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RESEARCH ARTICLE

# Total content, gas chromatography-mass spectrometry profiling and *in silico* studies of phytoconstituents from the ethanolic leaf extract of *Plumeria caracasana* J.R. Johnst.

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## Abstract

Plant material has been a major source of bioactive compounds for the treatment of various diseases and for drug development. The extracts of *Plumeria pudica* Jacq. have been studied for various diseases, but there have been no reports on the total content and docking studies related to the mechanistic antiepileptic action of the ethanolic extract of leaves of *P. pudica*. Hence, the study aimed to provide relevant information on the phytoconstituents present in the extracts of *P. Pudica* using gas chromatography-mass spectrometry (GC-MS) and in *in silico* studies. The leaves of *P. pudica* were ground into a fine powder and extracted using 6 different solvents. The total flavonoid, phenolic and alkaloid content was then determined following a preliminary qualitative analysis. To determine the presence of specific bioactive substances, such as flavonoids, alkaloids, terpenoids, phenolic compounds, steroids and fatty acids, preliminary phytochemical screening was conducted. In total content analysis, the total phenolic content was maximum in ethanolic extract (23.81 ± 0.148 mg GAE/g), methanol extract showed the highest alkaloid and flavonoid content of (30.93 ± 0.855 mg AE/g) and (12.02 ± 0.084 mg QE/g) respectively. The GC-MS profiling of the *P. pudica* leaf extract in 6 solvents revealed the presence of 16 phytoconstituents in the n-hexane extract, 8 in petroleum ether, 12 in chloroform, 17 in ethanol, 19 in methanol and 9 in the aqueous extract respectively. The phytoconstituents present in the ethanolic extract were screened for antiepileptic activity through *in silico* studies and the molecular docking scores were highest for 2-cyclohexen-1-one, 3-methyl-ethyl- and Pregnan-3,11-diol-20-one, with scores of -2.899 and -2.839 kcal/mol respectively, compared with the standard Diazepam, which was -3.295 kcal/mol. To gain a better understanding of the mechanism of action and toxicity profile, additional research is necessary for both *in vitro* and *in vivo* evaluations.

**Keywords:** GABA-A receptor; gas chromatography-mass spectroscopy; MM-GBSA; pharmacokinetic; *Plumeria pudica*; total content

## Introduction

Plants contain various bioactive compounds with important therapeutic effects, making it essential to study the active components in their different parts.This requires isolation, characterisation and knowledge of the presence of alkaloids, glycosides and phenols.

Plumeria species, *Plumeria obtuse* L. and *Plumeria rubra* L. exhibit central nervous system (CNS) depressant action such as sedative and hypnotic effects (1). *Plumeria obtusa* also showed reverse alloxan induced hyperglyceamic and ameliorated-Alzheimer related behavioural changes (2). *Plumeria alba* L. showed antidepressant action (3). The present study is based on *in silico* activity on the phytoconstituents observed in the ethanolic extract on the gas chromatography analysis of the ethanolic extract of *Plumeria pudica* Jacq. *Plumeria caracasana* J.R. Johnst. is recognised in botanical databases such as the Royal Botanical Gardens 'Kew, Plants of the World Online, as a synonym for *P. pudica* (4, 5). The leaves are alternate, dark green in colour and exhibit a fiddle-shaped or spoon-shaped morphology. The plant bears large clusters of bright white flowers, measuring up to 3 inches (7.5 cm) across, with a small yellow centre. Each flower has 5 petals arranged to resemble a beautiful bouquet, which is why the plant is commonly referred to as the "bridal bouquet" (5). It belongs to the family Apocynaceae (6, 7). It possesses different pharmacological actions such as analgesic (8), anti-inflammatory (9, 10), antiarthritic (11), antioxidant (12), antidiarrheal effect (13), antibacterial properties (14), cholinesterase inhibitor properties (15), free radical scavenging and larvicidal properties (16). The methanolic extract of *P. pudica* reduces acetylcholinesterase (AChE) activity and modulates GABAergic neurotransmission, reduces neuroinflammation and GABAergic inhibitory action (17); therefore, it may exhibit antiepileptic activity.

Epilepsy is a neurological disorder characterised by seizures, which affects people of all age groups. Due to the side effects associated with synthetic antiepileptic medications, there is an ongoing demand to discover new antiepileptic agents from natural plant sources. The rationale for screening the antiepileptic activity of ethanolic leaf extracts of *P. pudica* lies in the potential therapeutic benefits this plant may offer for the management of epilepsy. There are no reports regarding the *in silico* screening for antiepileptic activity of the leaf extract *P. pudica* to understand the pharmacokinetic (PK) properties like absorption, distribution, metabolism and excretion (ADME), toxicity profiling physicochemical properties and molecular interaction of phytoconstituents with target proteins hence, *in silico* studies were

conducted on the phytoconstituents identified in the ethanolic leaf extract of *P. pudica*, as determined by gas chromatography-mass spectrometry (GC-MS) analysis.

## Materials and Methods

*Plumeria pudica* leaves were collected from Manjeshwar in the Kasaragod district in June and July. The specimen (21PC005R) is kept in the college herbarium after being authenticated by a botanist from the Department of Botany at NUSCER (Nitte University Centre for Science Education & Research), Nitte (DU), Mangaluru, India.

