GC-MS based Metabolite profiling, and anti-inflammatory activity of Aqueous extract of *Myrica esculenta* through In vitro and In-Silico approach.

Amit Kumar Shrivastava[*1], Dipendra Chaudhary[2], Laxmi Shrestha[1], Maaweya E. Awadalla[3], Samia T. Al-Shouli[4], Anjan Palikhey[1], Wafa Ali Eltayb[5], Anamika Gupta[6], Pramodkumar P Gupta[*7], Mala Parab[7], Anchal Trivedi[8], Aditi Srivastava[8], Mohnad Abdalla[9*]

[1]Department of Pharmacology, Universal College of Medical Sciences, Bhairahawa, Rupandehi, Nepal, 32900. [2]Department of Pharmacy, Provincial Lumbini Hospital, Butwal, Rupandehi, Nepal. [3]Research Center, King Fahad Medical City, Riyadh, Saudi Arabia. [4]Immunology Unit, Pathology department, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia. [5]Biotechnology Department, Faculty of Science and Technology, Shendi University,Shendi 11111, Nher Anile, Sudan. [6]Cardiovascular Research Group, Sharjah Institute for Medical Research, University of Sharjah, Sharjah 27272, UAE. [7]School of Biotechnology and Bioinformatics, D Y Patil Deemed to be University, CBD, Belapur, Navi Mumbai, Maharashtra, India. [8]Department of Biochemistry, Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India. [9]Research Institute of Paediatrics, Children's Hospital Affiliated to Shandong University (Jinan Children's Hospital), Jinan, China.

Corresponding author: Amit Kumar Shrivastava (sr.akshri.ucms.np@gmail.com); Pramodkumar P Gupta, (pramodkumar785@gmail.com / pramod.gupta@dypatil.edu), Mohnad Abdalla (mohnadabdalla200@gmail.com)

Abstract:

The present study was to determine the anti-inflammatory activity of aqueous extract of bark and root of *Myrica esculenta* and their active phytoconstituents through *In-Vitro* and *In-Silico* studies. The bioactive phytoconstituent of *Myrica esculenta* determined by GC-MS spectroscopy techniques. After that total phenolic and flavonoid content of both bark and root extract was determined. Furthermore, In-vitro anti-inflammatory activity was determined in both extracts. The molecular docking analysis determined the binding affinity of bioactive compounds against inflammatory proteins COX-1, COX-2, IL-10, and TNF-α. The study revealed bark extract of *Myrica esculenta* has the highest total phenolic and flavonoid content compared with root extract (553.44±18.38mg GAE/g equivalent and 336.02±8.04mg quercetin/g equivalent respectively). Similarly, the bark extract showed good inhibitory activity with 5-LOX and HYA assay (IC_{50} 11.26±3.93 and 21.61±8.27 µg/mL respectively), but in 15-Lox inhibitory assay root extract showed the highest inhibitory activity, IC_{50} 16.95±5.92 µg/mL. The Docking result showed that myrecitin, Arjunolic acid, and myricanone have the highest binding affinity with all inflammatory proteins in respective order: myrecitin>arjunolic acid>celecoxib>myricanone>myricitrin>3-epi-ursonic acid. The MD simulation of COX-1 and myrecitin showed the highest stability and low deviation at 310K through RMSD values (1.07-2.3 Å) as compared with COX-1 and myricitrin (0.193-1.885 Å) and TNF-α and myricanone (1.377 to 3.457Å) respectively when analysed at 100 ns time frame. Extract and their active constituents showed good anti-inflammatory activity. Further study is essential to define their mechanism of action.

