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Original Article

In-silico Molecular Docking Studies of some Isolated Phytochemicals from *Biophytum veldkampii* against Cyclooxygenase-II Enzyme and *in vivo* Anti-inflammatory Activity

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ABSTRACT

Background: The current study estimate the anti-inflammatory activity of whole plant of *Biophytum veldkampii* on carageenan induced inflammation on rats compiled by molecular docking studies of phytocompounds from the plants with Cyclooxygenase II (PDB ID: 3LN1). **Methods:** In this research *Biophytum veldkampii* was subjected to extraction using methanol. *In-vivo* anti-inflammatory activity was assessed by using carageenan induced inflammation on rats and *in-silico* molecular docking studies was performed by using of Autodock 4.0. **Results:** The outcomes revealed that the methanolic extract has most prominenant anti-inflammatory activity at various doses. Among all the substances 2,6,3′,4′-Tetrahydroxy-2-benzylcoumaranone revealed the most effective docking rating of -6.8, which is near to Diclofenac, i.e. - 6.9, ensuring that 2,6,3′,4′-Tetrahydroxy-2-benzylcoumaranone has a strong binding fondness in between protein and ligand. **Conclusion:** From the results, a conclusion can be drawn that the anti-inflammatory activity of *Biophytum veldkampii* in both *in vivo* and *in silico* methods. This information sustains 2,6,3′,4′-Tetrahydroxy-2-benzylcoumaranone to be a useful anti-inflammatory compound beneficial to future clinical studies.

Key words: *In-silico*, Autodock4.0, Carageenan,2,6,3′,4′-Tetrahydroxy-2 benzylcoumaranone, Inflammation, Cyclooxygenase II, Phytochemical.

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INTRODUCTION

Medicinal plants are a source of valuable therapeutic assistance for the alleviation of human diseases. According to the World Health Organization (WHO), more than 80 per cent of the world's population, mainly in developing countries, relies on conventional plant-based medicines for their primary health needs.[1] Scientific confirmation concerns the screening of bioactive compounds from plants and has contributed to the creation of new medicines with successful roles in the defence and treatment of various diseases. Inflammation is the local reaction of living mammalian tissue to injury. It is a body's immune reaction to eliminate or limit the spread of the injurious agent. There are various components of an inflammatory reaction which may lead to the associated symptoms and tissue injury. Edema, leukocyte infiltration, and granuloma formation are all components of inflammation. It's a defence mechanism, though. Complex events and mediators involved in an inflammatory reaction can cause or aggravate a variety of reactions.[2] Nonsteroidal anti-inflammatory drugs (NSAIDs) are potent anti-inflammatory agents acting by inhibition of the enzyme Cyclooxygenase (COX) and subsequent inhibition of prostaglandins at the site of inflammation. Unfortunately, inhibition of gastrointestinal or renal prostaglandins is associated with mechanism-based sensitivity,that decreases the usefulness of these otherwise powerful and efficient drugs.[3] Two forms of COX enzyme have recently been identified: COX-1, which is constitutively expressed in many cells and tissues, and COX-2, which is selectively induced by proinflammatory cytokines at the site of inflammation.[4] The discovery of a second COX enzyme related to the hypothesis that the toxicity associated with clinically valuable NSAIDs was caused by inhibition of COX-1, while COX-1 was inhibited. In support of this assumption, the expression of the inducible COX-2 enzyme is selectively inhibited by the potent anti-inflammatory drug dexamethasone. Selective inhibition of COX-2 can produce superior anti-inflammatory drugs with considerable safety over existing NSAIDs.[5]

Biophytum veldkampii looks like a miniature palm and is native to India. The plants are usually found in wet lands (mostly plains) of tropical Africa, Asia and India, normally in the shades of trees and shrubs, in grasslands, open thickets, at low and medium altitudes.[6]

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The molecular docking process predicts ligand confirmation and orientation within the targeted binding site which holds great promise in the field of computer-based drug design.[7]

In present study we focus on the *in silico* docking studies of phytochemicals present in *Biophytum veldkampii* against cyclooxygenase-2 enzyme

MATERIALS AND METHODS

Plant material

Biophytum veldkampii whole plants were collected from the forest area of Rampachowdavaram, East Godavari and authenticated by Dr. P. Prasanna Kumari, Botany department, Dantuluri Narayana Raju College, Bhimavaram. A voucher specimen was kept at Pharmacology department, Shri Vishnu College of Pharmacy.

