Efficacy of some compounds isolated from *Nyctanthesarbor-tristis*Linn.on human and plant diseases as revealed from *in silico* analysis

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Abstract

Molecular docking analysis is used as an important tool in designing new drugs for different diseases. In the present study, GC-MS analysis of methanol extract of *Nyctanthesarbor-tristis*leaf yielded five compounds namely astragalin, nicotiflorin, nyctanthic acid, friedeline and lupeol. These molecules were subjected to molecular docking analysis against protein S-adenosyl-L-methionine decarboxylase involved in human liver cancer and against β-1,3-glucanase involved in wheat leaf rust diseases. Astragalin was the best inhibitory ligand for the enzyme S-adenosyl-L-methionine decarboxylase and friedeline was the best forβ-1,3-glucanase. Further in vivo study can confirm candidate molecules to be used in reality.

Keywords:*Nyctanthesarbor-tristis*, Phytochemicals, anticancer, anti-leaf rust, docking

Introduction

A plethora of knowledge regarding the benefits of herbal drugs was stored in ourearliest treatise of Indianmedicine, the CharakaSamhita (1000 B.C.), wherein the useof over 2000 herbs for medicinal purpose was mentioned (Cragg*et al*., 1997). According to a survey of WHO, 80% of the population living in the developingcountries depend almost exclusively on the traditional medicine fortheir primary health care needs.Exploration of the chemicalconstituents of the plants and pharmacological screening mayprovide us the basis for developing the leads for synthesis of novel agents. Among theestimated 400,000 plant species, only 6% have till now beenstudied for biological activity (Goyal*et al*., 2007).

*Nyctanthesarbor-tristis*Linn. (popularly known as nightjasmineor *parijaat*or *sephalika*)of familyOleaceae is a small tree, with a gray or greenish, rough and peeling bark. The shrub grows to a height of 10 meters. The simple leaves are opposite, with an entire edging about 6 to 12 cm long and 2 to 6.5 cm wide. The flowers are having snow white petals, fragrant with a five-to-eight lobed corolla and orange-red center, often seen in a cluster of two to seven. The fruit is plane, brown and heart-shaped to round capsule, around 2 cm in diameter with two sections, each containing a single seed (Bordoloi*et al*., 2016). It is traditionally used due to its extensive medicinal properties by the tribal rural people of India and also in Ayurveda, Siddha, and Unanimedicine systems (Sasmal*et al*., 2007). The whole plant and its parts are used for its effects in treating sciatica, arthritis, malaria, enlargement of spleenand as blood purifier; and the white flowers are used as stomachic, carminative,astringent, anti-bilious, expectorant, hair tonic and in the treatment of various skindiseasesandpiles; and in recent findings have been found to possess anti-spasmodic, anthelmintic,cytoprotective, anti-diabetic, anti-leishmanial, CNS depressant activity (Sandhar*et al*., 2011).

A number of bioactive compounds including flavanol glycosides, astragalin, nicotiflorin, oleanolic acid, nyctanthic acid, tannic acid,friedeline, lupeol, nyctanthin, nyctanthic acid, 3,4-secotriterpene acid, etc. have been isolated from the plant (Wikipedia, 2020). But reliable study on their efficacy against different human and plant diseases have not been carried out till date. In the present investigation molecular docking technology has been employed to find the effect of somecompoundsin controlling human and plant diseases.

Materials and methods

Plant collection and extract preparation

The leaves of *N. arbor-tristis*(Fig. 1. a) were collected from the campus of CUTM, Paralakhemundi, Odisha and were cleaned and dried under shade overnight. Then these were subjected to further drying in hot air oven at 40°C for 24 hours and subsequently ground into powder with Bajaj Maximix grinder. The powdered leaves were then extracted repeatedly with hot methanol (CH_3OH) using Soxhletapparatus(Fig. 1. b) fitted on a heating mantle keeping temperature set at 55°Cfor 12 hours following the method of Arulmozhi*et al.* (2019). The solvent was then removed at reduced pressure

and temperature (50°C) with a rotary vacuum evaporator to yield methanolic leaf extract to be used for further analysis.