Background

- *Myrica esculenta* Buch.-Ham. ex D. Don, also recognized as "Hairy Bayberry" and a member of the *myricaceae* family, is generally referred to as Kaiphal or Kataphala in the north Asian continent and is extensively utilized in Ayurveda for the treatment of several conditions such as asthma, diabetes, gout, arthritis, etc.
- Inflammation is the body's defensive mechanism in injured and infected tissues, triggered by immune cells and cytokines that release prostaglandins, interleukin-6, and tumor necrosis factor-α.
- Anti-inflammatory medicines are thought to be an effective way of mitigating the influence of chronic inflammation on the progression of degenerative diseases
- Difficulty in understating complex etiology and exacerbating mechanism leads to hinderance in emergent enchantment bullets for chronic inflammatory disorders. Subsequently, there is required for modern and secure anti-inflammatory agents extracted from plant origin
- Introducing protein-ligand docking, computational chemistry allows exploring the plant-inferred molecules as a drug candidate. The majority of recent computational docking approaches presume that the receptor structure is fixed.
- Thus, keeping in view the significance of the above explanations, the purpose of the current study is to assess the anti-inflammatory potential of root and bark extracts of *Myrica esculenta* through in-vitro evaluation as well in-silico molecular docking.

Materials and Method

Extraction: The bark and root were separately ground and made into porous powder. The root and bark powder were weighed 100 g and extracted with distilled water (1:10) separately for 72 hours using continuous hot Soxhlet apparatus. The extracted solvent was collected and dried under a controlled temperature of 70 °C ±5 °C in a rotatory evaporator. The semisolid consistency was obtained, both extracts were weighed separately, and the percentage yield was calculated. Both extracts were stored at 4 °C for future use.

In the present study different parameters were analyzed such as total phenolic content (TPC), total flavonoid content (TFC) and also characterization of different phytoconstituents of Myrica esculenta through GC-MS spectroscopy. After that identification of active phytoconstituents of aqueous extract of Myrica esculents in-vitro, and In-Silico anti-inflammatory activity was determined.

In-vitro anti inflammatory activity (LOX inhibition activity)
A 1 mL of 0.1 M sodium borate buffer, pH 8.8, 20,000 U of lipoxidase was dissolved. The aqueous extract of bark and root of *M. esculanta* at several concentrations was taken 1 mL from each stock in different test tubes. An equal volume of lipoxidase solution was added and incubated at 37 °C for 20 minutes. After incubation, 1 mL of linolic acid was added to each reaction mixture and mixed. The absorbance of the mixture was evaluated with a UV spectrophotometer at 234 nm. Indomethacin and etoricoxib were taken as standard.

In-Silico study of selected phytoconstituents of Myrica esculenta was carried out by using autodock 4.2 and autodock vina. In the present study the phytoconstituents were selected on the basis of their concentration present in the plant was reported in many previous literature. Also after the docking study the MD simulation at 100 ns was performed of selected ligand protein complexes to define their effects and stability by using Schrodinger software.

Results

Table 1: The mean TPC content of of Myrica esculenta bark and root extract was 553.44±18.38 and 421.17±5.34 mg GAE/g equivalent respectively. The mean TFC content of bark and root extract of Myrica esculenta was 336.02±8.04 and 421.17±5.34 mg quercetin/g equivalent respectively. The value was expressed in Mean±SEM.

Conc. (mg/mL)	Mean Absorbance od TPC at 765 nm and TFC at 510 nm											
	Bark Extract						Root Extract					
	Abs. of extract	Conc. of Gallic Acid (mg/mL)	TPC (mg GAE/g)	Abs. of extract	Conc. of Quercetin (mg/mL)	TFC (mg of Quercetin/g)	Abs. of extract	Conc. of Gallic Acid (mg/mL)	TPC (mg GAE/g)	Abs. of extract	Conc. of Quercetin (mg/mL)	TFC (mg of Quercetin/g)
0.1	0.332	28.63±1.14	286.34±11.40	0.238	15.84±0.09	158.40±0.98	0.245	21.06±0.05	210.69±0.50	0.168	11.11±0.12	111.10±1.25
0.2	0.459	39.70±2.06	397.07±20.61	0.353	23.94±0.26	239.48±2.65	0.317	27.38±1.10	273.88±11.06	0.289	19.28±0.30	191.28±3.03
0.4	0.560	51.67±2.26	484.89±22.63	0.452	29.10±0.05	291.05±5.85	0.462	39.96±0.77	399.68±7.78	0.379	25.34±0.28	253.44±2.81
0.8	0.688	56.54±2.01	596.20±20.15	0.571	38.31±0.64	387.455±6.41	0.533	46.14±0.28	461.42±2.85	0.477	32.01±0.21	320.11±2.14
1.0	0.841	71.15±1.47	729.24±14.74	0.682	45.97±2.90	459.75±29.07	0.627	54.31±0.27	543.15±2.76	0.529	35.52±0.45	355.06±4.52
2.0	0.953	82.69±2.07	826.92±20.76	0.714	47.97±0.33	480.02±3.31	0.736	63.82±0.71	638.23±7.10	0.645	43.31±0.07	433.17±0.78
Mean±SEM			**553.44±18.38**			**336.02±8.04**			**421.17±5.34**			**277.65±2.42**