### Extraction

The leaves of the plant were thoroughly washed, cleaned and dried, then the coarsely powdered leaves were subjected to a cold maceration process for the extraction of active compounds. The dried powder of leaves, around 50 g, was placed into 6 clean and labelled maceration chambers for 6 different solvents (500 mL each), which were selected based on polarity (chloroform, ethanol, methanol, n-hexane, petroleum ether and water). The maceration chambers were placed in the dark with occasional stirring for 7 days at room temperature, except the aqueous extract, which was kept only for 3 days to prevent microbial attack. After a week, using a muslin cloth, the extracts were filtered and collected in labelled china dishes and subjected to evaporation to get the dried extract and stored in desiccators to prevent any degradation. These extracts were investigated for phytochemical analysis, total content, followed by GC-MS analysis and isolation of 2 active constituents.

Qualitative analysis was carried out for carbohydrates, proteins, fats/oils, flavonoids, alkaloids, phenolic compounds, saponins, glycosides, steroids and terpenoids (18, 19) .

### Quantitative analysis

The Folin–Ciocalteu (FC) technique was used to assess the maximum phenolic content for 6 extracts (20, 21). The FC reagent undergoes reduction in the presence of phenols to produce molybdenum–tungsten blue complex. Gallic acid was used as a standard (1 mg/mL). Extract 1 mg/mL was dissolved in each of 6 solvents, 10–50 µg/mL working solutions were taken in five 10 mL volumetric flasks, 200 µL of FC reagent was added, shaken well and kept in the dark for incubation (30 min). To this, 2 mL of 7.5 % sodium carbonate ($Na_2CO_3$) was added and left at room temperature for 3 min; the absorbance was then measured at 650 nm, with results expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

Total alkaloid content (22): Bromocresol green (BCG (6.98 mg/100)) reacts with the sample, giving yellow coloured complex, which was extracted using $CHCl_3$ at pH 4.7. For solubilisation, 1mg of plant extract in 1mL of 2N hydrochloric acid (HCl) was dissolved and filtered. 1mL of this solution was taken into a separating flask, shaken and washed 3 times with 10 mL of $CHCl_3$ and neutralised with 0.1N sodium hydroxide (NaOH), then 5 mL of BCG and 5 mL of phosphate buffer were added and shaken well. Then extracted with 1, 2, 3, 4 mL of $CHCl_3$ and collected in 10 mL volumetric flasks to make up the volume with chloroform ($CHCl_3$). The absorbance was recorded at 470 nm and was compared against a standard atropine solution (1 mg/10 mL) with concentrations varying from 10 to 50 µg/mL.

Total flavonoid content (23, 24): In this method, aluminium chloride ($AlCl_3$)reacts with the hydroxyl group present in flavonoids and forms a coloured complex, which can be measured spectrophotometrically. 1 mg of plant extract was added to distilled water (4 mL) and 0.3 mL of 5 % sodium nitrite ($NaNO_2$)was combined in a 10 mL volumetric flask. Five minutes later, 0.3 mL of 10 % $AlCl_3$ was added and 6 min after, 2 mL of 1 M NaOH. The combination was quickly diluted with distilled water, thoroughly mixed and the final volume was adjusted to the desired level with distilled water. 1 mg of catechin equivalents per gram of dry extract was used to express the total flavonoid concentration, with catechin serving as the standard for the calibration curve.

The procedure for phenolic compounds, alkaloids and flavonoid content was carried out in triplicates and Graphpad Prism 8.0.2 software was used to calculate all data statistically.

### Gas chromatography-mass spectrometry

A Perkin Elmer Clarus 680 gas chromatograph fitted with a fused silica Elite-5MS column (5 % biphenyl, 95 % dimethylpolysiloxane; 30 × 0.25 mm ID × 250 µm film thickness) and linked to a Clarus 600 mass spectrometer running in electron ionisation (EI) mode was used to analyse the plant extracts obtained using different solvents by GC-MS. High-purity helium (99.9 %) served as the carrier gas at a steady flow rate of 1.0 mL/min. The transfer line and ion source temperatures were kept at 240 °C, while the injection temperature was maintained at 260 °C during chromatographic analysis (25, 26). The ionising energy was 70 eV. The oven's temperature was set to increase at a rate of 10 °C per min from 60–300 °C. Then 1 µL of the extract sample was injected with a split ratio. The acquisition parameters required for injection into the instrument are 10:1. The full scan mass spectra were collected within a range of 50–600 Da and the extracted constituents were quantified as percentages based on peak area. Phytoconstituents were identified and characterised by comparing GC retention times and matching mass spectra with those in standard mass spectral libraries. The spectra of the components were matched against the database of known spectra stored in the GC-MS NIST (2008) library for identification.

### *In-silico* studies

Maestro from the Schrödinger program was used to perform computational calculations. These ligands' smiles were produced using PubChem (27, 28). Ligprep: A new project was created in a selected working directory and the SMILES characters were moved into the workspace of Schrodinger Maestro. These ligands are subjected to the Schrodinger Suite 2020-4 LigPrep function. The OPLS-3 force field was employed for energy optimisation, providing accurate modelling of molecular conformations and interactions.