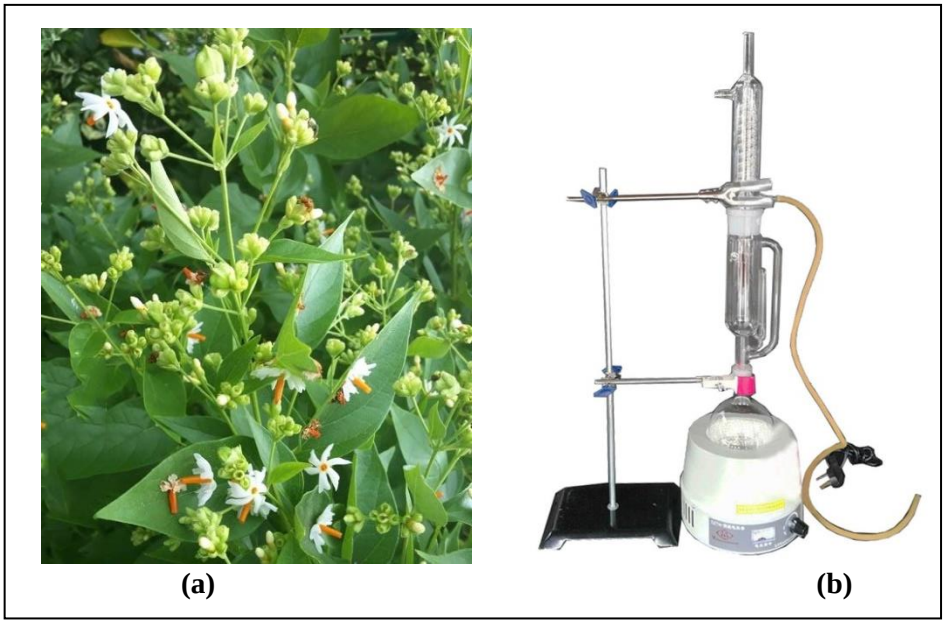

Fig. 1. (a) *N. arbor-tristis*plant, (b) Soxhlet extractor apparatus

Sample preparation for GC-MS analysis

The dried leaf extract (5 mg) was dissolved in 10 mL HPLC-grade methanol and was filtered through 0.22 µm PTFE membrane filter (Milipore, USA) and 1 mL of this was further diluted to 5 mL for GC-MS analysis.

GC-MS analysis

Thegas chromatography-mass spectrometry (GC-MS) analysisfor identification of the compounds was done as follows(Hemamalini*et al*., 2014). The GC-MS usedwas Varian CP 3800 GC coupled with Saturn 2200 MS andCombiPAL auto-sampler. The column used was Factor-Fourcapillary column (VF 5ms, 30m, 0.25 µm). The carrier gaswas helium at a flow rate of 1.0 mL min^{-1} and injection volumewas 1 µL. The temperature programming was as follows:injector temperature: 260°C, column oven temperatureprogramme: 50°C (1 min) to 270°C ramp at 10°C min^{-1}, heldfor 10 min.

Identification of compounds

Interpretation of mass spectrum in GC-MS 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. A total of five compounds were identified. 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 identified in the GC-MS analysis of methanolic extract of *N. arbor-tristis*leaf were used in the present study. The structures of the five compounds to be used as ligands were retrieved from PUBCHEM database.

Selection of enzymes with elevated activity during disease

In case of human beings, the activity of the enzyme S-adenosyl-L-methionine decarboxylase is found to be elevated in persons with liver cancer(Hemamalini*et al*., 2014). So the anticancer activity of these five compounds with S-adenosyl-L-methionine decarboxylase wasanalysed with molecular docking study. The same compounds were used to study the fungicidal efficacy against the fungal pathogen *Pucciniatriticina*,the causal organism of wheat leaf rust. This pathogen,an obligatory biotrophic parasite, is a severe fungal disease ofwheat causing substantial yield loss over a large part of theworld (Kolmer, 2005). Activity of one of the several pathogenesisrelated(PR) proteins, β-1,3-glucanase, is modulated due to *Puccinia* infection(Naz*et al*., 2014). So antifungal activity of the same five compounds isolated from *N. arbor-tristis*leaf was studied with this protein by utilizing molecular docking analysis.

Protein preparation

The target proteinsS-adenosyl-L-methionine decarboxylaseand β-1,3-glucanasewere retrieved from Protein Data Bank (www.rcsb.org), the ribbon structures of which are presented in Fig. 2.