Table 2: In-vitro anti-inflammatory activity of aqueous bark and root extract of Myrica esculenta

	5-LOX (IC_{50})	15-LOX (IC_{50})	HYA (IC_{50})
Bark extract	11.26±3.93	25.57±8.94	21.61±8.27
Root extract	23.024±8.04	16.95±5.92	40.24±15.41
Indomethacin	9.87±3.78	12.19±4.67	7.82±2.99
Etoricoxib	14.07±5.38	8.62±3.30	17.96±6.87

Table 3: Binding energies (kcal/mol) and dissociation constants (Kd) of Myricetin, Myricanone, 3-epi-ursonic acid, Myricitrin, Arjunolic acid, and celecoxib towards COX-1 and COX-2 using AutoDock 4.2.6

S.N.	Ligands	COX-1 (PDB ID: 4O1Z)				COX-2 (PDB ID: 4M11)			
		B.E. (kcal/mol)	Diss. Constant (Kd)	Interacting amino acid	H-atom	B.E. (kcal/mol)	Diss. Constant (Kd)	Interacting amino acid	H-atom
1.	Myricetin	-9.95	50.71 nM	Ser143, Arg374, Asn375, Gly533, Gly533, Asn537, Asn537, Asn53, Val228, Val228, Val228, Gly227	6	-6.97	7.79 µM	Phe361,Phe36,Lys360,Trp545, Arg61	4
2.	Myricanone	-7.65	2.46 µM	Ser143, Trp139, Arg376, Arg37	1	-6.51	16.78 µM	Trp545,Asp362, Asn560	2
3.	3-epi-ursonic acid	-6.88	9.08 µM	Arg376, Asn375, Gly225	2	-6.72	11.89 µM	Asp239,His242, Lys253	3
4.	Myricitrin	-7.64	2.51µM	Asp229, Trp139, Ser143, Arg376, Phe142, Arg374, Val145, Asn375	6	-4.37	624.03µM	Glu346,Arg109,Lys342,Glu553 ,Trp545,Asp362, Lys360	-
5.	Arjunolic acid	-9.25	165.34nM	Arg374, Asn375, Asn537, Val228, His226, Val145, Phe142	1	-6.92	8.5µM	Arg61, Asn560	-
6.	Celecoxib	-7.9	1.61 µM	Asn375, Asn37, Asn375, Trp139, Ser143, Arg374, Gly225	1	-5.72	64.32 µM	Lys342,Lys360,Lys557, Glu553, Glu553	1

Table 4: Binding energies (kcal/mol) and dissociation constants (Kd) of Myricetin, Myricanone, 3-epi-ursonic acid, Myricitrin, Arjunolic acid, and celecoxib towards TNF- α and IL-10 using AutoDock 4.2.6

