In Vivo Antinociceptive Effect of Methanolic Extract of Ipomoea marginata Desr. in Rodents as well as In Silico Molecular Docking of Some Phytoconstituents from the Plant

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Potluri et al.: Effect of Methanolic Extract of Ipomoea marginata Desr.

This research was performed to analyze the antinociceptive task of methanolic extract of Ipomoea marginata in addition to in silico evaluation of the antinociceptive task of the separated constituents from Ipomoea marginata versus cyclooxygenase-2 enzyme together with absorption, distribution, metabolism, excretion/toxicity analysis of separated substances. In vivo antinociceptive task of methanolic extract of Ipomoea marginata was examined by acetic acid-induced agonizing, tail immersion and the hot plate on rodents. In silico activity of the isolated substances, absorption, distribution, metabolism, excretion/toxicity assessment was carried out by Autodock 4.0 and data warrior software applications. The results revealed that methanolic extract of Ipomoea marginata has the greatest possible dose-dependent antinociceptive task at all doses. Amongst the substances, ipalbidine showed the very best docking score of -8.26, which was virtually better than standard diclofenac, i.e., -7.03, guaranteeing good binding compatibility among the ligand and the receptor than the standard and absorption, distribution, metabolism, excretion/toxicity evaluation using data warrior assures the compound has not breached Lipinski's guideline of five suggesting its safety consumption. To conclude, Ipomoea marginata can be a potent resource of antinociceptive activity and also additional simulation studies are needed to develop the performance of ipalbidine.

Key words: Ipomoea marginata, leucorrhoea, depression, analgesic

Pain is a beneficial tool for the body's immune system to safeguard the location harmed by various stimulations. To care for the pain, vast arrays of antinociceptive like nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal medicines in addition to opioid anaesthetics are utilized, which have a different harmful effect such as hepatic damage, cardio troubles, kidney failure, erectile dysfunction, manic depression, high blood pressure, aches as well as dizziness, look of inactive diabetes mellitus, skin degeneration, reduced bone density, intestinal system, abscess, reliance, constipation and also respiratory problems. So, it is crucial to the globe to make sure a resource of cost-abusing herbal-based antinociceptive medicines with more potent and less negative results may be acquired with the medicinal plant[1-4].

Molecular docking is an essential strategy of making plans and designing new drugs, where it is expected that a tiny molecule will certainly show affinity and bind experimentally to the binding site of the target receptor. Therefore, a practical docking approach must adequately forecast the native ligand model to the receptor-binding site and the linked physico-chemical molecular communications[5-8].

Ipomoea marginata (I. marginata) Verdc. (Family Convolvulaceae) is a perennial twiner with ovate-cordate acute leaves having reddish patches; light pink (having a dark eye), funnel-shaped flowers in pedunculate subumbellate cymes and ovoid, glabrous...
capsules are containing 2-4 grey seeds with silky pubescence. It is prevalent near water bodies throughout India\[^{[8,10]}\].

Traditionally, plant juice is used as a diuretic, hypotensive, deobstruent and antidote to poisoning. Tubers are used as an alternative, aphrodisiac, diuretic, uterine tonic, antidiabetic. Seeds mixed with milk taken as a vital tonic. Veterinary medicine, ground leaves applied to infected wounds\[^{[11]}\]. In Ayurveda, it is the only plant that can cure sterility in women. This is also believed to have the power of bestowing a male child. Its root is the correct part for promoting bodily strength and also acts as a tonic\[^{[9]}\]. The root decoction is taken internally to treat leucorrhoea and urinary complications/kidney stones\[^{[12]}\].

Consequently, this study aimed to examine the antinociceptive task of the methanolic extract of \textit{I. marginata} (MEIM) through \textit{in vivo} method and \textit{in silic}o recognition of possible phyto-constituents as an antinociceptive.

