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Alangium chinense (Lour.) Harms Leaves with Anti-inflammatory, Antioxidant Property: *in vitro* and *in silico* Study

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The present investigation aimed to find the *in vitro* antioxidant and anti-inflammatory potential of fractions of methanol extract of *Alangium chinense* (Lour.) Harms leaves followed by isolation and identification bioactive molecule and evaluate its possible mechanism through *in silico* study. A methanol extract of *Alangium chinense* (Lour.) Harms leaves subjected for chromatographic fractionation and pure isolates (F1-F8) were evaluated for their *in vitro* anti-inflammatory activity using albumin denaturation inhibition assay and *in vitro* antioxidant activity. Fraction F4 showed highest *in vitro* anti-inflammatory activity (IC_{50} = 70.02 µg/mL), whereas fractions F3, F4, F7 showed better *in vitro* antioxidant activity compare to other fractions. F4 fraction was screened for its tentative structure using FTIR, ^{1}H and ^{13}C NMR. The spectral analysis showed that the fraction F4 has a tentative structure of olean 19-ene-1yl-acetate. The molecular docking studies showed that test ligand significantly interact with different key active sites of amino acids and thus confirm possible COX-2 inhibitory activity of fraction F4. *In vitro* and *in silico* study confirmed the isolation of possible anti-inflammatory from *Alangium chinense* (Lour.) Harms leaves, which may further used as lead to a development of a new therapeutic agent.

Keywords: *Alangium chinense* (Lour.) Harms, Anti-inflammatory activity, Antioxidant activity, Phytomolecules.

INTRODUCTION

Inflammation has been considered as a well-known indication of many infectious diseases and other pathological conditions. Advance research increasingly established the close linkage between inflammation and oxidative stress. Free radical induced damage is considered as key molecular mechanism for inflammatory disorders like cardiovascular disease, cancer, CNS disorders, metabolic and other disorders [1,2]. Possibilities to find phytoconstituents for treatment and therapy of inflammatory diseases by blocking the inflammatory processes considered as a key approach in modern time. *Alangium chinense* (Lour.) Harms is mainly distributed throughout the tropical Asia from India to China, Japan, Thailand, Philippines and Indonesia. In India, it is mainly found in the parts of Bengal, Sikkim, Andhra Pradesh and Assam. *Alangium chinense* (Lour.) Harms belongs to the family Alangiaceae (APG Cornaceae). This species is closely related to *Alangium platinifolium* [3].

Till the date, studies have shown that *Alangium chinense* (Lour.) Harms has been used in the treatment of wound healing, rheumatism and traumatic injuries. The paste of the plant leaves is used for setting up of the dislocated bones. In addition, the root and shoots of the plant has also been used medicinally [4]. An ethnobotanical survey conducted in Guangxi, China revealed that the root, leave and flower of the plant used traditionally used to treat rheumatic arthritis and traumatic injury [5]. The areal parts of *Alangium chinense* (Lour.) Harms has been investigated for its anti plasmodial activity [6]. Different phytochemicals, such as benzyl, phenolic, flavonoid glycosides and alkaloids (venoterpine and dl-anabasine) were isolated from *Alangium chinense* (Lour.) Harms [7,8]. In addition, five novel phenolic glycosides (6′-O-galloylsalicin, 4′,6′-di-O-galloylsalicin, 4′,6′-O-(*S*)-hexahydroxyl diphenoyl salicin, 4′,6′,-O-(*R*)-hexahydroxyl diphenoyl salicin and pyrocatechol 1-O-β-D-xylopyranosyl(1-6)-β-D-glucopyranoside) has been isolated from *Alangium chinense* (Lour.) Harms [9]. To date,

few phytochemical and pharmacological studies have been reported related to plant leaves. In previous investigations, we have reported *in vivo* anti-inflammatory, *in vitro* antioxidant, antipyretic and analgesic effect of leave extract of *Alangium chinense* (Lour.) Harms. Methanol extract of leaf showed promising effect and contain significant amount of total phenolic and total flavonoid [3,4]. This study aimed to find antioxidant and anti-inflammatory activity of fractions of methanol extract and isolation, characterize anti-inflammatory molecule present in leaves of *Alangium chinense* (Lour.) Harms through the molecular docking study.