S.N.	Ligands	Tumor Necrosis Factor (TNF)- α (PDB ID: 2AZ5)				Interleukin (IL)-10 (PDB ID: 2H24)			
		B.E. (kcal/mol)	Diss. Constant (Kd)	Interacting amino acid	H-atom	B.E. (kcal/mol)	Diss. Constant (Kd)	Interacting amino acid	H-atom
1.	Myricetin	-7.3	4.42 µM	Lys11,Lys11	2	-5.78	57.5 µM	Arg110,Phe111, Phe56	1
2.	Myricanone	-7.78	2.0 µM	Gly121	2	-7.09	6.32 µM	Phe56, Phe111	2
3.	3-epi-ursonic acid	-6.97	7.84 µM	Leu120,Ser60	-	-4.51	491.31µM	Arg102,Arg102, Arg106,Gln70	1
4.	Myricitrin	-5.55	85.03 µM	Ser60,Gln61, Leu120	-	-5.09	184.36 µM	Glu74,Arg102, Glu115,Gln63	-
5.	Arjunolic acid	-7.34	4.2µM	Leu120, Leu57	-	-6.76	11.11µM	Gly61, Gly58, Cys62	-
6.	Celecoxib	-6.52	16.55 µM	Tyr59,Tyr59,Tyr151, Tyr151,Tyr151, Gln61	-	-5.44	102.12µM	Glu115,Asn116, Arg102,Arg102, Gln70, Glu74	1

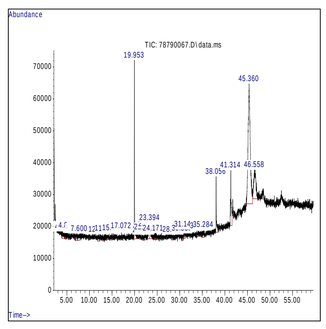

Figure 1: GC-MS analysis of *myrica esculenta* extract

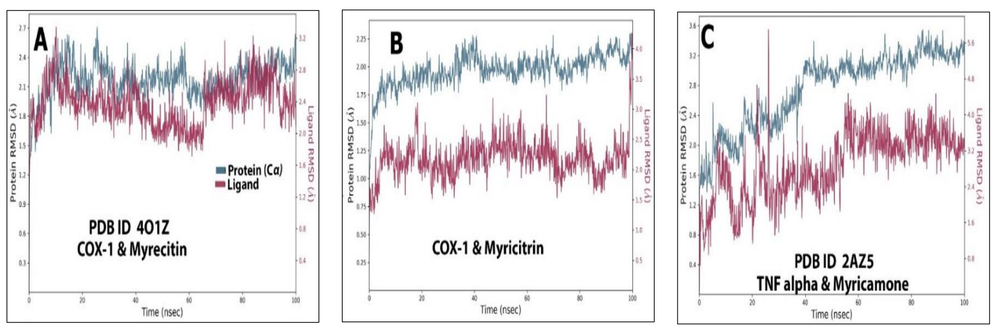

Figure 2: PDB ID-4O1Z COX-1 (A), COX-1 (B) and PDB ID 2AZ5 TNF alpha (C) with ligands Myrecitin, myricitrin and Myricanone *RMSD values plotted against simulation 100 ns*

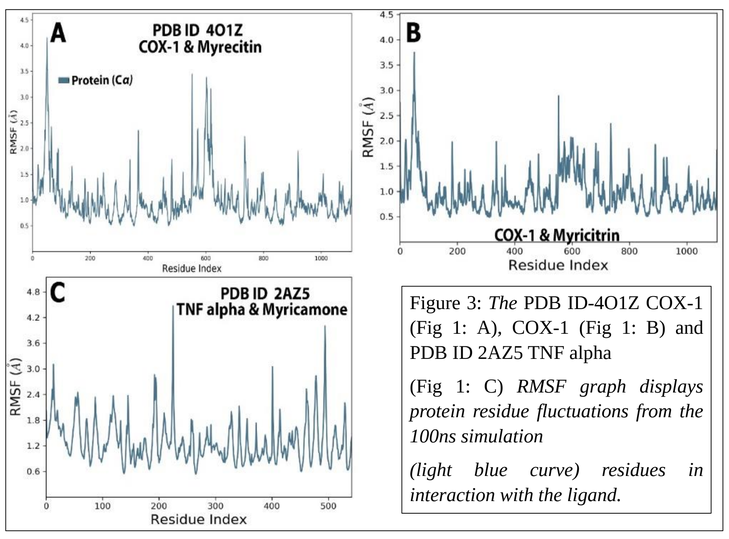

Figure 3: *The* PDB ID-4O1Z COX-1 (Fig 1: A), COX-1 (Fig 1: B) and PDB ID 2AZ5 TNF alpha

(Fig 1: C) *RMSF graph displays protein residue fluctuations from the 100ns simulation*

(light blue curve) residues in interaction with the ligand.