Preparation of plant extract

The whole plant was dried at room temperature. For removal of waste or fatty materials, the powder is extracted with petroleum ether for 7 days. Now the defatted marc is extracted with methanol by using soxhlet apparatus to get methanolic extract. This extract was dried under reduced pressure; brownish color of extract was obtained.[8] This extract was stored in desecrator for further use.

Phytochemical analysis

The methanolic extract was subjected to estimation of different phytochemicals as per literature.[9]

In vitro COX II inhibition assay

The assay was performed by using Colorimetric COX (human ovine) inhibitor Screening assay kit. Briefly, the reaction mixture contains, 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2), and 10 µl of plant sample (1 mg/ml). The assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. Diclofenac sodium 1mM was used as a standard drug. The percent COX inhibition was calculated using following equation:[10]

$$\text{COX II Inhibition activity (\%)} = 1 - T/G*100$$

Where T = Absorbance of the inhibitor well at 590 nm.

C = Absorbance of the 100 % initial activity without inhibitor well at 590 nm.

Animals

In this study male Wistar rats were used, animals were kept in animals house and maintained under standard quality conditions (RH 44-56%, temperature 27±2°C) and feed with standard diet and drinking water *ad libitum* for 1 week before and during study. Protocol of this study was approved by IAEC of Shri Vishnu college of Pharmacy and with the permission from CPCSEA Reg No. 439/PO/01/a/CPCSEA.

Assessment of anti-inflammatory activity
Carageenan-induced paw edema

The anti-inflammatory activity of MEBV was assessed in rats using carrageen-induced paw edema model as per the previously described method.[11] Carageenan suspension (0.1 ml of 1% w/v in normal saline) was injected into the sub-plantar region of right hind paw. Total 25 rats were randomly divided into 5 groups each containing 5 rats. Rats were pretreated with vehicle or MEBV (400 and 200 mg/kg, orally) or reference standard Diclofenac (70/mg/kg) at 1 h earlier to carageenan injection. The paw volume at 0h, 3h, 5h and 24 hr. After carageenan administration was measured in ml using Plethysmometer (Ugo Basile, Italy).

Formalin induced Inflammation

MEBV was assessed for evaluation of anti-inflammatory activity in formalin induced inflammation rat model.[12] Total 25 rats were randomly divided into 5 groups each containing 5 rats. After 1 h orally pretreated rats with vehicle (4% gum acacia) or MEBV (400 and 200 mg/kg) or Diclofenac (70 mg/kg) intraperitoneal administration of 0.05 ml of 2.5% commercially available 37% formalin in dorsal surface of left hind paw in rats. The paw volume at 0h,3h,5h and 24 hr. After carageenan administration was measured in ml using Plethysmometer (Ugo Basile, Italy).

Motility Test

The motility pattern of the rats was observed for a period of 5 min. If the rat was avoided touching the floor and walked difficulty, score is 0, if the rat walked with little difficulty but with toe touching the floor, score is 1 and if the rat walked easily score is 2.[13]

Sample Collection

After completion of experimental study, according to CPSCEA guidelines all animals were anesthetize by using ketamine and blood was collected by retriorbital puncture. Now blood samples were centrifuged for 15 m at 10000 rpm at 37°C collect serum and used for estimation of cytokines.