\section*{MATERIALS AND METHODS}

\textbf{Collection and identification of plant material:}

Fresh plants of \textit{I. marginata}, Convolvulaceae, were gathered from Tirumala, Tirupati, Andhra Pradesh (13° 37' 44.6340" N and also 79° 25' 28.0056" E), acknowledged and also confirmed by Prof. K. Madhava Chetty, Plant Taxonomist, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. They were washed after and cleaned by regular water, air-dried, ground into powder in a home appliance and preserved for pharmacognostical study.

\textbf{Preparation of extracts:}

The shade dried whole plant was powdered with the assistance of a miller and a crude powder was acquired. The coarse powder (1000 g) was extracted with methanol utilizing a soxhlet device. The extract was filtered, concentrated by vaporizing the solvent in a rotating evaporator and maintained in the refrigerator.

\textbf{Preliminary phytochemical screening:}

The methanolic extract of \textit{I. marginata} underwent initial phytochemical testing to recognize chemical components according to the standard operating procedures\[^{[13-16]}\].

\textbf{Experimental animals:}

Male albino Wistar rats (180–200 g) and male albino mice (18–25 g) were obtained from the Animal House of V. V. Institute of Pharmaceutical Sciences, Gudlavalleru. Before and after treatment, the animals were fasted for 12 and 10 h, respectively. However, water was made available \textit{ad libitum}.

\textbf{Acute toxicity studies:}

Some unfavourable impacts may result within 24 h from single or multiple exposures of products, which indicate acute toxicity. Lethal dose 50 \% (LD\(_{50}\)) of the examination of the extracts is found from this research complying with Organisation for Economic Co-operation and Development (OECD) 423 guidelines. Here, an indication of any mortality found after oral management of the test was checked approximately for 1 h. For the next 5–6 h, animals were observed on an hourly basis. Nonetheless, the animals were reserved under monitoring for 2 w.

\textbf{Evaluation of antinociceptive activity:}

\textbf{Acetic acid writing reflex:} This was executed, according to Gaertner \textit{et al.}\[^{[17]}\]. Male albino mice (6 per group) were infused intraperitoneally with 0.6 \% acetic acid at a dosage of 10 ml/kg. The extract (200, 400 mg/kg mouth (p.o.)), morphine (5 mg/kg subcutaneously (s.c.)), and also distilled water (p.o.) were carried out 30 min before treatment with acetic acid. The writhings induced by the acid, including stomach restrictions and also back limbs stretchings, were counted for 30 min after latency duration of 5 min. The percentage of antinociceptive activity was determined as complies with:

\[
\text{Percentage antinociceptive activity}=\frac{N-N'\times 100}{N}
\]

Where \(N\) is the average number of stretchings of control per group. \(N'\) is the average number of stretchings of tests per group.

\textbf{Hot plate method:} The rats were positioned on a warmer maintained at 55° within the restrainer. The reaction time was identified as the rats' reaction to react to the thermal pain by licking their paws or leaping. The response time was taped before (0 min) and at 15, 30, 45 and 60 min after the management of the therapies. The optimum response time was taken care of at 45 sec to stop any injury to the tissues of the paws. If the reading exceeds 45 s, it would certainly be thought about as optimal analgesia.
Tail flick method: The tail-flick approach assessed the antinociceptive task of the MEIM explained[9]. Concerning 5 cm from the distal end of the tail of each rat was placed in warm water kept at 50°. The reaction time (in s) was the time taken by the rat to flip its tail due to pain. The initial analysis was omitted and the response time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 15, 30, 45 and 60 min after administering the therapies. The optimum response time was repaired at 15 sec to stop any tail tissue injury. If the analysis goes beyond 15 sec, it would be taken into consideration as maximum analgesia. The maximum possible analgesia (MPA) was determined as adheres to:

\[
\text{Percentage antinociceptive activity} = \frac{\text{Reaction time for treatment} - \text{Reaction time for control}}{15\text{ s}}\times\frac{1}{\text{Reaction time for control}}\times 100
\]

Virtual screening: A structurally based virtual screening was then performed using PyRx software. If screening results with a binding affinity ≥10.0 kcal/mol were achieved, the corresponding compounds were used for subsequent molecular docking with AutoDock 4.0. Based on the virtual screening results, 25 identified candidate compounds were purchased from AnalytiCon Discovery GmbH (Potsdam, Germany) to verify the in silico results.