EXPERIMENTAL

Plant collection: Matured fresh leaves of *Alangium chinense* (Lour.) Harms were collected from the Kamrup district, India (Fig. 1). The plant leaves were identified and validated by the Botanical Survey of India, Shillong, India (Ref. No. BSI/ERC/Tech./Plant Iden./2015/44). The fresh leaves were dried in shed, powdered and passed through the sieve no. 22. Finally, leaves powder was kept in an airtight container and protected from the sunlight for further study.

Fig. 1. *Alangium chinense* (Lour.) Harms plant

Extraction and fractionation: Dried leaves powder was extracted successively using hot extraction method using hexane, ethyl acetate and methanol were used in order of their increasing polarity. Finally, the solvent was evaporated under reduced pressure to get concentrated extract. The extracts obtained from different solvents were used for the further studies.

In previous investigation, we reported the methanol extract of *Alangium chinense* (Lour.) Harms exhibited better activity [4]. Therefore, methanolic extract was further fractionated by column chromatography. Thin layer chromatography (TLC) was carried out to know the solvent system through which we can do the column chromatography [10]. TLC of the extract carried out on a pre-coated TLC plate and R_f (retardation factor) values was calculated. The maximum number of spots (12) with maximum R_f value (R_f = 0.95) were found with solvent system of hexane:chloroform:methanol (1:4:1). TLC result showed that hexane, chloroform, methanol are the solvent system through which maximum eluent can be collected. Based on the polarity, hexane was used earlier (low polar solvent) and methanol was used at the last (high polar solvent). Column chromatography was done in a borosilicate column (40 mm × 600 mm). For stationary phase, silica gel coarse powder was used (60-120 sieves). The column was prepared by wet packing technique. The column was packed up to 30 cm from the base and 4 cm width. The extract was charged in column by mixing methanolic extract (20 g) with methanol and silica gel coarse powder (30 g). Different solvents *viz*. hexane, chloroform and methanol were poured and the eluents (80 mL each) were collected. From the column chromatography, 84 eluents were collected. The eluents were combined based on the TLC data and eight major fractions (F1 to F8) were selected considering the purity and peak abundance in HPLC chromatogram. For HPLC analysis, Agilent 1220 infinity with C-18 reverse phase column (Kromasil, 250 mm × 4.6 mm, 5 µ particle size) was used. The HPLC method was run in an isocratic mode with solvent systems Milliq water and acetonitrile with 0.1% formic acid. The flow rate was 2 mL/min with an injection volume of 50 vL. Extract (1%) was prepared in HPLC grade methanol. Then the sample was sonicated using ultrasonicator for 10 min. Then extract was membrane filtered before injecting into the column.

***In vitro* antioxidant activity:** Eight fractions obtained from column chromatography were further studied for *in vitro* antioxidant activity using different methods.

DPPH radical scavenging assay: Methanolic solution of DPPH (1.0 mL, 0.1 mM) was mixed with 3.0 mL of sample solution of different concentrations. The reaction mixture was incubated in dark at room temperature for 30 min and the absorbance was recorded at 517 nm. The assay was carried out in triplicate for each sample. Methanol (1 mL) with 3.0 mL fraction solution (various concentrations) was used as a blank and DPPH solution (1.0 mL, 0.1 mM) with methanol (2.5 mL) served as negative control. The radical scavenging activity of ascorbic acid was also determined as positive control. The decrease in colour intensity on addition of test samples was used to calculate the antiradical activity, measured by taking absorbance and compared with negative control [11]. The activity was expressed by the inhibition (%) of DPPH radical, following eqn. 1:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where, A_c and A_s are the absorbance of control and of standard sample, respectively. From a plot of concentration against I%, a linear regression analysis was performed to determine the IC_{50} (extract concentration resulting in a 50% inhibition) value for each sample.