Protein preparation: The GABA-A receptor (PDB ID: 6X3X), the targeted protein, was imported from the RCSB PDB and shown on the Maestro interface. Water molecules or residues larger than 5 µm were removed. The OPLS_2005 force field was eventually used to maximise the protein's energy.

Receptor grid creation: Using the GLIDE grid production wizard, a receptor grid was made for the co-crystal ligands and diazepam. To make molecular docking experiments easier, the typical drug's binding site was chosen and an active site grid was created.

Molecular docking: The energy-minimised ligands are docked with the optimised crystal structure of the protein in this stage. This aids in determining the affinity with which the ligands interact with the protein. Docking predicts the preferred orientation of one molecule relative to another when they bind together, forming a stable complex

with the lowest overall energy and estimates the strength of their interaction or binding affinity. The extra precision (XP) algorithm in the GLIDE module of the Schrodinger 2020-4 suite device Maestro-11.7.012 was used after the ligands were docked into the protein and glide grid using the standard precision (SP) technique. The docked posture of ligands and their interactions were studied once molecular docking was completed.

The QikProp module (Schrödinger Suite 2020-4) was employed to perform ADME prediction for the ligands. This module predicts a molecule's PK properties in relation to its physicochemical parameters. The parameters like H-bond donors and acceptors, molecular volume, log P value and PSA are expected to be within an acceptable range when compared to standard values (29).

Molecular mechanics/generalised born surface area (MM/GBSA) simulations: The binding free energy between the protein and the collection of ligands was determined using Schrodinger Prime software in conjunction with the MM/GBSA approach.

GABA-A receptor (PDB ID 6X3X) for binding interactions was obtained from the Worldwide Protein Data Bank.

The prepared ligands were anchored into the active site of the receptors. The PK properties were used to analyse the oral effectiveness of the compound. Through examination of the drug-like characteristics, the best PK characteristics may be chosen and compliance-based predictions were made using Lipinski's five rules. Parameters, including molecular weight, the quantity of hydrogen bond donors and acceptors and the partition coefficient (log P), were assessed in accordance with the rule of five (30). Tables 3 and 4 describe toxicity investigations and Qikprop was used to predict the ADME features of the phytoconstituents. The ligand interactions with amino acid residues and MM/GBSA energy calculations are shown in Tables 5 and 6 respectively. MM/GBSA analysis yields a more accurate binding energy calculation than molecular docking energies and depicts a stronger binding of ligands to the receptor.

## Results

Flavonoids, alkaloids, terpenoids, phenolic compounds, steroids and fatty acids were among the phytochemicals that were found in the leaf extracts after a preliminary phytochemical examination using standard chemical testing techniques. The maximum yield was seen in aqueous extract (7.52 %). Ethanol, $CHCl_3$, methanol, n-hexane and petroleum ether showed the yields of 3.06 %, 2.12 %, 2.2 %, 2.4 % and 1.02 % respectively, as shown in Table 1 and the total content of phenols, alkaloids and flavonoids are also shown in Table 1 and the values (triplicates) are expressed in mean ± SD.

A total of 63 phytoconstituents were found in 6 extracts of *P. pudica* leaves, according to GC-MS analysis. Fig. 1–3 display the GC-MS spectra of *P. pudica* in 6 solvent extracts, while Table 2 displays the phytoconstituents found in the ethanol extract.

The GC-MS evaluation carried out on the ethanolic fraction of *P. pudica* confirmed 17 bioactive compounds. The target protein

## Discussion

Quantitative analysis revealed the ethanolic extract to exhibit a maximum total phenolic content of 23.81 ± 0.148 mg of gallic acid equivalent (GAE)/g. The standard absorbance of gallic acid was plotted to obtain a calibration curve and thereby the equation of $y = 0.0046x + 0.0999$ with the $R^2$ value of 0.9832 was obtained. Among the solvents, the methanol extract showed the highest total alkaloid content of 30.93 ± 0.855 mg of ascorbic acid equivalent (AE/g) and the standard absorbance of atropine was plotted to obtain a calibration curve and equation of $y = 0.0033x + 0.0069$ with an $R^2$ value of 0.9902. The methanolic extract showed maximum flavonoid content of 12.02±0.084 mg of quercetin equivalent (QE)/g of extract. Standard absorbance of quercetin was plotted to obtain a calibration curve and equation of $y = 0.0082x - 0.014$ with the $R^2$ value of 0.9939.

The GC-MS profiling of the ethanolic extract was selected as phenolic compounds, particularly flavonoids, are reported to be

**Table 1.** The percentage yield and total content of phenols, alkaloids and flavonoids present in leaf extracts in different solvents

| Solvent | n-hexane | Petroleum ether | Chloroform | Ethanol | Methanol | Aqueous |
|---|---|---|---|---|---|---|
| Percentage yield (%) | 2.4 | 1.02 | 2.12 | 3.06 | 2.2 | 7.52 |
| Phenolic content mg of GAE/g | 20.23±0.311 | 18.57±0.148 | 21.83±0.148 | 23.81±0.148 | 22.40±0.304 | 20.66 ±0.311 |
| Alkaloid content mg of AE/g | 18.10±0.424 | 17.09±0.424 | 21.84±0.424 | 11.33±1.923 | 30.93±0.855 | 30.53 ±0.212 |
| Flavonoid content mg of QE/g | 8.08±0.084 | 3.77±0.091 | 11.37±0.091 | 10.2±0.084 | 12.02±0.084 | 11.21±0.176 |

GAE: gallic acid equivalent; AE: ascorbic acid equivalent; QE: quercetin equivalent.