Computational study:

The molecular docking simulation was done utilizing on cyclooxygenase-2 (COX-2) enzyme (protein data bank ID: 4PH9 (PDB ID: 4PH9) versus the potent substances gathered from the literary works testimonial.

Preparation of ligand structures:

Structures of selected ligands (fig. 1) were downloaded in the structure data file (SDF) documents style from the PubChem compound database. Physicochemical abilities of the ligands satisfied the standards of Lipinski’s guideline of five or else understood as Lipinski’s guideline of drug-likeness[18,19].

Chemical structures in the .SDF layout was transformed into the .PDB layout making use of Discovery Studio Biivia (DSB). After that, AutoDock tools (ADT) used to check out ligand structure regarding amalgamations with nonpolar hydrogens, enhancements of Gasteiger modifications and rotatable bonds. Structures in the ligand .PDB style was after transformed into the ligand .PDBQT style utilizing ADT, making it possible for users with AutoDock4[20].

Preparation of COX-2 protein:

The protein COX-2 (4PH9) was computed and installed from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database and was inscribed with the PDB code 1SA0 (fig. 2). ADT software application was used to prepare the called for declaring AutoDock 4.0 by accrediting hydrogen polarities, computing Gasteiger charges to protein and transforming protein structure .PDB documents style to .PDBQT layout[21-23] (fig. 2).

Docking methodology:

Molecular docking was carried out utilizing the AutoDock program. Ligands were anchored independently to the receptor with grid collaborates (grid facility) and grid boxes of particular dimensions for each receptor. The arrangement data was involved by opening up the note pad to run AutoDock .ADT was needed to prepare the input .PDBQT documents for beta (β)-Tubulin and also to establish the dimension and even the facility of the grid box. The grid dimension was set at 60×60×60 (x, y as well as z) factors and also the grid facility was assigned at x, y as well as z measurements of 124.37, 96.859 as well as 14.124, precisely, with a grid spacing of 0.375 Å. Post-docking evaluations were pictured using DSB, which revealed the dimensions, places of binding sites, hydrogen-bond communications, hydrophobic communications and bonding, ranging as communication distance <5 Å of from the placement of the docked ligand[20] (fig. 3).
Drug likeliness properties:
Our research evaluated the drug likeliness of the separated compounds from *I. marginata*; we used OSIRIS Data warrior v 4.6.1 software[24].

Statistical analysis:
All information was revealed as the mean±standard deviation; information went through one-way analysis of variance (ANOVA) adhered to by Tukey examination. The analytical evaluation executed with Graphpad Prism (Version 3, USA) software program. p<0.05 was taken into consideration statistically considerable.

RESULTS AND DISCUSSION
Initial phytochemical testing of *I. marginata* was exposed to different phytoconstituents detailed in Table 1. The antinociceptive task of MEIM was carried out by the acetic acid method (Table 2), hot plate method (Table 3) and also tail-flick method (Table 4).

In the acetic acid approach, the diclofenac sodium was revealed 69.8 % inhibitions of analgesia. Simultaneously, 50, 100, 200 and 400 mg/kg *I. marginata* showed 10.8, 20, 27 and 50.72 % inhibitions of analgesia, specifically when contrasted with the acetic acid control team.

In the hot plate method, at 120 min, the mean response time for tramadol of analgesia effect revealed 19.18±1.63, while 50, 100, 200 and 400 mg/kg *I. marginata* showed significant analgesic effect (14.55±1.69, 16.22±2.58, 20.19±2.22 and 21.16±1.69), respectively. In the tail-flick method, at 90 min, the mean response time for tramadol of analgesia effect revealed 7.26±0.36, while

| Phytoconstituents       | Method                | Methanolic extract (MEIM) |
|-------------------------|-----------------------|---------------------------|
| Shinoda test            |                       | +                         |
| Zn. Hydrochloride test  |                       | +                         |
| Lead acetate test       |                       | +                         |
| Stain test              |                       | -                         |
| Wagner test             |                       | +                         |
| Hager's test            |                       | +                         |
| FeCl3 test              |                       | +                         |
| Potassium dichromate test |                   | +                         |
| Foaming test            |                       | +                         |
| Salkowski test          |                       | +                         |
| Molish test             |                       | +                         |
| Litmus test             |                       | -                         |
| Keller-Killani test     |                       | +                         |
| Ninhydrin test          |                       | +                         |
| Biuret test             |                       | +                         |