Hydroxyl radical scavenging activity: An amount of 0.2 mL of 100 mM KH_2PO_4–KOH, 0.2 mL of 15 mM deoxyribose, 0.2 mL of 500 mM $FeCl_3$, 0.1 mL of 1 mM ethylene diamine tetraacetic acid (EDTA), 0.1 mL of 1 mM ascorbic acid and 0.1 mL of 10 mM H_2O_2 were mixed with 0.1 mL sample with different concentration. The mixture was incubated for 1 h at 37 ºC. After incubation, 1.0 mL of 1% w/v TBA and followed

by 1.0 mL of 2.8% w/v TCA was added to the mixture. The resultant mixture was heated for 20 min at 80 ºC on a water bath results in development of pink colour which was measured at 532 nm. Quercetin was used as the positive control [11]. The scavenging activity (I %) was calculated using eqn. 1.

Nitric oxide radical scavenging activity: An aliquot of extract solution (4 mL) at different concentrations were mixed with 1.0 mL of 25 mM sodium nitroprusside solution in a test tube and incubated for 2 h at 37 ºC. Incubated solution (2 mL) was mixed with 1.2 mL Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthyl ethylenediamine dihydrochloride), which results in diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride to form a chromophore. The absorbance of chromophore was measured immediately at 570 nm. Control experiment was also carried out in similar manner taking same volume of distilled water in the place of sample solution [11]. The experiment was performed in triplicate, ascorbic acid was used as positive control and percentage scavenging activity was calculated using eqn. 1.

Anti-inflammatory activity of fractions: The *in vitro* anti-inflammatory activity of the selected fractions was carried out as mentioned in the literature [12]. The test sample (5 mL) was prepared by adding egg albumin (0.2 mL) and phosphate buffer saline (2.8 mL). The final concentrations (50, 100, 150 µg/mL) were prepared accordingly. Indomethacin was kept as the reference drug. Both the test solution and methanol solution were incubated for 15 min at 37 ± 2 ºC in a BOD incubator. After incubation, the solutions were heated at 70 ºC for 5 min and then allowed to cool. Later, the absorbance was seen at 660 nm by using the methanol solution as a blank. The inhibition of protein denaturation percentage was measured by using the formula:

$$\text{Inhibition (\%)} = \left(1 - \frac{V_t}{V_c}\right) \times 100$$

where, V_t = absorbance of the test, V_c = absorbance of the control.

Spectral analysis: One selected fraction (F4) was sent for spectral analysis as it showed better *in vitro* antioxidant and anti-inflammatory activity (inhibition of albumin denaturation) compare to other fractions. Spectral studies, such as FTIR, ^{1}H NMR and ^{13}C NMR were performed to analyze the possible structure of constituent.

Molecular docking: The structure of the ligand was drawn using Sanjeevani online program that supports Marvin sketch tool. The 2D structure of the ligand was later introduced to Discovery Studio 4.5 workplace (Visualizer), where it was converted to a corresponding 3D structure. Hydrogens were added to the ligand and energy was minimized by using Char mM force field. Possible ligand bindings were confirmed further by initiation of prepare ligand protocol where an *in silico* pH of 7.0-7.4 was considered. The lowest energy possessing ligand was further selected for docking. The docking was done at the active site of the enzyme protein. For protein structure, crystal structure of COX-2 enzyme bound with naproxen and other co-factors was obtained from RCSB protein data bank (PDB entry code: 3NT1). Water molecules were removed and other cofactors were allowed to retain. Autodock Tools (ADT) did optimization of the receptors and ligand molecules. The receptor molecule was prepared using the kollman charges, polar hydrogen's. The atoms of AD4 were also added. Maximum numbers of active torsions was given and gasteiger charges were added on the ligands. Grid map of interaction energies was made by AutoGrid4 around Leu1040, Gln267, Leu263, Phe264, Ile981, Ala985, Ile977, Asn1039, Asn1042 and Ile1041 with a grid box of 90 Å × 90 Å × 90 Å centered on X, Y, Z = 52.27, 16.45, 11.48 with a grid spacing of 0.375 Å. Lamarckian genetic algorithm (LGA) was used to do the molecular docking. At first, the docking parameters should be kept in default mode to obtain a barrier free molecular docking. During the docking simulation process the molecule and receptor binding was kept rigid for establishing a proper binding. Post docking analysis was done in Discovery studio 4.5 visualizer.

