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IN SILICO SCREENING OF PHYTOCHEMICALS IDENTIFIED FROM *ACACIA NILOTICA* BY GCMS METHOD FOR ITS ANTICANCER ACTIVITY

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ABSTRACT: In present study the phytochemical analysis of the plant *Acacia nilotica* was done using GCMS method and seven compounds were identified as 3-picoline-2-nitro, 1-acetyl beta carboline, Hydroxy citronellal, Trans decalone, Propionic acid-2-chloro, ethyl ester, Lavandulyl acetate and D-Glucoronic acid. The anticancer activity of these seven compounds were analyzed with molecular docking study against the protein S-adenosyl-L-methionine decarboxylase which is found elevated in person with liver cancer. Among the seven phytochemicals identified, hydroxy citronellal followed by 1-Acetyl ß carboline was observed to have maximum score with S-adenosyl-L-methionine decarboxylase in molecular docking study, Glucoronic acid scores maximum in number of Hydrogen bonds. And taking into consideration all the scoring parameters, Glucoronic acid is considered as the best fit. Further studies are needed for the isolation of this particular phytochemical and subject it to further cell line testing and animal testing for anticancer activity and can be processed to be an effective and cheaper drug due to higher biomass availability of *Acacia nilotica* plant.

INTRODUCTION: Cancer is a major public health burden in both developed and developing countries. Major research programs are being undertaken in number of laboratories in various parts of the world for screening plant extracts for anticancer activity.

Some 3000 - 4000 plant samples representing 2000 species are screened each year for anticancer property [1].

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There are more than 2, 70, 000 higher plants existing on this planet. But only a small portion has been explored phytochemically. So, it is anticipated that plants can provide potential bioactive compounds for the development of new 'leads' to combat cancer diseases [2].

Acacia nilotica (L.) Del. belongs to the family Mimosaceae and is widely distributed in tropical and sub-tropical countries. Ayurvedic medicine practices use of natural medicinal plants to promote self-healing, good health and longevity, have declared that *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions [3]. Traditionally the bark, leaves, pods and flowers are used against

cancer, cold, congestion, cough, diarrhea, dysentery, fever, hemorrhoid, opthalmia, sclerosis, smallpox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual problem [4]. And recently the light has been thrown upon this plant because of its higher biomass availability, so that it can prove effective and cheaper drug for cancer. Recent studies also revealed that the plant extract has Antibacterial activity, Antimalarial activity, Antifungal activity, Antibiotic activity, Anti-Diarrhea activity, Molluscidal activity, Anti-hypertensive activity, Anthelmentic activity, Anti denaturation property, Antioxidant and Anticancer property [5].

Carcinogenesis is a multi-step process characterized by progressive changes in the amounts or activity of proteins that regulate cellular proliferation, differentiation and survival[6]. S-adenosyl-L-methionine decarboxylase is one of such identified protein that is found in excess in cancer patients. This enzyme is responsible for the conversion of putrescine to spermidine and then to spermine. Increased biosynthesis and subsequent accumulation of the polyamines like putrescine, spermidine and spermine have been shown to occur in tissues after various growth stimuli [7]. These polyamines are ubiquitous intracellular bases required for normal and neoplastic growth. Among their other functions, they facilitate transcription, translation, and initiation of protein synthesis [8]. In a number of animal systems, the accumulation of spermidine parallels that of RNA [9]. So, a control in the enzyme S-adenosyl-L-methionine decarboxylase can indirectly control the massive cell growth and RNA synthesis by controlling the spermidine synthesis. Hence, the cell growth can be controlled preventing it to become a cancer cell.

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. Insilico technique is an inexpensive technique that shortens the length of time spending in testing the efficacy of drug. The present study focuses on the screening of Phytochemicals from the plant *Acacia nilotica* and to test its potential of its phyto-chemicals as an anticancer agent by molecular docking studies with S-adenosyl-L-methionine decarboxylase.

MATERIALS AND METHODS:

Collection of plant material: The leaves of *Acacia nilotica* collected from T M Palayam, Coimbatore.

Dry powder preparation: The plant leaf sample was dried in hot air oven at 40°C for 24 hours and ground into powder.

Sample preparation for GCMS analysis: About 5g of powdered material of plant was taken in a clean, flat-bottomed glass container and soaked in 25mL of 80% methanol. The container with its content was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then, it was filtered through Whatmann filter paper. The filtrate (methanolic extract) obtained for the plant was evaporated under ceiling fan and in a water bath until dried.

