shares higher number of hydrogen bonds (6), the least score was by cyclooxygenase 2, but it has highest PLP score that indicates the stronger receptor-ligand binding. So Malic acid proved good docking result towards all the five target proteins that bring conformational changes, hence, the virulence of cancer may be reduced. Each target protein has a definite role in progression of cancer and act as a target for anticancer treatment.

Gamma-glutamyl transferase (GGT) is a key enzyme involved in glutathione metabolism and whose expression is often significantly increased in human malignancies. In the past years, several studies focused on the possible role of GGT in tumor progression, invasion and drug resistance. Alessandro *et al.*, (2010)[7] focused on the potential role of GGT as a diagnostic/prognostic marker, as well as a target for anticancer treatments.

Normal breast cells and most breast cancer cells have receptors that attach to circulating estrogen and progesterone. Estrogen and progesterone bind to the receptors and may work with growth factors (e.g., oncogenes and mutated tumor suppressor genes) to cause cancer cell growth and proliferation Breast cancers that are estrogen and progesterone receptor positive (i.e., ER+ and PR+) have prognosis of cancers.[8] So, these receptors act as a target for cancer treatment.

Kinase inhibitors are among the fastest growing class of anti-cancer therapies. One family of kinases that has recently gained attention as a target for treating malignant disorders is the Tec kinase family. Evidence has been published that one member of this family, the Bmx kinase, may play a role in the pathogenesis of glioblastoma (Primary brain tumour), prostate, breast and lung cancer.[9]

Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. COX-2, undetectable in normal tissues, and induced during inflammation, hypoxia and Wnt-signalling, is present in many cancers.[10]

Neuron-specific enolase (NSE) is a well-known marker of small cell lung cancer[11] assessed the clinical value of NSE in non-small cell lung cancer (NSCLC).

As a single molecule Malic acid proves best docking score with all the above target proteins, thus it may serve as a best anticancer drug.

Table 2: Summary of docking information of the Top ranked poses of each cancer cell target proteins with Malic acid.

S.No	Target proteins	Protein ID	Ligand score 1	Ligand score 2	PLP1	PLP2	JAIN	PMF	Dock score
1	Gamma glutamyltransferase	3CRY	2.05	1.13	-2.19	3.67	-2.21	39.44	56.703
2	Human estrogen receptor	2IOK	2.63	2.44	18.35	15.65	-1.71	35.99	55.227
3	BMX non receptor tyrosine kinase	3SXR	1.51	0.56	-6.78	2.1	-1.51	32.41	55.809
4	Cyclooxygenase 2	6COX	2.75	1.82	41.59	45.95	1.3	18.49	33.246
5	Neuron specific enolase	1TE6	4.08	3.38	38.9	43.63	0.01	41.3	63.026

Ligand score 1 and 2: Protein ligand affinity energy

PLP1 and PLP2: Steric and hydrogen bonding intermolecular intermolecular function

JAIN: Sum of five interaction terms (Lipic interaction, polar attractive and repulsive interaction, solvation of protein and ligand)

PMF: Developed based on statistical structures of protein ligand complex.

Table 3: Details of Hydrogen Bond interactions between the cancer cell target protein and the ligand Malic acid.

Target protein	PDB ID	Amino acid	Atom in amino acid	Position of amino acid	Atom in ligand	Hydrogen bond length (A°)
Gamma glutamyltransferase	3CRY					
		ARG	HH11	123	O5	2.41
		ARG	HH12	123	O3	2.44
		GLU	OE1	109	H14	1.00
		GLU	OE1	109	H15	0.90
Human estrogen receptor	2IOK					
		PRO	O	325	H13	2.06
		GLU	OE2	353	H14	1.18
		GLU	OE2	353	H15	1.14
BMX kinase	3SXR					
		ASP	OD1	563	H14	1.22
		ASP	OD2	563	H15	0.87
Cyclooxygenase enzyme (COX2)	6COX					
		LEU	HN	503	O3	2.08
		LEU	HN	503	O5	2.31
		GLU	OE1	502	H14	1.03