Cytokines Assay

Cytokines like IL- 6, TNF – α and IL-10 (pg/ml) were estimated by using cytokines ELISA kits and assays were conducted as per ELISA kits manufacturer's guidelines.[14]

Molecular Docking
Compounds from *Biophytum veldkampii*

The following phytochemicals like 2, 6,3',4'-Tetrahydroxy-2-benzylcoumaranone, 2(1H)-Quinazolinone, 2(1H)-Quinazolinone, Phenylephrine, 4-_fluro_histamine, Phthalic_acid, Tenamfetamine, 3-Hydroxy-4-methoxybenzoic_acid, 3-Methoxyamphetamine, Phytol, n-Hexadecanoic_acid and 9,12-Octadecadienoic_acid_(Z,Z) were subjected to docking studies. The structures and the physiochemical properties of these compounds were taken from the PubChem database (www. ncbi. nlm. nih.gov/pubchem.)[15]

COX-2 enzyme protein structure: The three-dimensional (3D) structure of the COX-2 enzyme was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb) The PDB acts as a repository for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. The PDB ID of COX- 2 enzyme is **(PDB ID: 3LN1).**

Docking studies: The docking studies of compounds were carried out using Auto dock 4.0 and Discovery studio Biovia 2017 software to find out the interaction between ligands and the target protein.[16,17]

ADMET Analysis

By using of admetSAR, ADMET properties of ligands were studied it is very important to know the pharmacokinetic properties of ligands to establish their function inside of the body.[18]

Statistical analysis

All the data were expressed as mean ± S.D and performed by using one- way analysis of variance followed by Tukey test. $p<0.05$ is considered as statistically significant.

Table 1: Phytochemicals present in methanolic extract of *Biophytum veldkampii*

S.NO	Phytochemical tests	MEBV
1	Alkaloids	-
2	Glycosides	++
3	Tannins	++
4	Phenols	++
5	Flavonoids	++
6	Saponins	-
7	Steroids	++

Where (+) Indicates presence, and (-) Indicates absence

Table 2: Effect of MEBV on COX II inhibition.

Group	Inhibition of COX II (%)
Group I Normal control	-
Group II: Carageenan control	-
Group III: Standard Diclofenac	86.3
Group IV MEBV	78.3
Group V MEBV	60.34

RESULTS

Phytochemical studies of methanolic extract revealed the presence of various phytochemicals shown in Table 1.

Effect of MEBV on COX II inhibition

The result of COX II inhibition by using methanolic extract of *Biophytum veldkampii* are summarized in Table 2. The average COX II inhibition was calculated by taking the mean values. Diclofenac show 86.3 % of COX II inhibition, Whereas MEBV at dose of 200 mg/kg exhibit 60.34% and at dose of 400 mg/kg show 78.3 % inhibition of COX II.

Effect of *Biophytum veldkampii* on paw thickness of rats (cm) on Carageenan induced inflammation

Injection of carageenan into the hind paws a progressive edema reaching it maximum at 5 hr. In case of Group I animals had shown (Normal control) paw thickness was found 3.028 ± 0.12 at 0 hr and this remains constant up to 24 hr. Group II animals (Carageenan induced group) had showed an increase in a paw thickness for each hr, at 0 hr.028 ± 0.12cm, at 5 hr 3.59±0.56 cm, and at 24 hr 4.01 ±0.18 cm. The paw thickness of Group III (Diclofenac) animals was 3.35±0.41 cm which showed a mild increase at end of 3 hr, after end of 3 hr it decreased the paw thickness to 3.04 ±0.58 cm and 2.99 ±0.74 cm at the end of 5 and 24 hr respectively. Group IV (MEBV 400 mg/kg) shows decreases the paw thickness to 3.014 ±0 cm at the end of 24 hr. Group V (MEBV 400 mg/kg) show decreases the thickness of paw to 3.42±0.75cm at the end of 24 hr. Figure 1

Effect of Biophytum *veldkampii* on paw thickness of rats (cm) on Formalin induced inflammation

Injection of formalin into the hind paws a progressive edema reaching it maximum at 5 hr. In case of Group I animals had shown (Normal control) paw thickness was found 3.028 ± 0.12 at 0 hr and this remains constant up to 24 hr. Group II animals (Formalin induced group) had showed an increase in a paw thickness for each hour, at 0 hr 3.028 ± 0.12cm, at 5 hr 3.12 ±0.08 cm, and 3.89 ±0.58** cm at 24 hr. The paw thickness of Group III (Diclofenac) animals was 3.66 ±0.89 cm which