Note: "+"=Present; "-"=Absent

**TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACTS OF THE WHOLE PLANT OF I. marginata**
I. marginata showed significant analgesic effect (3.56±0.26, 4.78±0.26, 5.68±0.28, 6.91±1.58) respectively. The difference was statistically significant compared with the control group.

In today's examination, to evaluate the possibility of substances being accountable for antinociceptive activity, the docking score was used to verify the prospective binding energy. The molecules were also based on intelligent learning object guide (iLOG).

Note: The results are presented as a mean±standard deviation, (n=5). One-way ANOVA was used to evaluate the results, followed by Dunnett’s examination. *p<0.05 vs. control; *p<0.05 vs. standard group
predictors utilizing online tools to identify their absorption, distribution, metabolism, excretion/toxicity (ADME/T) properties. 13 out of 22 substances strictly adhered to Lipinski’s Rule of 5 and were picked for more analyses and also remainder were eliminated. ADMET properties of the picked 13 substances revealed the capacity of effective compounds (Table 5).

Docking researches revealed that out of all isolated substances consisted of in the study; Ipalbidine had the most excellent docking rating of -8.26 kcal/mol, which showed both hydrogen bond interactions (ASN383) and also electrostatic interactions (ALA200, HIS389, LEU392, ALA203, TRP388, HIS208 and TYR386) with the COX-2 enzyme. Except for swainsonine, no other compounds form hydrophobic interactions. However, all compounds had significant electrostatic communications. The standard diclofenac revealed a possible docking rating of -7.03, which was virtually lesser than ipalbidine. The outcomes obtained by the autodock 4.0 are shown in Table 6 and Table 7, and the protein-ligand interactions revealing hydrogen bonding and binding settings, which are also published in fig. 4-fig. 6.

The significant addition of therapeutic herbs in medicine growth and commercial medications resources has been incredible. However, pharmaceuticals currently used in

### Table 5: Drug Likeliness Analysis of Separated Phytoconstituents from I. marginata

| Compound         | Total Mol weight | cLogP | H-Acceptors | H-Donors | Molar refractivity | Rotatable bonds | No. of deviations |
|------------------|------------------|-------|-------------|----------|--------------------|-----------------|-------------------|
| Actinidol        | 194.273          | 1.509 | 2           | 1        | 56.61              | 1               | 0                 |
| Agroclavine      | 238.333          | 3.0185| 2           | 1        | 79.18              | 0               | 0                 |
| Arctigenin       | 372.416          | 2.8465| 6           | 1        | 100.6              | 7               | 0                 |
| Beta amyrin      | 426.726          | 7.3406| 1           | 1        | 134.88             | 0               | 1                 |
| Betulinic acid   | 456.708          | 6.3706| 3           | 2        | 136.91             | 2               | 1                 |
| Caffeic acid     | 180.159          | 0.7825| 4           | 3        | 47.16              | 2               | 0                 |
| Elymoclavine     | 254.332          | 2.0918| 3           | 2        | 80.34              | 1               | 0                 |
| Ergometrine      | 325.411          | 0.8747| 5           | 3        | 98.56              | 3               | 0                 |
| Esculetin        | 178.143          | 0.806 | 4           | 2        | 46.53              | 0               | 0                 |
| Friedelin        | 426.726          | 7.5888| 1           | 0        | 134.39             | 0               | 1                 |
| Glochidone       | 422.694          | 7.5152| 1           | 0        | 133.71             | 1               | 1                 |
| Isoclorogenic acid A | 516.454     | 0.7977| 12          | 7        | 83.5               | 9               | 3                 |
| Isoclorogenic acid B | 516.454     | 0.7977| 12          | 7        | 83.5               | 9               | 3                 |
| Isoclorogenic acid C | 516.454     | 0.7977| 12          | 7        | 83.5               | 9               | 3                 |
| Lysergol         | 254.332          | 1.4317| 3           | 2        | 81.14              | 1               | 0                 |
| Penniclavine     | 270.331          | 0.6083| 4           | 3        | 82.34              | 1               | 0                 |
| Swainsonine      | 173.211          | -1.0292| 4        | 3        | 46.64              | 0               | 0                 |
| Taraxerol        | 426.726          | 7.3406| 1           | 1        | 134.88             | 0               | 1                 |
| Umbelliferone    | 162.144          | 1.1517| 3           | 1        | 44.51              | 0               | 0                 |
| Ipalbidine       | 231.338          | 2.0733| 2           | 1        | 74.43              | 0               | 0                 |
| Scopoletin       | 192.17           | 1.0817| 4           | 1        | 51                 | 1               | 0                 |