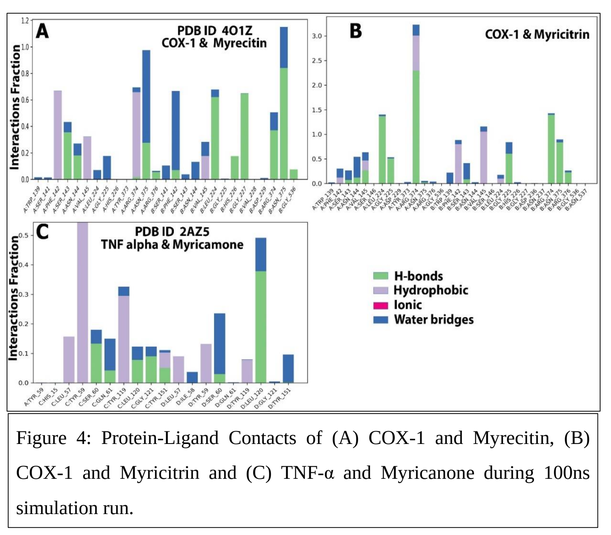

Figure 4: Protein-Ligand Contacts of (A) COX-1 and Myrecitin, (B) COX-1 and Myricitrin and (C) TNF-α and Myricanone during 100ns simulation run.

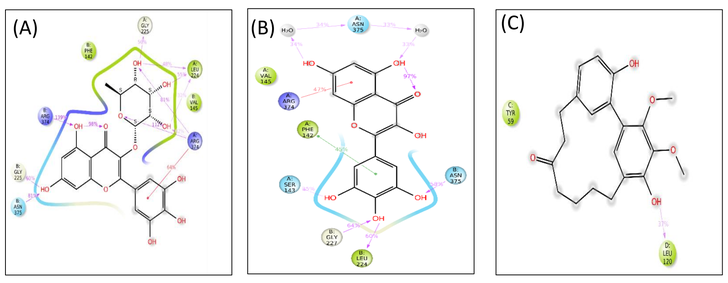

Figure 5: Ligand-Protein Contact Analysis of (A) Myricitrin with COX-1 protein(B) Myrecitin with COX-1 protein and (C) Myricanone with TNF alpha protein.

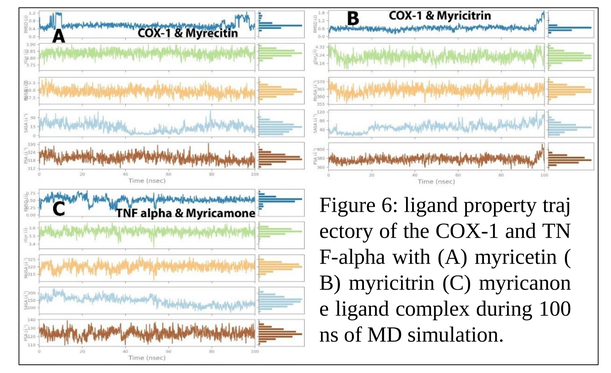

Figure 6: ligand property trajectory of the COX-1 and TNF-alpha with (A) myrecetin (B) myricitrin (C) myricanone ligand complex during 100 ns of MD simulation.

Conclusion: In the present study the GC-MS analysis showed good concentration of bioactive phytoconstituent present in bark extract of Myrica esculenta. Also, the In-Vitro and In-Silico study revealed that Myrica esculenta and their active phytoconstituents have good anti-inflammatory activity.

Reference:

https://doi.org/10.7717/peerj.6012
https://doi.org/10.1016/j.cell.2010.02.043