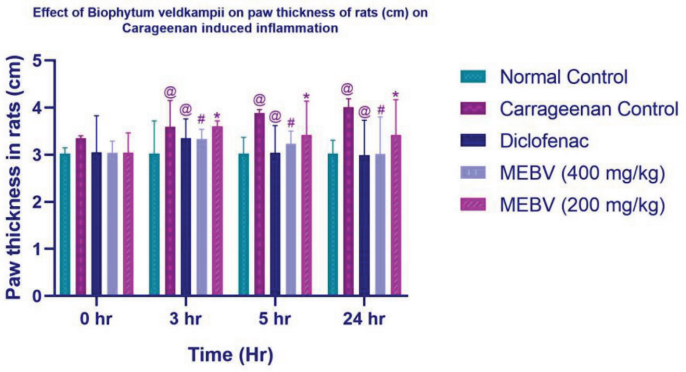

Figure 1: Effect of *Biophytum veldkampii* on paw thickness of rats (cm) on Carageenan induced inflammation. All values are expressed as mean ± S.D. @, $p<0.0001$ vs Normal Control; %, $p<0.01$ vs Normal Control; #, $p<0.05$ vs Normal Control; **, $p<0.01$ vs Carageenan Control, *, $p<0.05$ vs Carageenan Control.

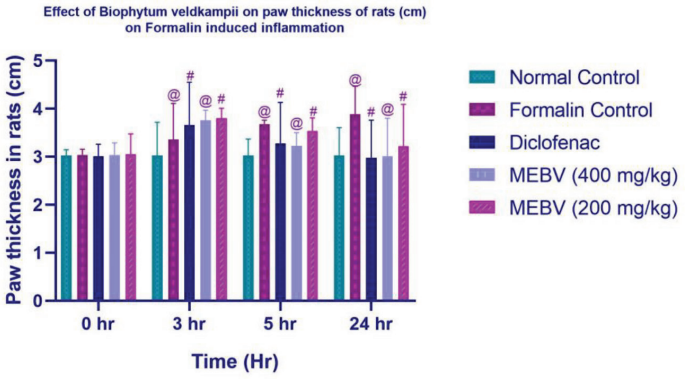

Figure 1: Effect of *Biophytum veldkampii* on paw thickness of rats (cm) on Carageenan induced inflammation. All values are expressed as mean ± S.D. @, $p<0.0001$ vs Normal Control; %, $p<0.01$ vs Normal Control; #, $p<0.05$ vs Normal Control; **, $p<0.01$ vs Carageenan Control, *, $p<0.05$ vs Carageenan Control.

showed a mild increase at end of 3 hr, after end of 3 hr it decreased the paw thickness to 3.28±0.85 cm and 2.98 ±0.78cm at the end of 5 and 24 hr respectively. Group IV (MEBV 400 mg/kg) shows decreases the paw thickness to 3.014 ±0.79 cm at the end of 24 hr. Group V (MEBV 200 mg/kg) show decreases the thickness of paw to 3.22±0.87 cm at the end of 24 hr. Figure 2

Effect of *Biophytum veldkampii* on motility of rats

Walking ability of the rats to climb the staircase at the time of peak inflammation was checked by score of motility. Group I (normal control) animals showing the motility score is 2. Group II (carageenan) animals show 0.16 ± 0.35 motility score. Whereas Group IV (MEBV 400mg/kg) and V (MEBV 200mg/kg) animals exhibits motility score like 1.58 ±0.78 and 1.22 ±0.48 respectively. This indicates MEBV decrease the inflammation and increase the walking ability of animals.

Effect of *Biophytum veldkampii* on cytokines expression (pg/ml)

In Group II (Carageenan treated animals) shows highest levels of serum IL-6 (82.4±1.25 pg/ml), TNF- α (779 ±0.68 pg/ml) and lowest values of IL-10 (18.4 ±089 pg/ml) compared to normal group. On the contrary, Diclofenac sodium treated animals show statistically decreased the IL-6