GCMS analysis: The GCMS analysis was conducted at the south Indian textile research association, Coimbatore. 2uL aliquot was injected into a fisons GC8000 series GC coupled to a MD800 MS with quadrapole mass analyzer (fision instrument, Milano, Italy). The chromatography was performed by using the DB5-MS column. Injection temperature was 230°C. Helium flow was 1mL/min. After a 5 min solvent delay time at 70°C; the oven temperature was increased at 5°C/min to 310°C, 1min isocratic, and cooled to 70°C, followed by the additional 5min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks.

Identification of Components: Interpretation of mass spectrum GCMS was conducted using data base of National Institute Standard and Technology (NIST) and Wiley spectra libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library. The molecular weight, molecular formula, and the number of hits used to identify the name of the compound from NIST and Wiley spectra libraries were recorded.

Ligand Preparation: The compounds reported in Gas Chromatography and Mass Spectrum (GCMS) analysis of methanolic extract of leaf of *Acacia nilotica* were used in the present study. The structures of the seven compounds (ligands) were retrieved from PUBCHEM database.

Protein preparation: The target protein S-adenosyl-L-methionine decarboxylase was retrieved from protein Data Bank (www.rcsb.org) and crystallographic water molecules were removed from the protein.

Docking Analysis: Molecular docking continues to hold a great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in binding site of a protein. The interaction study was carried out in Ligandfit of Accelrys Discovery Studio software. The binding sites of the protein were predicted using find cavities from the receptor site parameter of the tool. The determination of the ligand binding affinity was calculated using Dock scores, the Dock score for each ligand is calculated by the software itself. The number of Hydrogen bonds involved in the interaction along with amino acids involved in the hydrogen bonding and the distance between the hydrogen bonds were also estimated using Accelrys Discovery Studio software. Here, the seven phytochemicals identified in GCMS analysis is docked with the target protein S-adenosyl-L-methionine decarboxylase.

RESULT AND DISCUSSION: GC-MS analyzed results which include the active principles with their molecular formula and molecular weight are presented in Table 1. Here, seven compounds were identified and they are reported as 3-picoline-2-nitro, 1-acetyl beta carboline, Hydroxy citronellal, Trans decalone, Propionic acid-2-chloro, ethyl ester, Lavandulyl acetate and D-Glucoronic acid. On further study of each compound, it was found that they individually have its own biological importance. These phytochemicals were treated as ligands and it is docked with target protein S-adenosyl-L-methionine decarboxylase. The list of the seven phytochemicals identified along with Compound ID as in pubchem is shown in **table1.**

TABLE 1: PHYTOCHEMICALS IDENTIFIED BY GC-MS METHOD

S. No.	Name of the compound	Compound ID	Molecular formula	Molecular weight
1	3-picoline-2-nitro	564205	$C_6H_6O_2N_2$	138
2	1-acetyl beta carboline	638667	$C_{13}H_{10}ON_2$	210
3	Hydroxy citronellal	7888	$C_{10}H_{20}O_2$	172
4	Trans decalone	98501	$C_{10}H_{16}O$	152
5	Propionic acid-2-chloro,ethyl ester	10807	$C_5H_9O_2Cl$	136
6	Lavandulyl acetate	30247	$C_{12}H_{20}O_2$	196
7	D-Glucoronic acid	444791	$C_6H_{10}O_7$	194

Importance of Ligands: Indole alkaloids are pharmacologically active natural products that have been shown to possess a wide range of biological activities, including cytotoxic, antiviral, antimicrobial, antiinflammatory, antiserotonin, and enzyme inhibitory activities [10]. One important subclass of indole alkaloids is β-carbolines, which possess a common tricyclic pyrido [11] indole ring structure [12].

The β-carbolines skeleton is an important structure in drug discovery [13], and drugs on the market such as tadalafil possess this indole nucleus. 1-Acetyl-β-carboline was isolated as an anti-MRSA (Methicillin-Resistant *Staphylococcus aureus*) agent. Combination of 1-acetyl-β- carboline with ampicillin exhibited synergistic antibacterial activity against MRSA [14].

In the animal body, glucuronic acid is often linked to the xenobiotic metabolism of substances such as drugs, pollutants, bilirubin, androgens, estrogens, mineralocorticoids, glucocorticoids, fatty acid derivatives, retinoids, and bile acids [15]. D-Glucuronic acid exists as a component of glycosaminoglucans such as hyaluronan, heparin and chondroitin sulphate present in mammalian connective tissue such as cartilage.In all plants and mammals other than guinea pigs and primates-glucuronic acid is a precursor of ascorbic acid, also known as vitamin C [16]. Tissue repair and wound healing are complex processes that involve a series of biochemical and cellular reactions, beginning with inflammation and followed by the repair and remodeling of the injured tissue When there is damage to connective tissue it is important to address the nutritional requirements for the

synthesis of both the collagen fibers and the proteoglycans [17]. Many nutrients are involved in connective tissue repair and wound healing: D-glucuronic acid is one of those wound healing compound [18].