### Table 6: Binding Affinities of Isolated Compounds at the Active Site of COX-2

| Ligands               | Highest to the Lowest mode of conformation with corresponding RMS binding affinities in ΔG (Kcal/mol) |
|-----------------------|----------------------------------------------------------------------------------------------------|
|                       | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          | 9          |
| Actinidol             | -6.99      | -6.99      | -6.97      | -6.97      | -6.96      | -6.96      | -6.93      | -6.62      | -6.4       |
| Arctigenin            | -5.79      | -3.31      | -1.8       | -1.12      | -0.37      | -         | -         | -          | -          |
| Glochidone            | -2.91      | -2.5       | -2.33      | 31.58      | 31.59      | 31.71      | 32.33      | 33.54      | 33.61      |
| Swainsonine           | -6.16      | -6.1       | -6.1       | -6.07      | -6.03      | -5.96      | -4.92      | -4.86      | -4.77      |
| Chlorogenic acid      | -6.07      | -3.87      | -3.45      | -2.62      | -1.28      | -0.62      | 4.96       | -          | -          |
| Ipalbidine            | -8.26      | -8.23      | -7.52      | -7.43      | -7.03      | -6.98      | -6.94      | -6.13      | -6.11      |
| Scopoletin            | -6.91      | -6.91      | -6.86      | -6.81      | -5.85      | -5.82      | -5.8       | -5.8       | -5.78      |
| Diclofenac            | -7.03      | -6.94      | -6.91      | -6.9       | -6.49      | -6.41      | -5.16      | -5.14      | -4.6       |
| Ligands    | Binding Affinity, $\Delta G$ (Kcal/mol) | Hydrogen Binding Interactions | Amino acids involved and Distance (Å) | Hydrophobic Interactions | Electrostatic Interactions |
|-----------|------------------------------------------|------------------------------|--------------------------------------|--------------------------|---------------------------|
| Actinidol | -6.99                                    | GLY527 (3.28; 3.45), SER531 (3.97) | VAL350 (3.99, 5.06, 5.12), LEU353 (3.85, 4.63, 4.02) | ALA203 (5.35, 6.11), LEU391 (4.20, 5.48), ALA200 (4.47, 4.77), TRP388 (4.35), PHE201 (3.40, 5.47), LEU392 (4.78, 5.92), HIS389 (3.90), VAL448 (5.45), HIS387 (4.84, 4.63, 5.73) |
| Glochidone| -2.91                                    |                              |                                      |                          |                           |
| Swainsonine| -6.16                                   | TYR386 (3.68; 4.73)          | ALA200 (4.29)                        | LEA200 (4.29), LEU391 (4.28), LEU392 (5.29) |
| Chlorogenic acid| -6.07                                  | PHE530 (5.60)                |                                      |                          |                           |
| Ipalbidine| -8.26                                    | ASN383 (3.83)                |                                      |                          |                           |
| Scopoletin | -6.91                                    | THR207 (3.25)                |                                      |                          |                           |
| Diclofenac| -7.03                                    | TYR386 (5.16)                |                                      |                          |                           |