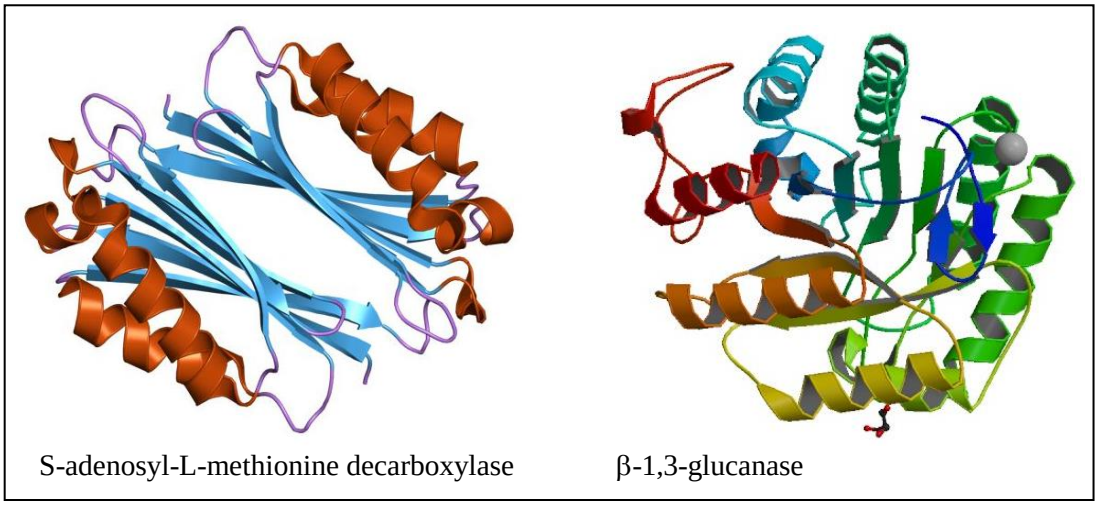

Fig. 2. Ribbon structures of the two enzymes under study retrieved from RCSB PDB

Molecular 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 bondsinvolved in the interaction along with amino acids involved in the hydrogen bonding and the distance between the hydrogen bonds were also estimated using LigandfitAccelrys Discovery Studio software. Here, the five phytochemicals identified in GC-MS analysis is docked with the target proteins S-adenosyl-L-methionine decarboxylase and β-1,3-glucanase.

Results and discussion

GC-MS identification of compounds

The results of GC-MS analysisof the active principles with their molecular formula and molecular weight are presented in Table 1. Here, five compounds were identified which are reported as astragalin, nicotiflorin, nyctanthicacid, friedeline and lupeol; structures of which are given in Fig. 3.

Table 1. Compounds identified from *N. arbor-tristis* by GC-MS

Sl. No.	Name of compound	Molecular formula	Molecular weight (g/mol)
1.	Astragalin	$C_{21}H_{20}O_{11}$	448.4
2.	Nicotiflorin	$C_{27}H_{30}O_{15}$	594.5
3.	Nyctanthicacid	$C_{30}H_{48}O_2$	440.7
4.	Friedeline	$C_{30}H_{50}O$	426.7
5.	Lupeol	$C_{30}H_{50}O$	426.7