3-picoline-2-nitro is also known as 3-Methyl-2-nitropyridine. 3-Picoline is usually useful precursor to agrochemicals, such as chlorpyriform [19], and also a main precursor to niacin, one of the B vitamins [20].

Hydroxy citronellal and Lavandulyl acetate are the floral compounds; it is used to impart the pleasant odour to numerous consumer products [21]. These volatile aroma compounds are also known as essential oils. There are various evidences reporting that essential oils has many biological activity such as anticancer, anti-nociceptive, anti-inflammatory, anti-viral and also anti oxidative property [22].

Trans-1-decalone is used as the substrate for enzyme assays.

Thus, each compound identified in leaf extract of *Acacia nilotica* has its own biological importance and the study of this plant's phytochemical by insilico method can prove its medicinal importance in future and can be an effective and efficient drug source in cheaper rate as it has higher biomass availability. So Phytochemical analysis is proceeded with molecular docking studies.

Lipinski Properties: Lipinski's rule of five also known as the Pfizer's rule is to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. All the ligands satisfy Lipinski rule as shown in **Table 2.**

TABLE 2: PROPERTIES OF LIGANDS FOLLWING LIPINSKI RULE

S. No.	Compound name	Molecular weight <500Daltons	H-bond donor <=5	H-bond acceptor <=10	Log p <5
1	3-picoline-2-nitro	138	0	3	1.4
2	1-acetyl beta carboline	210	1	2	2.3
3	Hydroxy citronellal	172	1	2	1.6
4	Trans decalone	152	0	1	2.6
5	Propionic acid-2-chloro,ethyl ester	136	0	2	1.5
6	Lavandulyl acetate	196	0	2	3.6
7	D-Glucoronic acid	194	5	7	2.3

Lipinski rule of five is used as the first step filter to perform virtual screening of compound libraries in an effort to quickly eliminate lead candidates that have poor physico chemical properties, but satisfactorily all the seven compounds identified as ligand passed the initial screening test.

Validation of docking result: Docking procedures aim to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, docking describes a process by which two molecules fit together in three-dimensional space.

Molecular docking has contributed important proceedings to drug discovery for many years. Here, the seven phytochemicals identified in GCMS analysis is docked with the target protein S-adenosyl-L-methionine decarboxylase.

The validation process consists of two parts:

(i) Prediction of binding energy between the docked ligand and the protein using various score calculated using discovery studio (PLP1, PLP2, JAIN, Ligand internal energy and PMF), these scores were taken for analysis.

The Dock score generated on the basis of the binding energy and binding forces between the docked ligand and the protein using and

(ii) Hydrogen bond details of the top ranked docked pose.

The summary of docking information of the top ranked poses in each compound is given in **table 3**.

TABLE 3: SUMMARY OF DOCKING INFORMATION

S. No.	Compound name	PLP1	PLP2	JAIN	Ligand internal energy	PMF	Dock Score
1	3-picoline-2-nitro	41.90	41.23	1.76	-0.914	55.32	43.565
2	1-acetyl beta carboline	47.86	58.07	2.77	-2.89	93.47	45.226
3	Hydroxy citronellal	52.68	50.46	-1.81	-1.86	59.69	45.276
4	Trans decalone	67.82	65.96	1.9	-1.913	76.09	26.857
5	Propionic acid-2-chloro,ethyl ester	30.24	35.54	-0.83	-0.887	36.10	29.024
6	Lavandulyl acetate	67.91	60.49	1.02	0.118	81.61	43.397
7	D-Glucoronic acid	43.06	42.65	0.68	-0.151	54.39	39.500

As a result of docking there were 10 different conformations were generated for seven ligands, Only for top ranked docked complex the scores were copied from the table browser view of Discovery studio for binding affinity analysis. **Table 3** shows the different score values of top ranked ligands. The score values include PLP1, PLP2 (Steric and H-bonding intermolecular function, Higher PLP scores indicate stronger receptor-ligand binding [23], JAIN (sum of five interaction terms namely Lipophilic interactions, Polar attractive interactions ,Polar repulsive interactions, Solvation of the protein and ligand ,An entropy term for the ligand) [24], Lower internal energy means better docking stability. PMF(developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex, a higher score indicates a stronger receptor-ligand binding affinity) [25] and Dockscore (Candidate ligand poses are evaluated and prioritized according to the DockScore function) .