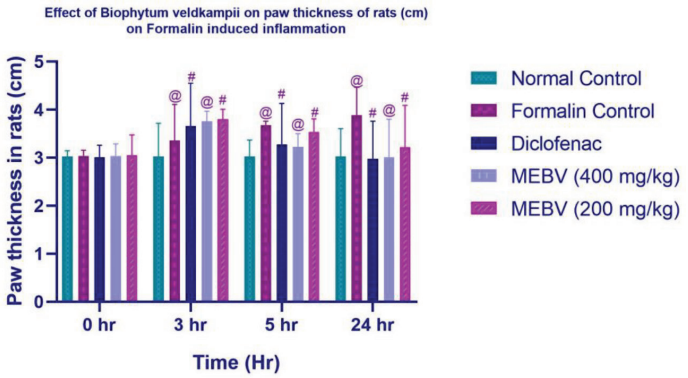

Figure 2: Effect of *Biophytum veldkampii* on paw thickness of rats (cm) on Formalin induced inflammation. All values are expressed as mean ± S.D. @, $p<0.0001$ vs Normal Control; %, $p<0.01$ vs Normal Control; #, $p<0.05$ vs Normal Control; **, $p<0.01$ vs Carrageenan Control, *, $p<0.05$ vs Carrageenan Control.

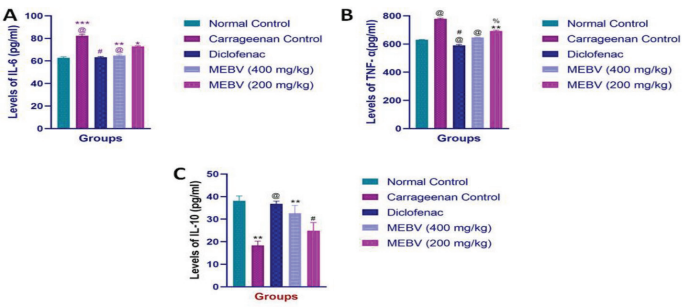

Figure 3: Effect of *Biophytum veldkampii* on cytokines expression (pg/ml). All values are expressed as mean ± S.D. @, $p<0.0001$ vs Normal Control; %, $p<0.01$ vs Normal Control; #, $p<0.05$ vs Normal Control; **, $p<0.01$ vs Carageenan Control, *, $p<0.05$ vs Carageenan Control.

and TNF-α and increases levels of IL-10.Whereas *Biophytum veldkampii* treated groups like Group IV(MEBV 400mg/kg) and Group V (MEBV 200mg/kg) exhibits low levels of IL-6 (64.8 ±0.69 pg/ml; 72.8 ±0.62 pg/ml) and TNF- α(646 ±2.75 pg/ml; 692±0.36pg/ml) and elevated levels of IL-10 (32.6 ±0.85 pg/ml; 24.9 ±0.63 pg/ml). Figure 3

Computational Study

In silico studies of compounds present in *Biophytum veldkampii,* using Auto Dock 4.0 showed the following results. Of 11 compounds studied, 2, 6, 3', 4'-Tetrahydroxy-2-benzylcoumaranone, 2(1H)-Quinazolinone satisfy the Lipinski's rule of five for drug-likeness. The other compounds which do not follow the Lipinski's properties were not considered for further docking studies. The structures of compounds from *Biophytum veldkampii* are shown in Figure 4. The binding energy for each chosen compound with the COX-2 enzyme is given in Table 3.

The amino acid residues at active site of COX-2 are cysteine, tyrosine, proline, aspargine, arginine, lucine and glycine. Docking studies show that the ligands bind to the active site region of COX-2 enzyme with good binding energy in the same Diclofenac hydrophobic pocket. The docking models of the selected compounds (1) Diclofenac, (2) 2, 6,3',4'-Tetrahydroxy-2-benzylcoumaranone, 2(1H)-Quinazolinone in 3D view are shown in Figure 2-4. The hydrogen contacts of the ligands are given in Table 2. Docking researches revealed that 2, 6,3',4'-Tetrahydroxy-2-benzylcoumaranone had the most excellent docking rating of -6.8(Kcal/mol)which showed three hydrogen bond interactions (CYS A:32 (2.67),