RESULTS AND DISCUSSION

Isolation of chemical compound: Extract of *Alangium chinense* (Lour.) Harms ssp. triangulare (stem) showed significant NF-5B inhibitory activity at 50 and 25 µg/mL concentration [13]. Another study reported that salicin obtained from *Alangium chinense* (Lour.) Harms is useful in ameliorate rheumatoid arthritis that may link with modulation of oxidative stress and Nrf2-HO-1-ROS 54 pathways [14]. Herein, for the isolation of the compound, silica gel coarse 60-120 mesh was used as column stationary phase. Hexane, chloroform, methanol (1:4:1) was used as mobile phase. Total 84 eluents each of 80 mL were collected (Table-1). TLC was done for all the eluents and a total 11 fractions were collected and subjected for purity analysis using HPLC. Fractions with pure single peak were selected. Fractions showed multiple peaks were considered as an impure compound. Finally, based on the HPLC data, eight fractions were selected for further analysis.

TABLE-1
TOTAL FRACTION COLLECTED IN COLUMN CHROMATOGRAPHY

Solvents	Fractions				
Hexane	9-15#	17-19*			
Chloroform	23-27**	30-33*	35-42*	44-47**	50-53#
Methanol	57-63*	65-71*	74-79**	81-83#	

#: Several peaks with less abundance and considered impure.
*: Single peak appeared in HPLC. **: More than one peak with high abundance and considered for pencil column.

***In vitro* antioxidant activity:** Fractions (F1-F8) of *Alangium chinense* (Lour.) Harms exhibited strong DPPH radical scavenging activity. In this experiment, all fractions exhibited potent antioxidant activity exhibited through DPPH radical, nitric oxide radical and hydroxyl radical scavenging activity (Table-2). F4 showed highest DPPH radical scavenging effect (IC_{50} = 6.10 ± 0.16 µg/mL) followed by F7 (IC_{50} = 6.50 ± 0.09 µg/mL). IC_{50} value of standard ascorbic acid was found to be 4.21 ± 0.10 µg/mL. Fractions also exhibited strong nitric oxide radical scavenging effect. IC_{50} value of ascorbic acid was found to be 21.91 ± 0.16 µg/mL. F3 and F4 fractions showed better results

TABLE-2
In vitro ANTIOXIDANT ACTIVITY OF FRACTIONS OF METHANOL EXTRACT OF LEAVES OF *Alangium chinense* (Lour.) Harms.

Fractions/ Standards	IC_{50} value (µg/mL)		
	DPPH• scavenging assay	NO• scavenging assay	OH• scavenging assay
F1	10.05 ± 0.17	24.99 ± 0.20	24.02 ± 0.15
F2	8.20 ± 0.11	23.21 ± 0.19	27.10 ± 0.22
F3	6.66 ± 0.16	18.50 ± 0.14	26.36 ± 0.24
F4	6.10 ± 0.16	18.79 ± 0.17	22.88 ± 0.19
F5	8.41 ± 0.14	26. 01 ± 0.23	26.83 ± 0.21
F6	7.34 ± 0.10	21.65 ± 0.19	25.80 ± 0.30
F7	6.50 ± 0.09	23.99 ± 0.20	20.23 ± 0.27
F8	8.12 ± 0.14	25.91 ± 0.18	23.88 ± 0.10
Standard	4.21 ± 0.10 (Ascorbic acid)	21.91 ± 0.16 (Ascorbic acid)	20.22 ± 0.14 (Quercetin)

Results were calculated as mean ± SEM (n = 3).

than ascorbic acid in scavenging nitric oxide radical (in terms of IC_{50} value) which was 18.50 ± 0.14 and 18.79 ± 0.17 µg/mL, respectively. Positive control quercetin showed a high scavenging activity with IC_{50} of 20.22 ± 0.14 µg/mL. F7 was showing IC_{50} of 20.23 ± 0.27 µg/mL, followed by F4 (IC_{50} 22.88 ± 0.19 µg/mL). Fractions of methanolic extract of *A. chinense* (Lour.) Harms significantly scavenge hydroxyl radical and NO radical, which further expands the role of this plant as a potent antioxidant.