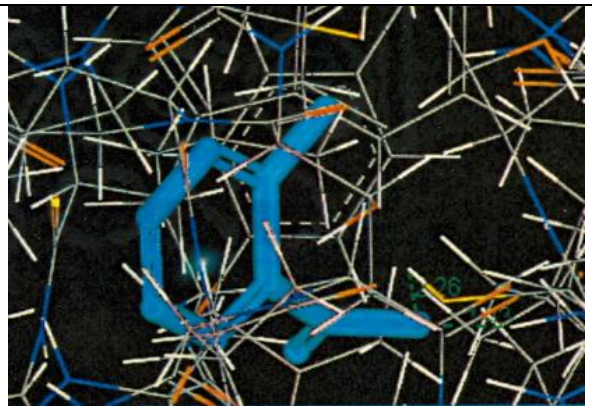
Regarding the Dock score value, Hydroxy citronellal and 1-acetyl beta carboline have similar scores of 45.276 and 45.226 and shows best binding affinities towards the protein. The order of ligands based on Dock score is Hydroxy citronellal>1-acetyl beta carboline>3-picoline-2-nitro> Lavandulyl acetate> D-Glucoronic acid> Propionic acid-2-chloro, ethyl ester> Trans decalone. Among the seven ligands, Lavandulyl acetate he maximum has PLP value. 1-acetyl beta carboline takes the greatest PMF value. It was quiet a minute difference between each Ligand taking up the scores.

By enlarging the interaction analysis hydrogen bond analysis is contributed as the major parameter. Results were analyzed using Hbond monitor of Accelrys Discovery Studio software. 3-picoline-2-nitro shares two hydrogen bonds with the protein S-adenosyl-L-methionine decarboxylase. 1-acetyl beta carboline, Hydroxy citronellal, Trans decalone, Propionic acid-2-chloro,ethyl ester, Lavandulyl acetate all share only one hydrogen bond with the protein and finally D-Glucoronic acid share six hydrogen bonds and scores the maximum value. The details of hydrogen bonds are given in **table 4**, and the docking model of the seven ligands with the protein is shown in **fig 1** (the hydrogen bond is shown in green dotted line).

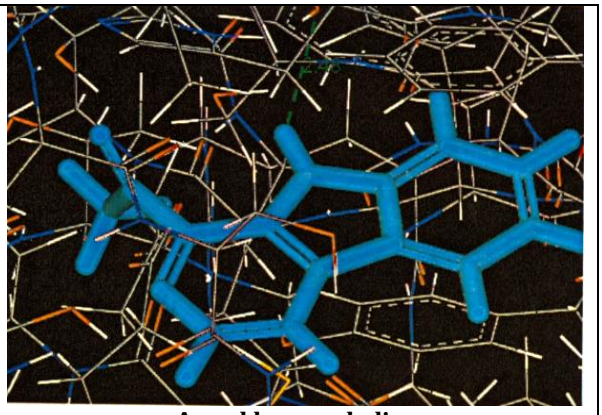
TABLE 4: DETAILS OF HYDROGEN BONDS

S. No.	Ligand name	No. of hydrogen bonds	Amino acid residues involved in bonding	Distance of Hydrogen bonds
1	3-picoline-2-nitro	2	A:CY582:HN-2nitro 3 picoline A:CY582:HG-2nitro 3 picoline	2.26 1.43
2	1-acetyl beta carboline	1	Acetyl ß carboline H17-A:SER229	2.48
3	Hydroxy citronellal	1	A:CY582:HN-hydroxy citronellal	1.96
4	Trans decalone	1	B:GLU67:HN-trans decalone	2.17
5	Propionic acid-2-chloro, ethyl ester	1	A:CYS582:HG- Propionic acid-2-chloro,ethyl ester	1.91
6	Lavandulyl acetate	1	A:THR245:HG1-Lavandulyl acetate	2.16
7	D-Glucoronic acid	6	B:GLU67:HN-D-Glucoronic acid:O5 A:CY582:HG- D-Glucoronic acid:O7 A:SER229:HG- D-Glucoronic acid:O3 A:HIS243:HD1- D-Glucoronic acid:O3 D-Glucoronic acid:H20-A:SER229:O5 D-Glucoronic acid:H23-B:LEU65:O3	1.92 2.49 2.15 2.46 1.44 1.41

Hydroxy citronellal has the leading docking score with 45.276, but has shared only one hydrogen bond with the protein moreover it does not have any specific biological importance. D-Glucoronic acid share six hydrogen bonds with the protein, that is really a good number for best interaction and has the docking score of 39.5 which is high enough to inhibit the protein, it also did not show any unsatisfactory results in any of the scoring parameters such as PLP, JAIN, PMF, Ligand internal energy etc,. D-Glucoronic also has various biological importances which are previously discussed in this paper. Thus D-Glucoronic acid shall be considered as the best ligand that can successfully inhibit S-adenosyl-L-methionine decarboxylase, thus can be used as an anticancer agent. This has to be confirmed in further studies with cell line testing and animal testing, and if it gives the better result than an anticancer drug can be formulated at cheaper rate with the plant *Acacia nilotic*a.