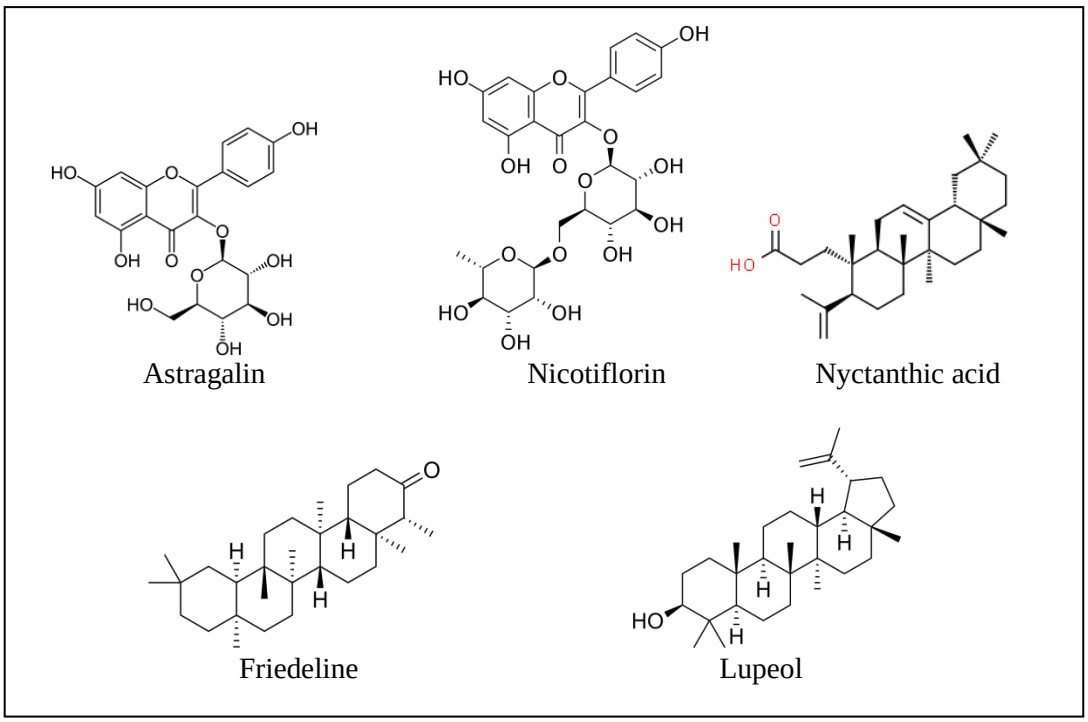

Fig. 3. Structures of the compounds identified by GC-MS analysis from *N. arbor-tristis*Linn.

Lipinski properties

Lipinski's rule of five also known as the Pfizer's rule is applied to evaluate drug-likeness 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. 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 five compounds identified as ligand passed the initial screening test.

Table 2: Properties of ligands following Lipinski rule

Sl. No.	**Name of compound**	**Molecular weight (g/mol)**	**H bond donor ≤ 5**	**H bond acceptor ≤ 10**	**log p < 5**
1.	Astragalin	448.4	1	4	2.3
2.	Nicotiflorin	594.5	0	2	1.8
3.	Nyctanthicacid	440.7	2	3	3.4
4.	Friedeline	426.7	0	6	1.7
5.	Lupeol	426.7	1	3	3.1

Validation of docking result

Docking describes a process by which two molecules fit together in three-dimensional space. Molecular docking has contributed important inputs to drug discovery for many years. Here, the five phytochemicals identified through GC-MS analysis is docked with the target proteins S-adenosyl-L-methionine decarboxylase and β-1,3-glucanase. 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), and (ii) hydrogen bond details of the top ranked docked pose(Hemamalini*et al*., 2014). The summary of docking information of the top ranked poses in each compound is given in Table 3 and Table 4 for the target proteins S-adenosyl-L-methionine decarboxylase and β-1,3-glucanase respectively.

Table 3. Summary of docking information of the compounds for S-adenosyl-L-methionine decarboxylase enzyme

Sl. No.	**Compound name**	**PLP1**	**PLP2**	**JAIN**	**Ligand internal energy**	**PMF**	**Dock Score**
1	Astragalin	40.81	40.53	1.83	-0.93	54.32	45.87
2	Nicotiflorin	48.47	59.27	2.65	-2.95	94.58	42.26
3	Nyctanthicacid	51.68	49.75	-1.74	-1.77	60.59	36.47
4	Friedeline	68.43	67.39	1.88	-1.83	74.28	26.85

5	Lupeol	31.28	37.74	-0.85	-0.89	37.71	29.04

Table 4. Summary of docking information of the compounds for β-1,3-glucanase

Sl. No.	Compound name	PLP1	PLP2	JAIN	Ligand internal energy	PMF	Dock Score
1	Astragalin	38.63	41.37	1.74	-0.91	56.47	43.565
2	Nicotiflorin	47.52	58.87	2.55	-2.76	92.63	41.226
3	Nyctanthicacid	52.49	48.56	-1.78	-1.83	61.46	37.276
4	Friedeline	67.34	66.59	1.72	-1.37	75.33	48.65
5	Lupeol	33.82	38.45	-0.83	-0.87	39.46	28.24

The score values include PLP1 and PLP2 (steric and H-bonding intermolecular function);higher PLP scores indicate stronger receptor-ligand binding (Gehlhaar*et al*., 1995), JAIN (sum of five interaction terms),lower internal energy means better docking stability. Scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex (higher score indicates stronger receptor-ligand binding affinity, Muegge, 2006) and Dockscore (candidate ligand poses are evaluated and prioritized according to the Dock score function).