***In vitro* inflammatory activity:** Eight pure fractions were evaluated for their anti-inflammatory activity using of albumin denaturation inhibition assay. The eight fractions showed the anti-inflammatory activity in a concentration dependent manner. All the fractions has showed significant inhibition of heat induced albumin denaturation. However, the percentage inhibition was high in the fraction F4 (IC_{50} value 70.20 µg/mL), which is better compared to the standard drug indomethacin. Figs. 2 and 3 showed *in vitro* anti-inflammatory activity (percentage inhibition and IC_{50} value) of fractions of *A. chinense* (Lour.) Harms leaves extract. Fractions of *A. chinense* (Lour.) Harms showed potent *in vitro* anti-inflammatory activity, among them fraction F4 exhibited the highest protein denaturation inhibition effect.

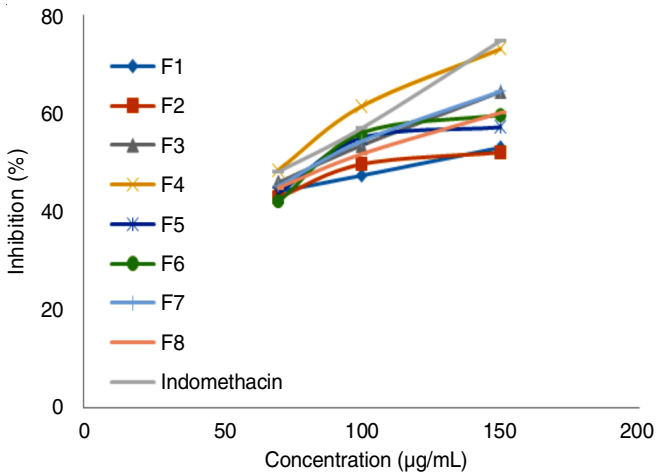

Fig. 2. *In vitro* anti-inflammatory activity (percentage inhibition) of fractions of *Alangium chinense* (Lour.) Harms leaves extract

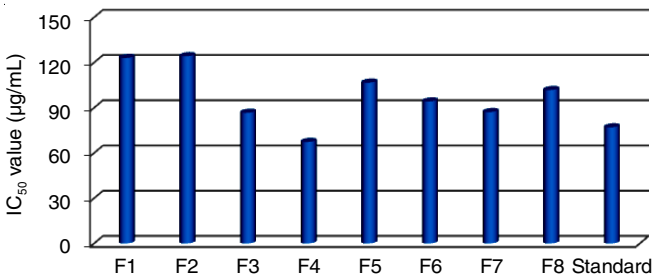

Fig. 3. IC_{50} value of fractions of *Alangium chinense* (Lour.) Harms leaves extract in albumin denaturation inhibition assay

Structural characterization: The IR spectral analysis showed characteristic peaks at 958 cm^{-1} (C=C outlaying bending), 1051 cm^{-1} (C-O stretching), 1376 cm^{-1} [(CH bending)CH_3], 1462