**Table 2.** Phytoconstituents present in the ethanolic extract of *Plumeria pudica* leaves obtained from gas chromatography-mass spectrometry analysis in identification through NIST database

| Sl. No. | IUPAC name of the constituent | Molecular formula | Molecular weight | Retention time (Rt) |
|---|---|---|---|---|
| 1 | Allyl chloride | $C_3H_5Cl$ | 76 | 1.309 |
| 2 | 2-Cyclohexen-1-one,3-methyl-ethyl)- | $C_{10}H_{16}O$ | 152 | 8.622 |
| 3 | 2-Pentadecanone-6,10,14-trimethyl- | $C_{18}H_{36}O$ | 268 | 15.560 |
| 4 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 17.060 |
| 5 | Phytol | $C_{20}H_{40}O$ | 296 | 18.181 |
| 6 | 9,12-Octadecadien-1-ol(z,z) | $C_{18}H_{34}O$ | 266 | 18.616 |
| 7 | 1-Hexyl-1-nitrocyclohexane | $C_{12}H_{23}O_2N$ | 213 | 18.676 |
| 8 | Pentanoic acid,10-Undecenyl ester | $C_{16}H_{30}O_2$ | 254 | 18.776 |
| 9 | Octadecanoic acid,ethyl ester | $C_{20}H_{40}O_2$ | 312 | 18.901 |
| 10 | Pregnan-3,11-diol-20-one | $C_{21}H_{34}O_3$ | 334 | 27.124 |
| 11 | Beta amyrin | $C_{30}H_{50}O$ | 426 | 28.555 |
| 12 | Hop-22(29)-EN-3beta-ol | $C_{30}H_{50}O$ | 426 | 29.145 |
| 13 | 2,5,9-Tetradecatriene,3,12-diethyl- | $C_{18}H_{32}$ | 248 | 29.640 |
| 14 | 4,22-Stigmastadiene-3-one | $C_{29}H_{46}O$ | 410 | 29.705 |
| 15 | Dichloroacetaldehyde | $C_2H_2OCl_2$ | 112 | 1.229 |
| 16 | N-hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 16.940 |
| 17 | Betasitosterol | $C_{29}H_{50}O$ | 414 | 28.060 |

**Table 3.** Physicochemical properties of phytoconstituents present in the ethanolic extract of *Plumeria pudica* leaves

| Code | MW | Log p (QlogPo/w) | Donor HB | Acceptor HB | Rule of five |
|---|---|---|---|---|---|
| **Acceptable range** | **≤ 500** | **>5** | **≤5** | **≤10** | **<5** |
| Betasitosterol | 414.713 | 7.525 | 1 | 1.700 | 1 |
| N-hexadecanoic acid | 256.427 | 5.270 | 1 | 2 | 1 |
| Dichloroacetaldehyde* | 112.942 | 0.506 | 0 | 2 | 0 |
| 4,22-Stigmastadiene-3-one | 410.682 | 7.567 | 0 | 2 | 1 |
| 2,5,9-Tetradecatriene,3,12-diethyl- | 248.450 | 8.352 | 0 | 0 | 1 |
| Hop-22(29)-EN-3beta-ol | 426.723 | 7.012 | 1 | 1.700 | 1 |
| Beta amyrin | 426.723 | 7.158 | 1 | 1.700 | 1 |
| Pregnan-3,11-diol-20-one* | 334.497 | 2.931 | 2 | 5.400 | 0 |
| Octadecanoic acid,ethyl ester | 312.535 | 7.080 | 0 | 2 | 1 |
| Pentanoic acid,10-Undecenyl ester | 254.412 | 5.442 | 0 | 2 | 1 |
| 1-Hexyl-1-nitrocyclohexane* | 213.318 | 3.343 | 0 | 2 | 0 |
| 9,12-Octadecadien-1-ol(z,z) | 266.466 | 5.628 | 1 | 1.700 | 1 |
| Phytol | 296.535 | 6.323 | 1 | 1.700 | 1 |
| Hexadecanoic acid,ethyl ester | 284.480 | 6.253 | 0 | 2 | 1 |
| 2-Pentadecanone-6,10,14-trimethyl- | 268.482 | 5.716 | 0 | 2 | 1 |
| 2-Cyclohexen-1-one,3-methyl-ethyl)-* | 138.209 | 1.854 | 0 | 2 | 0 |
| Allyl chloride* | 76.525 | 1.989 | 0 | 0 | 0 |
| Diazepam* | 284.744 | 2.996 | 0 | 4 | 0 |

*indicates compounds that have violated one rule.