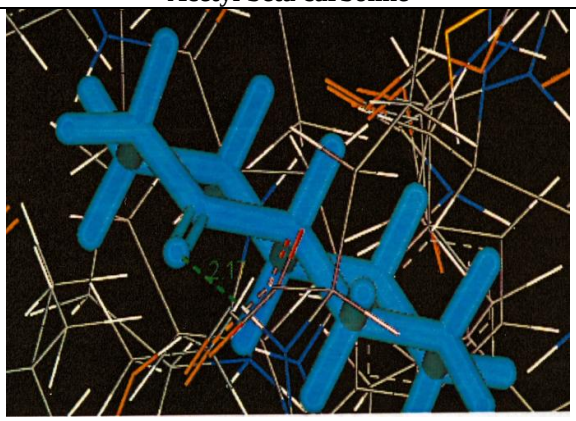


3 picoline-2-nitro

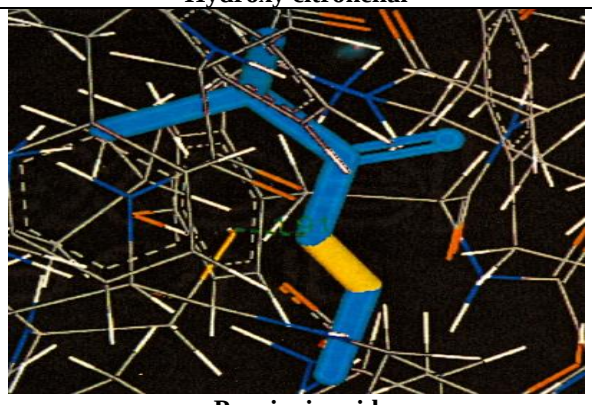


Acetyl beta carboline

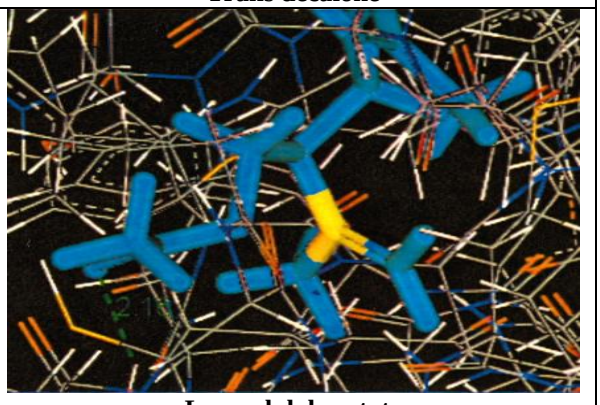
Hydroxy citronellal



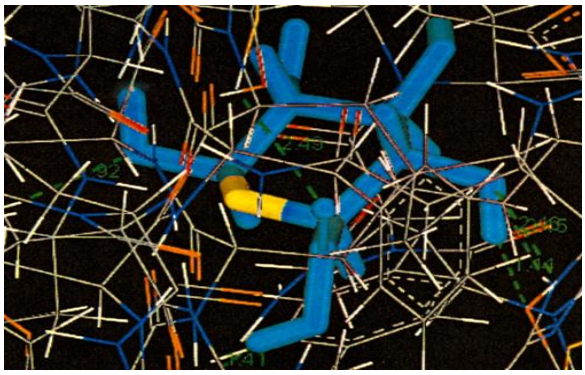
Trans decalone



Propionic acid



Lavandulyl acetate



D-Glucoronic acid

FIG. 1: DOCKED IMAGE OF EACH LIGAND WITH S-ADENOSYL-L-METHIONINE DECARBOXYLASE

CONCLUSION: Each compound identified by GC-MS in leaf extract of *Acacia nilotica* has its own biological importance, and the molecular docking study reveals the locking mechanism of each compound identified (Ligand) with the protein S-adenosyl-L-methionine decarboxylase, which is found excess with liver cancer patients. Glucoronic acid is found to have maximum docking effect. This study can be expanded to cell line testing and animal testing for screening anticancer activity and its side effects. An efficient drug can be synthesized at cheaper rate as *Acacia nilotica* has higher biomass availability.

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