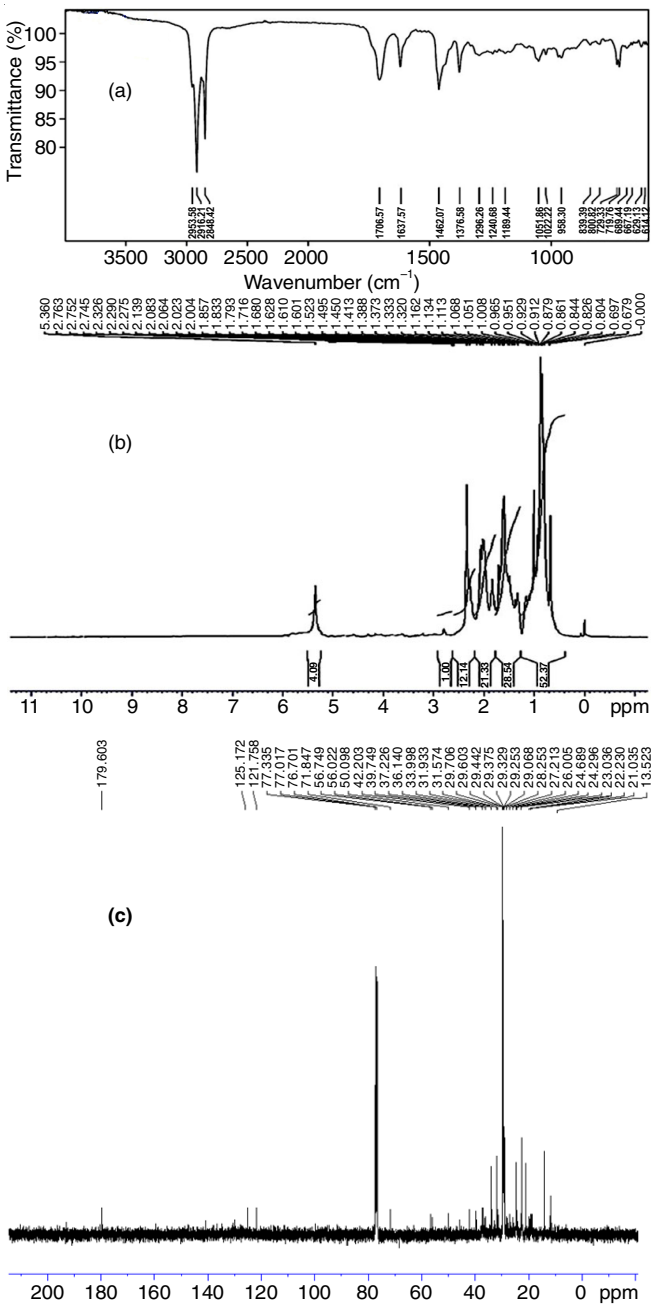

Fig. 4. (a) FTIR, (b) ^1H NMR and (c) ^{13}C NMR spectra of F4

cm^{-1} [(CH_2 bending)CH_2], 1637 cm^{-1} (C=C non-conjugated stretching), 1706 cm^{-1} [(C=O *str.*) acid/ester], 2848 cm^{-1} (-CH *str.*), 2916 cm^{-1} (CH_3 *str.*) and 2953 cm^{-1} (C-H *str.*) (Fig. 4a). ^{1}H NMR spectroscopy analysis showed the δ value at 0.679-1.320 (24 protons of CH_3), 1.495-1.857 (18 protons of CH_2), 2.023-2.139 (4 proton of CH), 2.290-2.326 (proton present at C_{19} and C_{20}), 2.752 (proton present at C_1), 5.360 (proton present at C_{24}) (Fig. 4b). The ^{13}C NMR spectroscopy data showed δ value at 13.523 (C_{30}), 21.035 (C_{29}), 22.230 (C_{28}), 23.036 (C_{27}), 24.296 (C_{31}, C_{32}), 26.005 (C_{26}), 27.213 (C_{25}), 28.253 (C_{24}), 29.068-29.706 (C_{11}, C_{12},C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{21}, C_{22}), 31.574 (C_{04}), 31.933 (C_{10}), 33.998 (C_{07}), 36.140 (C_{06}), 37.226 (C_{13}), 39.479 (C_{02}), 42.203 (C_{09}), 50.098 (C_{03}), 56.022 (C_{05}), 71.847 (C_{01}), 121.758 (C_{19}), 125.172 (C_{20}) and 179.603 (C_{23}) (Fig. 4c).

Depending upon the results of spectral data, the tentative structure of the chemical fraction F4 is found to be olean 19-ene-1yl-acetate (Fig. 5).