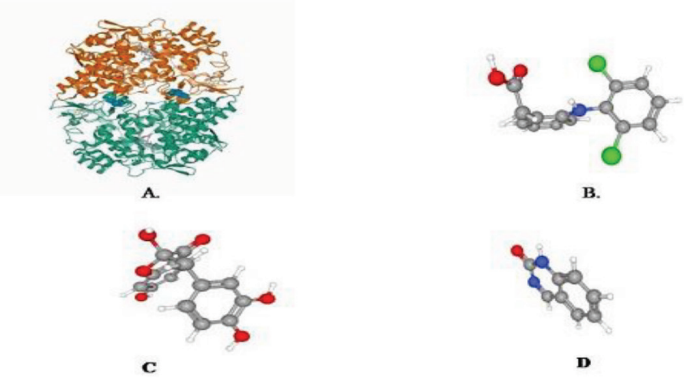

Figure 4: The Three Dimensional structures of target and ligands. (a) Cyclooxygenase -II (PDB ID: 3LN1), (b) Diclofenac sodium (c) 2, 6, 3', 4'-Tetrahydroxy-2-benzylcoumaranone (d) 2(1H)-Quinazolinone.

Table 3: Binding affinities of isolated compounds at the active site of COX II.

Sr. No	Ligands	Dock Score (Kcal/mol)
		3LN1
1	Diclofenac	-6.9
2	2,6,3',4'-Tetrahydroxy-2-benzylcoumaranone	-6.8
3	2(1H)-Quinazolinone	-6.2
4	Phenylephrine	-5.8
5	4-_fluro_histamine	-5.7
6	Phthalic_acid	-5.7
7	Tenamfetamine	-5.7
8	3-Hydroxy-4-methoxybenzoic_acid	-5.5
9	3-Methoxyamphetamine	-5.5
10	Phytol	-5.5
11	n-Hexadecanoic_acid	-5.1
12	9,12-Octadecadienoic_acid_(Z,Z)	-4.8

TYR A:116 (5.81)), hydrophobic interactions (PRO A:139 (4.95)) and also electrostatic interaction (ASP A:111 (7.25), ARG A:29 (4.05), GLU A:31 (4.90)) with COX-II enzyme, where as 2(1H)-Quinazolinone also show good docking rating of -6.2, which showed three hydrogen bond interactions (ASN A:24 (3.22), GLN A:447 (5.37)), hydrophobic interactions (ARG A:455 (4.85), ARG A:29 (5.70), LEU A:138 (6.04)) with amino acids of COX-2.The standard Diclofenac revealed the highest possible docking rating of -6.9(Kcal/mol).The outcomes gotten by the auto dock 4.0 are shown in Table 2, as well as the protein-ligand interactions revealing hydrogen bonding and also binding settings are additionally published in Tables 3 (Figure 5-7).

ADME/T evaluation by using admetSAR

In this research by using admetSAR, ADMET properties of ligand were estimated. All ligands shows excellent human intestinal solubility, Blood brain barrier infiltration (BBB).All ligands were AMES negative. The

Table 4: Interactions COX II amino acid residues with ligands at receptor site.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (A°)		
		Hydrogen Binding Interactions	Hydrophobic Interactions	Electrostatic Interactions
Diclofenac	-6.9	GLY A:30 (4.35)	PRO A:139 (4.97, 6.15), CYS A:32 (4.88), LEU A:138 (5.20), ARG A:29 (4.29), ARG A:455 (4.16)	-
2,6,3',4'-Tetrahydroxy-2-benzylcoumaranone	-6.8	CYS A:32 (2.67), TYR A:116 (5.81)	PRO A:139 (4.95)	ASP A:111 (7.25), ARG A:29 (4.05), GLU A:31 (4.90)
2(1H)-Quinazolinone	-6.2	ASN A:24 (3.22), GLN A:447 (5.37)	ARG A:455 (4.85), ARG A:29 (5.70), LEU A:138 (6.04), GLU A:451 (4.64)	-