		GLN	O	461	H15	1.23
Neuron specific enolase	ITE6					
		LYS	HZ1	192	O2	2.13
		LYS	HZ1	192	O4	2.11
		LYS	HZ3	192	O5	1.97
		ASN	OD1	205	H13	2.26
		ASP	OD1	208	H14	1.18
		ASP	OD2	208	H14	1.19
		ASP	OD1	208	H15	2.38

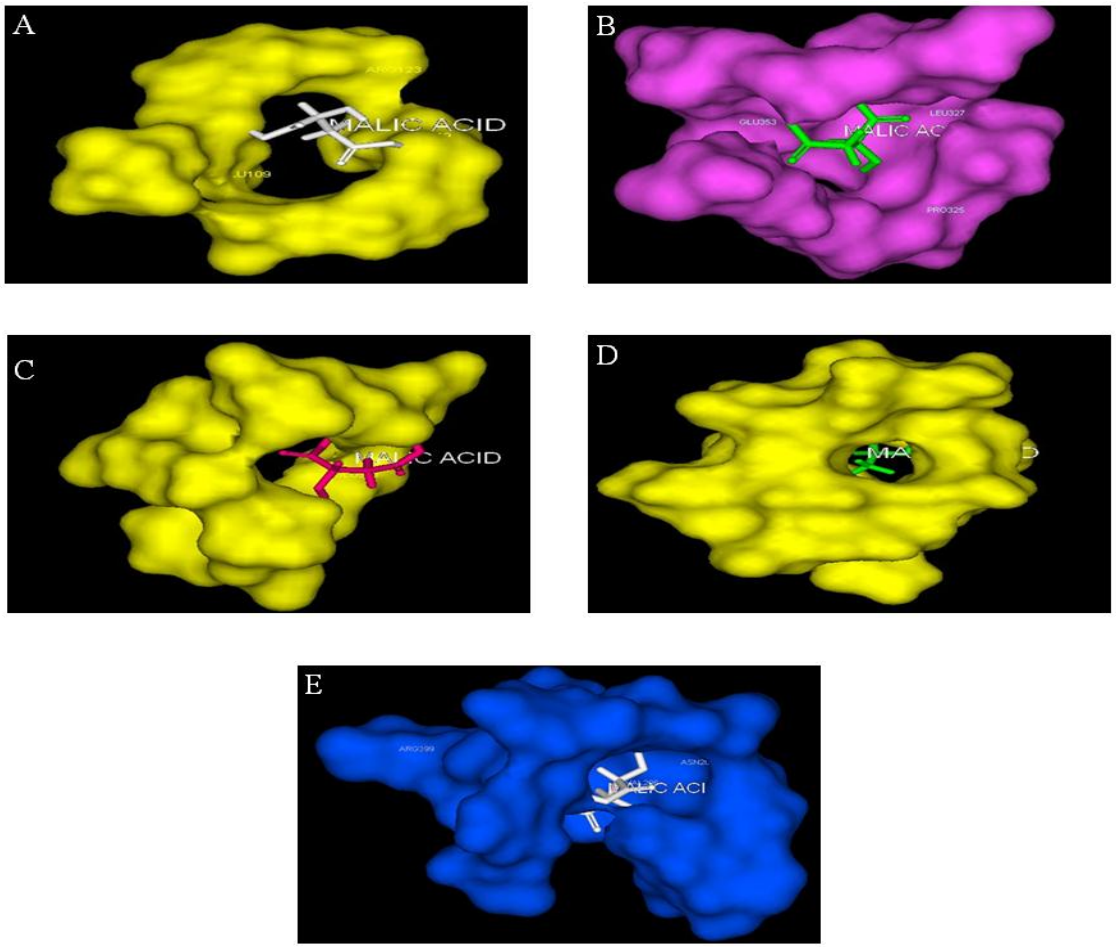

Fig. 4: Solid surface view of Malic acid complex with cancer receptor proteins.

A: Malic acid complex with Gamma glutamyltransferase

B: Malic acid complex with Human estrogen receptor

C: Malic acid complex with BMX non receptor tyrosine kinase

D: Malic acid complex with Cyclooxygenase 2

E: Malic acid complex with Neuron specific enolase

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