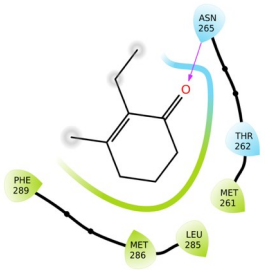
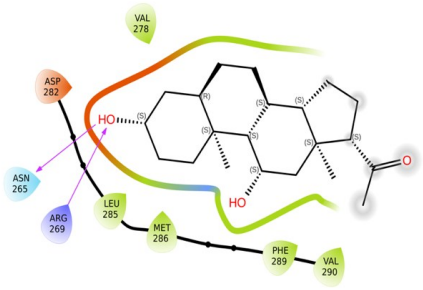
**Table 4.** Absorption, distribution, metabolism, excretion and toxicity (ADMET) studies of the top three phytoconstituents of the ethanolic extract of *Plumeria pudica* leaves

| Compounds | HOA (%) | QPlogS | QPPCaco | QP logBB | Qpp MDCK | Qplog | #Metab | QP logKp |
|---|---|---|---|---|---|---|---|---|
| **Acceptable range** | **> 80 % high < 25% low** | **-6.5to -0.5** | **> 500 great < 25 poor** | **-3.0 to 1.2** | **> 500 great < 25 poor** | **-1.5 to 1.5** | **(1 to 8)** | **-8.0 to -1.0** |
| 2-Cyclohexen-1-one,3-methyl-ethyl)- | 100 | -2.053 | 4045.418 | 0.179 | 2240.813 | -0.241 | 4 | -2.164 |
| Pregnan-3,11-diol-20-one | 100 | -4.252 | 1088.263 | -0.47 | 542.07 | 0.378 | 3 | -3.096 |
| 4,22-Stigmastadiene-3-one | 100 | -9.325 | 1856.702 | -0.537 | 965.669 | 2.207 | 4 | -2.358 |
| Diazepam | 100 | -3.806 | 2577.159 | 0.184 | 3388.097 | 0.133 | 1 | -1.716 |

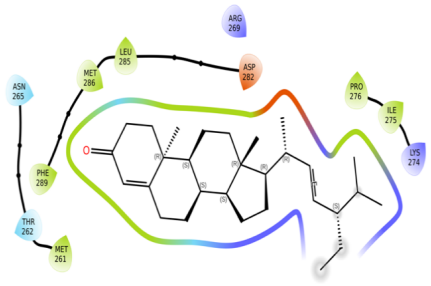
| Compounds | Drug relevant properties | | | | Toxicity | | | |
|---|---|---|---|---|---|---|---|---|
| | C log P | Solubility | Drug likeness | Drug score | Tumgn | RepE | IrE | Mutgn |
| 2-Cyclohexen-1-one,3-methyl-ethyl)- | 2.4 | -2.09 | -3.16 | 0.49 | Green | Green | Green | Green |
| Pregnan-3,11-diol-20-one | 3.13 | -4.36 | 0.28 | 0.34 | Green | Red | Green | Green |
| 4,22-Stigmastadiene-3-one | 7.75 | -6.49 | 1.39 | 0.15 | Green | Red | Green | Green |
| Diazepam | 2.98 | -4.67 | 5.98 | 0.15 | Red | Red | Green | Red |

HOA: Hydroxyl radical scavenging activity; MDCK: Madin-Darby canine kidney.

**Table 5.** Ligand interactions with amino residues of top three phytoconstituents of the ethanolic extract of *Plumeria pudica* leaves

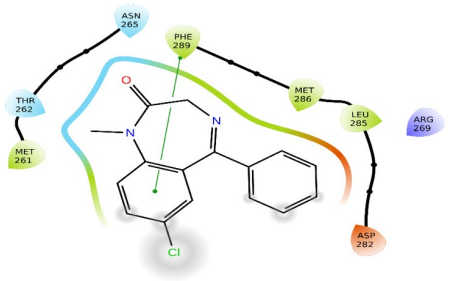
| Compounds | Ligand-interaction diagram | Interactions |
|---|---|---|
| 2-Cyclohexen-1-one,3-methyl-ethyl)- |  | Hydrogen bond: ASN-265<br>Hydrophobic Interaction: THR-262, MET-261,LEU-285,MET-286, PHE-289 |
| Pregnan-3,11-diol-20-one |  | Hydrogen bond :ASN-265,ARG-269<br>Hydrophobic interaction:VAL-278, ASP-282,LEU-285,MET-286, PHE-289,VAL-290 |

4,22-Stigmastadiene-3-one



No hydrogen bond
Hydrophobic Interactions : ASN-265,THR-262,MET-261,
PHE-289,MET-286,LEU-285,
ASP-282,ARG-269,PRO-276,
ILE-275,LYS-274

Diazepam



No hydrogen bond
Pi-Pi interactions : PHE-289
Hydrophobic interactions:MET-261,
THR-262,ASN-265,MET-286,LEU-285,ASP-282,ARG-269