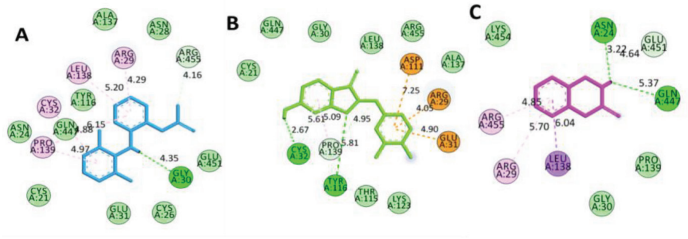

Figure 5: 2D Interactions of ligands with Cyclooxygenase -II (PDB ID: 3LN1) (A) Diclofenac (B) 2, 6, 3', 4'-Tetrahydroxy-2-benzylcoumaranone(C) 2(1H)-Quinazolinone

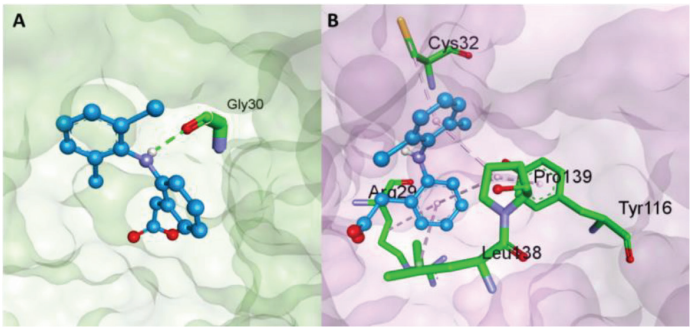

Figure 6: 3D Interactions of Diclofenac with COX-II (PDB ID: 3LN1) (A) Hydrogen Bonding (B) Hydrophobic Interactions.

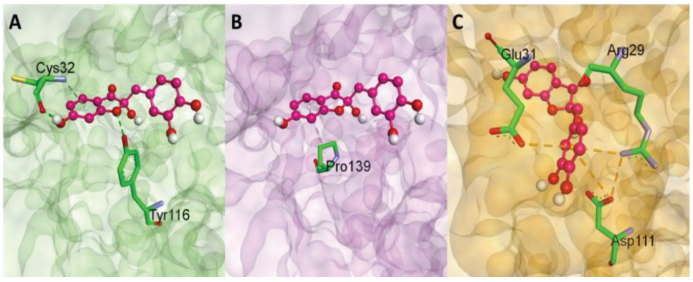

Figure 7: 3D interactions of 2, 6, 3', 4'-Tetrahydroxy-2-benzylcoumaranone with COX-2 (PDB IS: 3LN1) (A) Hydrogen Bonding (B) Hydrophobic Interactions and (C) Electrostatic Interactions.

results of HIA, BBB and LD50 values of the ligands are shown in Table 4 and 5.

DISCUSSION

For screening of anti- inflammatory activity of drugs, carageenan induced rat paw edema model is suitable and commonly used method.

Table 5: ADME/T properties of different compounds from *Biophytum veldkampii*.

Compound	HIA	BBB	AMES toxicity	Carcinogenicity	LD_{50} in rats (mol/kg)
Diclofenac	0.99455	0.9481	Non toxic	Non carcinogenic	2.085
, 6, 3', 4'-Tetrahydroxy-2-benzylcoumaranone	0.9648	0.8245	Non toxic	Non carcinogenic	1.4228
2(1H)-Quinazolinone	0.9786	0.9246	Non toxic	Non carcinogenic	1.8246

Carageenan is a polysaccharide it cause the inflammation by release of pro inflammatory mediators like prostaglandins, leuckotrienes, histamine and TNF alpha.[19] The inflammation occurs in two phase. In first phase started with release of histamine, 5HT and kinins after the injection of carageenan in the first few hours. In second phase related to release of prostaglandins in next 2-3 hr. Prostaglandins are main chemical agents responsible for inflammation. Second phase is the most target phase for steroidal and non-steroidal anti-inflammatory drugs.[20]

In this research *Biophytum veldkampii* had very consistent anti-inflammatory action and exhibit significant decreases thickness of paw. Although, the Cyclooxygenase and lipoxygenases pathways play a vital role in the inflammatory process, the inhibition of Cyclooxygenase is more effective in inhibiting carageenan-induced inflammation than lipoxygenases inhibitors.[21]