The order of ligands based on Dock score for the enzyme S-adenosyl-L-methioninedecarboxylase is: astragalin>nicotiflorin>nyctanthic acid >lupeol>friedeline; and that for β-1,3-glucanase is: friedeline>astragalin>nicotiflorin>nyctanthic acid >lupeol.

So, astragalinwas observed to have maximum score (45.87) with S-adenosyl-L-methionine decarboxylase in molecular docking study and friedeline has maximum score (48.65) withβ-1,3-glucanase. The docking model of the five ligands with the protein S-adenosyl-L-methioninedecarboxylase is shown in Fig. 4.

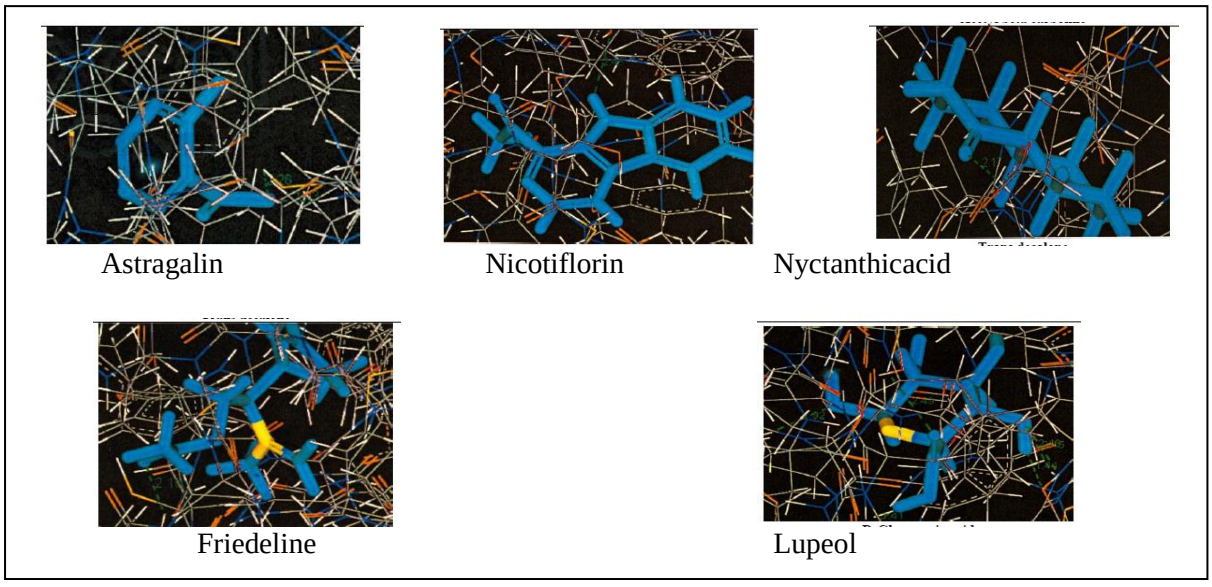

Fig. 4. Docking model of five ligands with S-adenosyl-L-methioninedecarboxylase

By studying the interaction between protein and the ligands and also considering the H-bond interaction it was found that out of the five molecules under study, astragalinisthe best ligand that can

successfully inhibit S-adenosyl-L-methionine decarboxylase, and thus can be taken as one candidate anticancer agent. In case of β-1,3-glucanase,friedeline showed maximum possible interaction with the protein and so this molecule can be considered as a candidate fungicide for controlling the wheat leaf rust.

Conclusion

Molecular docking analysis is used as an important tool in designing new drugs for different diseases. It saves expenses, manpower and time to a very good extent and so has become popular among the concerned scientists. In the present study astragalinwas found to be the best ligand that could inhibit the enzyme S-adenosyl-L-methionine decarboxylasesuccessfully and friedeline was the best one to inhibit the function of β-1,3-glucanase. So these two compounds can be considered as candidate molecules for drug of human cancer and wheat leaf rust respectively. Further *in vitro*and *in vivo*analysis will confirm the possibilities to be real.

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