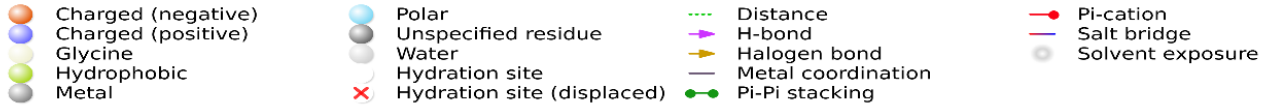



**Table 6.** Docking score and PRIME-MM/GBSA calculations of top three phytoconstituents of the ethanolic extract of *Plumeria pudica* leaves

| Bioactive | Docking score for 6X3X | ΔGbind (Kcal/mol) | ΔGbind coulomb | ΔGbind covalent | ΔGbind vander | ΔGbind Hbond | ΔGbind lipophilic |
|---|---|---|---|---|---|---|---|
| 2-Cyclohexen-1-one,3-methyl-ethyl)- | -2.899 | -24.320 | -5.089 | 1.260 | -15.935 | -0.406 | -10.106 |
| Pregnan-3,11-diol-20-one | -2.839 | -27.687 | -6.851 | 1.979 | -16.647 | -1.123 | -11.316 |
| 4,22-Stigmastadiene-3-one | -2.803 | -33.741 | -2.624 | 2.419 | -32.868 | -0.002 | -13.256 |
| Diazepam | -3.691 | -40.918 | -4.489 | 0.287 | -28.466 | -0.019 | -14.744 |

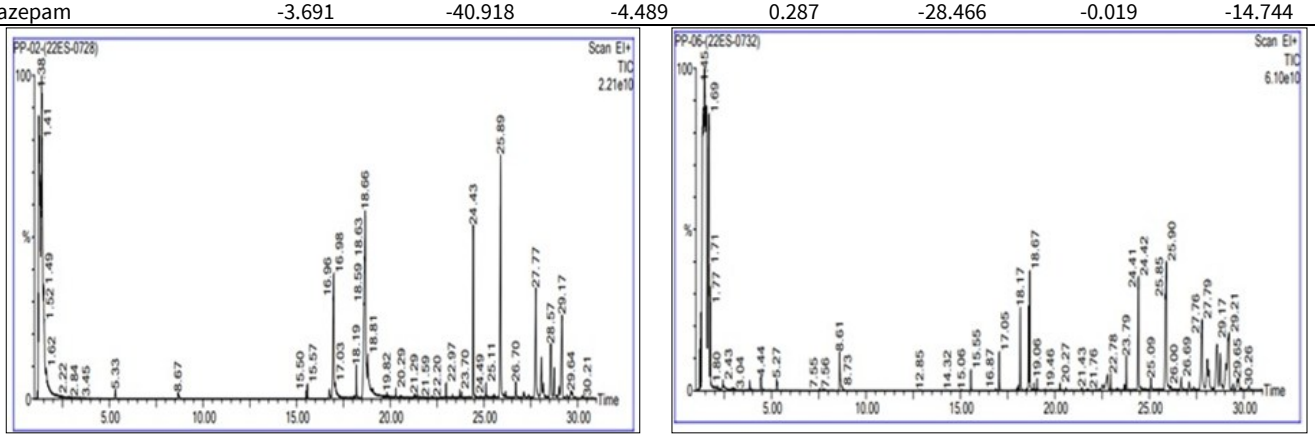



**Fig. 1 A, B.** Gas chromatography-mass spectrometry spectrum of leaf extract of *Plumeria pudica* in n-hexane and chloroform respectively.

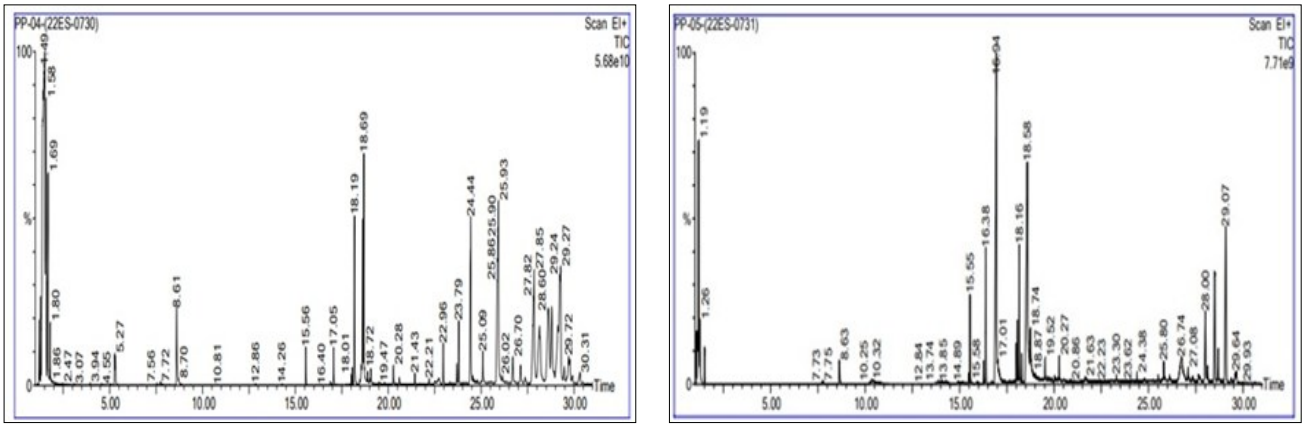



**Fig. 2 A, B.** Gas chromatography-mass spectrometry spectrum of leaf extract of *Plumeria pudica* in petroleum ether and methanol