**TABLE 7: INTERACTIONS OF COX-2 AMINO ACID RESIDUES WITH LIGANDS AT RECEPTOR SITES**

**Fig. 4:** Various two dimensional interactions of ligands with COX-2 complex (4PH9) (a) Actindol; (b) Glochidone; (c) Ipalbidine; (d) Scopoletin; (e) Swainsonine; (f) Diclofenac
Fig. 5: Various three-dimensional interactions of ligands with COX-2 complex (4PH9) via Hydrogen bonding (a) Actindol with GLY527 and SER531; (b) Ipalbidine with ASN383; (c) Scopoletin with THR207; (d) Swainsonine with TYR386; (e) Diclofenac with TYR386

Fig. 6: Various three dimensional interactions of ligands with COX-2 complex (4PH9) via electrostatic interactions (a) Actindol; (b) Glochidone; (c) Ipalbidine; (d) Scopoletin; (e) Swainsonine; (f) Diclofenac
therapy and swelling like narcotics and non-steroidal anti-inflammatory drugs possess a higher risk of toxicity[25]. Conversely, natural medicines used, given that the old times keep lower toxicity, have much better absorption and are abundant[26]. Therefore, scientists worldwide remain searching for plants that possess such biological activities, which will undoubtedly help develop existing clinical strategies providing more affordable and efficient therapy.

In the writhing induced model, the stomach constriction approach helps to review the outer antinociceptive task. As soon as acetic acid is infused, intraperitoneally (i.p), the body raises the generation of Prostaglandin E2 and Prostaglandin F2α (PG E2 and PG F2α), accountable for the swelling effect and agony understanding through the COX pathway[27]. The outcome mentioned above suggests that the I. marginata extract significantly prevented the writing, which can be discussed by disrupting the path, causing analgesia.

The hot plate and tail immersion techniques are strategies for establishing central antinociceptive task[28]. These two techniques better review centrally acting analgesics and opioid receptor agonists. Table 2 and Table 3 for the antinociceptive activity of I. marginata in tail immersion and hot plate taste.

The docking research disclosed an intriguing perspective regarding the communication between the provided ligands and the COX-2 enzyme. Protein-ligand communication showed that actindol, swainsonine, ipalbidine, scopoletin and diclofenac created hydrogen bonds to GLY527, SER531; TYR-386; ASN383; THR207 and TYR386, respectively. Lesser negative energy shows a stable system, leading to the most likely binding synergy that describes why ipalbidine has the most effective docking score.

In the current research study, we anchored six ligands of I. marginata right into the energetic site of the COX-2 enzyme and also, the docking score was gauged, making use of Autodock 4.0. As a result, the ipalbidine had the highest docking rating of -8.26 kcal/mol, recommending a strong communication between ipalbidine and the COX-2 enzyme. It is comprehended that the evaluation of ADMET in the exploration phase considerably lowering the faltering connected to pharmacokinetics in the scientific stage[32]. Therefore, the computer-based prognosis of ADME/T from the molecular structure is a legitimate choice for experiential methods[33]. In this research, ipalbidine has the greatest "drug-likeness" for oral delivery due to its low molecular weight, which enhances the permeation of medicine[34], reduced lipophilicity (log Po/w) showing far better oral absorption as well as bioavailability[35], as well as reduced hydrogen-bonding ability connecting to more significant permeability in the structure and also absorption[18]. For this reason, ipalbidine is an ideal choice for antinociceptive medicine.

The research demonstrated that MEIM possesses a considerable analgesic task, as verified by three designs in this research study. First, high-throughput screening using molecular docking and ADME/T evaluation resulted in the verdict that ipalbidine revealed the best fitness score and appropriates human intake. Even more, a complicated measurable SAR model is needed to guarantee its safety and security and bioefficacy. Finally, the outcomes confirm the ethnomedicinal use of I. marginata to pain condition, which recommend this plant, might be a prospective resource for advancing a new analgesic agent.

Conflict of interests:

The authors declared no conflicts of interest.

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