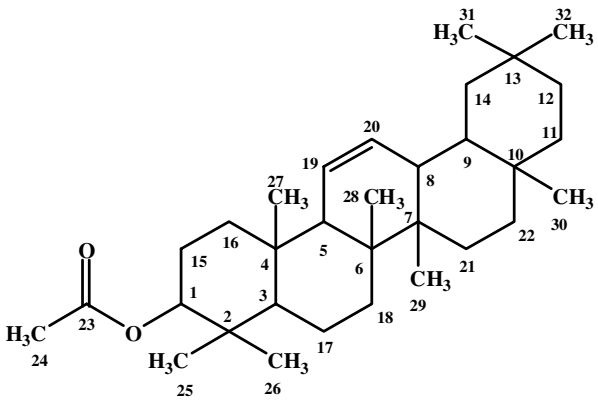

Fig. 5. Tentative structure of chemical compound from fraction F4

Molecular docking study: Upon successful completion of molecular docking of the ligand at the selected protein active site, results were analyzed using Discovery Studio 4.5 visualizer. The test ligand was found to interact with different key active site amino acids *via* nine alkyl bonds (LEU A: 408, LEU A: 408, LEU A: 408, VAL A: 444, VAL A: 447, TYR A: 409, TYR A: 409, HIS A: 214, HIS A: 214) and one hydrogen bond at (ARG A:222) (Fig. 6). Spectral analysis revealed that fraction F4 is a triterpenoid (olean 19-ene-1yl-acetate), which was isolated for the first time from this plant leaf. The binding pattern of this triterpenoid (olean 19-ene-1yl-acetate) shows a close relation with the binding pattern of established COX-2 inhibitor. Present findings suggest that the test ligand (F4) can probably be potent NSAIDs and a future candidate for development of NSAIDs. Though future studies are required to investigate the isolated molecule and to find other bioactive molecules from the plant.

Conclusion

The present study demonstrates that fractions of methanol extract of *Alangium chinense* (Lour.) Harms leaves exhibited *in vitro* anti-inflammatory and antioxidant activity. A triterpenoid (olean 19-ene-1yl-acetate) was isolated from the plant leaf, which also demonstrated COX-2 inhibitory property through molecular docking study. The isolated molecule can be a future candidate to be developed as anti-inflammatory therapeutics or preventatives though extensive studies are required.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