Biophytum veldkampii might have inhibited the synthesis of PGs mediated by Cyclooxygenase. Oral administration of methanolic extract of *Biophytum veldkampii* significantly decreases the pro inflammatory cytokines and elevated the anti-inflammatory cytokines. COX enzyme increases the prostaglandins synthesis which causes the decreases the anti-inflammatory cytokines like IL-10, IL-4 and IL-13. Our present findings *Biophytum veldkampii* shows elevated levels of IL-10 hence IL-10 has been found as a potent macrophage deactivator, which blocked TNF-α, IL-1, IL-6, IL-8, and GM-CSF by human monocytes.[22]

In silico molecular docking studies is one of the significant method to development of new medications for various pathological conditions, advantage of this method is less time and required less budget compared to standard lab experiments.

In this research, we used a docking method making use of open software programs as well as virtualized. Interactions of ligands 2, 6, 3', 4'-Tet-

rahydroxy-2-benzylcoumaranone and 2(1H)-Quinazolinone with anti-inflammatory protein COX-II enzyme. ΔG indicates informative of ligand docking in the active site of a protein, kind of molecular communications, such as hydrogen bond, hydrophobic, as well as likewise electrostatic interactions, with necessary amino acid, which is a step of ligand docking in favorable conformations. Hydrophobic synergy is the main aspect of the firmness of proteins. Hydrogen bonding furthermore maintains protein firmness, yet to a minimized degree than hydrophobic synergy. Our results disclose that electrostatic, hydrophobic and hydrophilic communications are regulated by numerous amino acid deposits in each ligand-protein communication.

2,6,3',4'-Tetrahydroxy-2-benzylcoumaranone exhibits hydrogen bond, hydrophobic bond and electrostatic interactions whereas 2(1H)-Quinazolinone exhibits only hydrogen bond, and hydrophobic bond interactions.

CONCLUSION

To conclude with the compounds from the *Biophytum veldkampii* showed better binding features with the COX-2 enzyme. Thus, these compounds can be effectively used as drugs for treating inflammation which is predicted on the basis of docking scores. The insights gained in this work can be further used in experimental studies for designing anti-inflammatory drugs with novel targets and mechanism of action.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HIA: Human Intestinal Absorption; **B.B.B.:** Blood- Brain Barrier; **LD_{50}:** Lethal Dose 50%; **MEBV:** Methanolic extract of *Biophytum veldkampii*.

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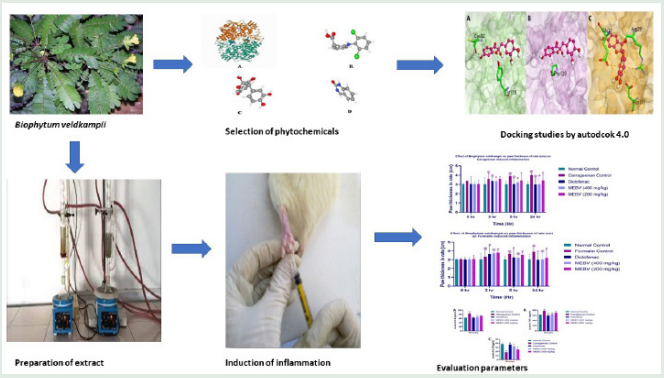
GRAPHICAL ABSTRACT



SUMMARY

In this research Biophytum veldkampii was subjected to extraction using methanol. *In-vivo* anti-inflammatory activity was assessed by using carageenan induced inflammation on rats and *in-silico* molecular docking studies was performed by using of Autodock 4.0. The outcomes revealed that the methanolic extract has most prominenant anti-inflammatory activity at various doses. Among all the substances 2,6,3',4'-Tetrahydroxy-2-benzylcoumara none revealed the most effective docking rating of -6.8, which is near to Diclofenac, i.e. - 6.9, ensuring that 2,6,3',4'-Tetrahydroxy-2-benzylcoumaranone has a strong binding fondness in between protein and ligand. From the results, a conclusion can be drawn that the anti-inflammatory activity of *Biophytum veldkampii* in both *in vivo* and *in silico* methods. This information sustains 2,6,3',4'-Tetrahydroxy-2-benzylcoumaranone to be a useful anti inflammatory compound beneficial to future clinical studies.

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