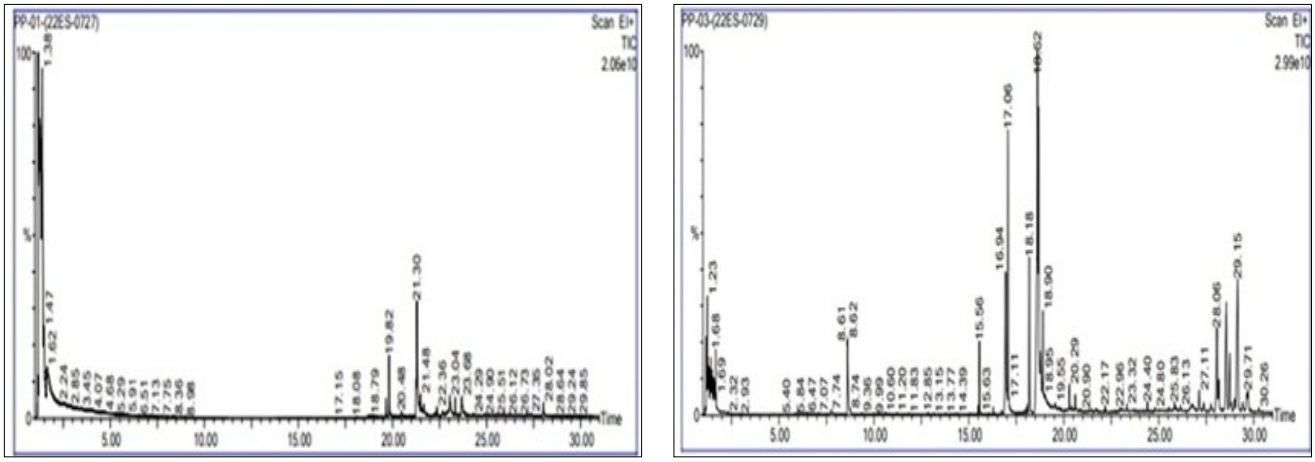


**Fig. 3 A, B.** Gas chromatography-mass spectrometry spectrum of leaf extract of *Plumeria pudica* in aqueous ethanol.

associated with anti-epileptic effects as their mechanism of action is shown to be binding to GABA-A receptor to increase the inhibitory action of GABA, so a total of 17 phytoconstituents, which were investigated for their potential to screen for antiepileptic effects in silico (30).

The GC-MS results from Table 3 show few metabolites violated one rule and the remaining constituents are within the permissible limits and have obeyed the Lipinski RO5 and Jorgensen RO3 without any violation. All the compounds were analysed and the top 3 compounds that obeyed Lipinski's rule and based on physicochemical properties are reported.

ADME prediction for the phytoconstituents was done using Qikprop in Schrödinger software. Toxicity was calculated with OSIRIS property explorer (31). The predicted ADMET for all tested compounds is listed in Table 4. Toxicity scores are colour coded, green shows low probable activity and compounds with toxic effects are shown in red. Activities analysed are tumorogenic effect, mutagenicity, skin irritant and reproductive effect. 2-Cyclohexen-1-one,3-methyl-ethyl), Pregnan-3,11-diol-20-one, 4,22-Stigmastadiene -3-one did not show any tumorogenic, mutagenic or skin irritancy but Pregnan-3,11-diol-20-one, 4,22-Stigmastadiene-3-one showed undesirable reproductive effect. It was discovered that the BBB penetration for the top three compounds out of 17 was expected to be very good. Additionally, all the designed compounds were predicted to have a high percentage of human oral absorption. The QPCaco models evaluate the apparent permeability of Caco-2 cells. The Madin-Darby canine kidney (MDCK) cells are considered a valuable model for studying the blood-brain barrier. The Qplog KHSA results indicate that the compounds have a binding affinity to human serum albumin (HSA), allowing us to examine how strongly a drug binds to plasma proteins (30). The metabolism results show that the compounds are metabolised at an appropriate rate. The tumourigenicity prediction concludes that the compounds are not tumourigenic. Computational analysis of the synthesised compounds revealed that they are lipophilic.

The compounds with the highest docking scores are shown in Table 6 after the produced phytoconstituents were docked into the receptors active sites. From the docking results, it is observed that the best-docked score of ligands interacting with 6X3X was 2-Cyclohexen-1-one,3-methyl-ethyl-and Pregnan-3,11-diol-20-one with scores of -2.899 and -2.839 respectively, compared with standard Diazepam and their 2-dimensional and 3-dimensional (2D and 3D)interactions are shown in Fig. 4, 5. The binding free energy between a protein and its ligand is frequently determined using the MM/GBSA approach (32, 33), which offers a practical and theoretically valid estimate of the intensity of their interaction. To assess the comparative binding energies of these constituents to receptor, MM/GBSA analysis was utilised. A more negative free energy value denotes stronger ligand-protein interactions and enhanced complex stability, indicating a greater energy release during complex formation. Due to its balance of computational efficiency and accuracy in estimating binding free energies, MM/GBSA is widely favoured for re-scoring over other computational methods.

All phytoconstituents exhibited a significant affinity for 6X3X binding, accompanied by highly favourable free binding energies. (34). The binding energy values, ΔGbind, indicate the overall binding affinity of the compounds, the more negative the value, the stronger the interaction. According to our data, van der Waals energy and non -polar solvation GLipo are crucial for stabilising the ligands at the binding pocket of 6X3X because they consistently show notable negative values for all compounds.