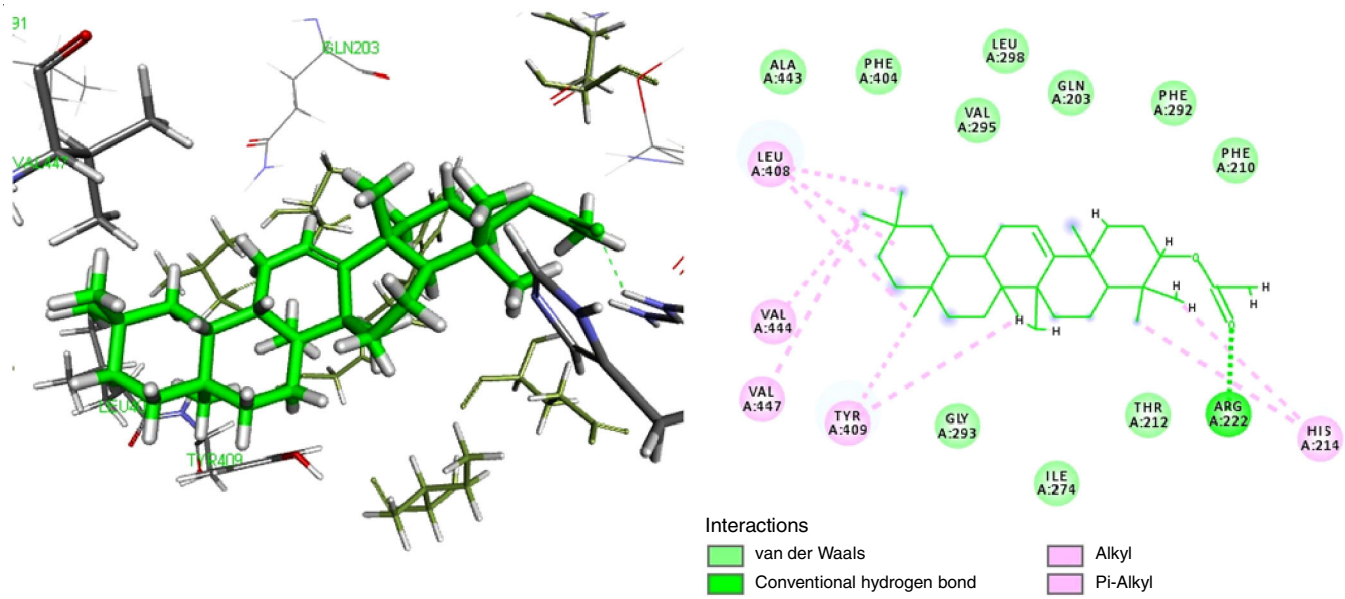

Fig. 6. Docking pose of the test ligand (LEU: Leucine; VAL: Valine; TYR: Tyrosine; HIS: Histidine; ARG: Arginine) visualized by Studio 4.5 workplace and used Autodock tool for docking

REFERENCES

1. P. Hunter, *EMBO Rep.*, **13**, 968 (2012); https://doi.org/10.1038/embor.2012.142
2. S. Biswas, R. Das and E.R. Banerjee, *AIMS Biophys*., **4**, 596 (2017); https://doi.org/10.3934/biophy.2017.4.596
3. M.K. Deka, R. Chakraborty, S. Sen and C.C. Kandar, *CIBTech J. Pharm. Sci.*, **5**, 52 (2016).
4. R.C. Laloo, L. Kharlukhi, S. Jeeva and B.P. Mishra, *Curr. Sci.*, **90**, 225 (2006).
5. R. Hu, C. Lin, W. Xu, Y. Liu and C. Long, *J. Ethnobiol. Ethnomed*., **16**, 40 (2020); https://doi.org/10.1186/s13002-020-00387-z
6. S. Beourou, A.C.L. Lamer, S.M. Chevalley, P.B.C. Mutis, F. Souard and C. Moulis, *Faslnamah-i Giyahan-i Daruyi*, **2**, 219 (2016).
7. A. Itoh, T. Tanahashi, N. Nagakura, K. Inoue, H. Kuwajima and H.X. Wu, *Chem. Pharm. Bull. (Tokyo)*, **49**, 1343 (2001); https://doi.org/10.1248/cpb.49.1343
8. Y. Zhang, Y.B. Liu, Y. Li, S.G. Ma, L. Li, J. Qu, D. Zhang, J.-D. Jiang and S.-S. Yu, *Chin. Chem. Lett.*, **28**, 32 (2017); https://doi.org/10.1016/j.cclet.2016.05.012
9. A. Itoh, T. Tanahashi, S. Ikejima, M. Inoue, N. Nagakura, K. Inoue, H. Kuwajima and H.-X. Wu, *J. Nat. Prod.*, **63**, 95 (2000); https://doi.org/10.1021/np990391z
10. S. Mehta, R.P. Singh and P. Saklani, *Int. J. Pharmacog. Phytochem. Res.*, **9**, 523 (2017).
11. S. Sen, B. De, N. Devanna and R. Chakraborty, *Chin. J. Nat. Med.*, **11**, 149 (2013).
12. S.N. Heendeniya, W.D. Ratnasooriya and R.N. Pathirana, *J. Pharmacogn. Phytochem.*, **7**, 1759 (2018).
13. C. Fan, H. Jin, L. Wu, Y. Zhang, R.D. Ye, W. Zhang and Y. Zhang, *Evid. Based Complement. Alternat. Med.*, **2017**, 1 (2017); https://doi.org/10.1155/2017/1231820
14. K.F. Zhai, H. Duan, G.J. Khan, H. Xu, F. Han, W. Cao, G. Gao, L. Shan and Z.-J. Wei, *J. Agric. Food Chem.*, **66**, 6073 (2018); https://doi.org/10.1021/acs.jafc.8b02241