## Conclusion

The present study showed the presence of a number of medicinally useful active constituents in the leaves of *Plumeria pudica* through GC-MS analysis and *in silico* studies and predicted their possible mechanism of action at the molecular level. Based on the results obtained, the active bioconstituents show significant interaction with the 6X3X receptor with a good docking score. To gain a better understanding of the mechanism of action and toxicity profile, additional research is necessary to understand multi-targeted mechanism by network pharmacology and *in vitro* and *in vivo* evaluations of the top compounds, 2-Cyclohexen-1-one,3-methyl-ethyl), Pregnan-3,11-diol-20-one and 4, 22-Stigmastadiene-3-one of the leaves of *P. Pudica* after isolation and characterisation.

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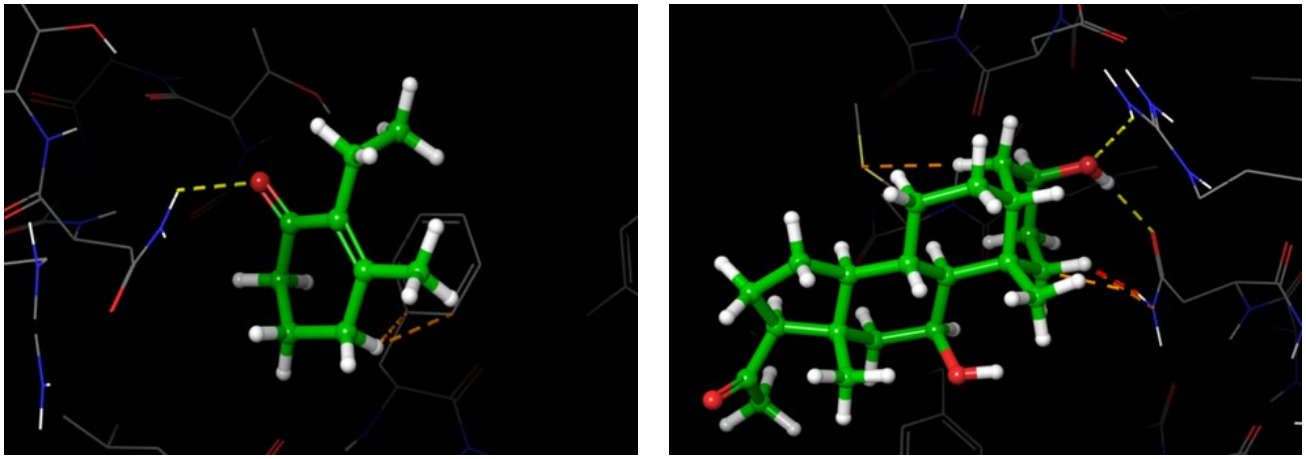


**Fig. 4.** 3D interaction of 2-Cyclohexen-1-one,3-methyl-ethyl)- and Pregnan-3,11-diol-20- one with target protein (PDB ID:6X3X).

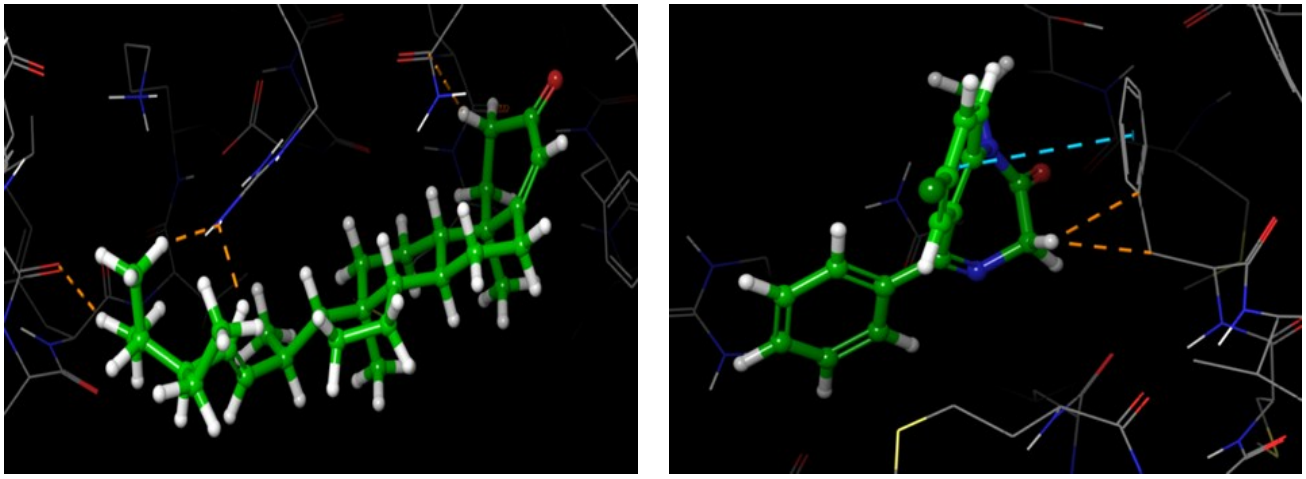


**Fig. 5.** 3D interactions of 4,22-Stigmastadiene-3-one and Diazepam with target protein (PDB ID:6X3X).

## Authors' contributions

MBS carried out the phytochemical analysis, literature search and draft preparation. JBM participated in the conceptualisation of the idea, supervision, draft editing and sequence alignment. SVP and SS performed *in silico* studies and interpretation of data. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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