JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/49929
RESEARCH ARTICLE

Integrative Network Pharmacology and *In Vitro* Wound Healing Potential of Two Neglected Invasive Weed from Mizoram, India

Anima Debbarma[1,2], Probin Kr Roy[1], Sonjit Das[1], Lalhlenmawia Hauzel[1] and Lalhriatpuii Tochhawng[1*]

[1]Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Zemabawk - 796017, Mizoram, India; tclalhriatpuii@ripans.ac.in

[2]School of Medical and Paramedical Sciences, Mizoram University, Tanhril - 796004, Mizoram, India

Abstract

Background: Wound healing is undoubtedly a challenging clinical problem since regenerating healthy and functional skin is difficult, and modern allopathy-based formulations have their limitations in treating wounds. **Aim:** The current objective of the study aims to explore the network pharmacology and *in vitro* wound healing potential of two weeds of Mizoram, India, namely, *Chromolaena odorata* (L.) R.M. King and H. Rob. (CO) and *Mikania micrantha* Kunth (MM). **Methods:** Plant leaves were extracted successively, and methanolic extracts of both plants were used to carry out the experiment. Quantitative phytochemical screening, like Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), was performed. A network pharmacology approach was performed using various computational tools. *In-vitro* albumin denaturation inhibition, proteinase inhibition, and heat-induced haemolysis were performed to assess the *in-vitro* anti-inflammatory activity, followed by an angiogenesis study using the Chorioallantois Membrane (CAM) assay. **Results:** Phenolic and flavonoid content was found in both extracts, and network pharmacology revealed the involvement of both plants in wound healing through signalling pathways like EGFR and HIF1 signalling pathways. These pathways help in the reduction of inflammation and angiogenesis, and both *in vitro* anti-inflammatory and angiogenesis studies support the computational claims. **Conclusion:** Our study concludes that CO and MM may have the potential benefits for faster wound healing and may be further evaluated for *in vivo* efficacy in animals.

Major Findings: TPC and TFC were present in both plant extracts, and the network pharmacology revealed interaction between the phytoconstituents with various inflammatory genes. This evidence shows the faster wound healing potential of CO and MM.

Keywords: Angiogenesis, Anti-inflammatory, Medicinal Plants, Network Pharmacology, Wound Healing

1. Introduction

A wound is an injury, most commonly a break in the skin or body tissue, such as a cut, puncture, or scratch *etc*. Therefore, skin damage or loss of larger areas of the skin due to any kind of physical trauma or illness may be life-threatening[1,2]. The wound can range from a simple epithelial integrity break to subcutaneous tissue damage along with other structural damages such as tendons, muscles, vessels, nerves, parenchymal organs, and even bone[3,4]. Wound healing is distinctive in nature as it includes several processes like multiple cell populations, the extracellular matrix, and the action of soluble mediators such as growth factors and cytokines[5]. Healing of wounds involves a physiological response to the injury by replacing the damaged tissue with connective tissue, which then forms a scar to restore tissue integrity. The mechanisms of the wound healing process are coagulation, haemostasis, inflammation, proliferation, and wound remodelling

*Author for correspondence

Article Received on: 23.05.2025 Revised on: 31.07.2025 Accepted on: 14.08.2025

with scar tissue formation[6]. Wound healing continues to be a challenging clinical problem worldwide, as the regeneration of healthy and functional skin tissue is very difficult due to its multilayer structure and the presence of different cell types within the extracellular matrix in an organized way[7]. Modern medicine fails to achieve most of the wound healing due to delayed wound healing, as there are increased chances of infection, hypersensitivity, and local erythema[8]. Due to these limitations of modern synthetic allopathy-based medicines and the high cost of the treatments, scientists and wound care professionals must consider alternative approaches and validate their use using modern technology for wound treatment[9].

Herbal-based formulations have gained attention in the modern era, especially in wound healing therapy, due to multiple beneficial effects, including the ability to target multiple molecular pathways, low microbial resistance, lower cost, and sustainability *etc*[10-12]. Plants are a diverse source of phytoconstituents responsible for curing infections and promoting wound healing. Plants rich with polyphenols and flavonoids help in the wound healing process *via* their antimicrobial and antioxidant properties. Therefore, people from rural areas are still using medicinal plants to cure or treat wounds[13].

The north eastern state of India is rich in flora and fauna, and Mizoram is one of the hotspots for herbal drugs. Traditional healer of Mizoram practices different herbs for wound healing. *C. odorata* (L.) R.M. King and H. Rob. (CO) and *M. micrantha* Kunth (MM), belonging to the *Asteraceae* family, these 2 plants are extensively used by the traditional healer of Mizoram, to treat or cure fresh cuts and infections[14]. However, scientific evidence is yet to be established for the wound-healing potency of the plants.

Therefore, the aim of this research is to establish the scientific basis of the wound healing potency of *C. odorata* (L.) R.M. King and H. Rob. (CO) and *M. micrantha* Kunth (MM) *via* network pharmacology to understand the interaction between the phytoconstituents present in the plant and the human body. Network pharmacology is a computational tool that helps in finding lead molecules and disease targets rather than conducting experiments blindly[15]. Furthermore, TPC, TFC, *in vitro* anti-inflammatory and angiogenesis studies were performed to support computational claims on the wound healing potential of the plants.

2. Materials and Methods

2.1 Drugs and Chemicals

Gallic acid monohydrate (CAS number: 5995-86-8), quercetin (≥95% HPLC, CAS number: 117-39-5), diclofenac sodium salt (CAS number:15307-79-6), bovine Serum albumin (CAS number: 9048-46-8), and trypsin (CAS number: 9002-07-7) were procured from Sigma-Aldrich Pvt. Ltd. Petroleum ether (60-80° C, CAS number:8032-32-4) extra pure; chloroform (CAS number: 67-66-3) and methanol (CAS number: 67-56-1) were analytical grade and obtained from Merck Pvt. Ltd. All other chemicals, reagents, and solvents used were of analytical grade.

2.2 Collection, Authentication and Extraction of the Plants

CO and MM leaves were collected in the months of March-June 2022, from Aizawl, Mizoram, India. Plants were authenticated from the Botanical Survey of India (BSI), Shillong, with reference No: BSI/ERC/Tech/2022-23-163. CO and MM plants leaves were shade-dried and coarsely powdered. Successive extraction methods were carried out using a Soxhlet apparatus. At first, the powdered leaves were extracted according to the polarity of solvents, starting with petroleum ether, then chloroform, and finally with methanol. Different solvent extracts were filtered, concentrated, and stored separately at 4° C for further evaluation. Since the target study was to extract the phenolic and flavonoid compounds, hence methanolic extract was selected further for the study.

2.3 Determination of Total Phenol Content (TPC)

TPC of both methanolic plant extracts was determined using a modified Folin-Ciocalteu colorimetric method. Different concentrations of standard Gallic Acid (GA) (10-50 µg/mL) in methanol were prepared. To each concentration of 1 mL of GA, 5 mL of 10% Folin Ciocalteu reagent, along with 4 mL of 7% sodium carbonate were mixed, and the mixture was incubated for 30 minutes at 40° C in a water bath. The same procedure was repeated for the plant extract concentration (1 mg/mL), and the

absorbance was recorded at 760 nm using a UV-visible spectrophotometer (Model: FM12038A2HSL, Motras Scientific) against a blank. The total quantity of phenol in the samples was determined from the calibration curve of GA and was expressed as mg GA equivalent phenol per g of extract (mg/GAE)[16].

2.4 Determination of Total Flavonoid Content (TFC)

TFC was determined spectrophotometrically using a modified aluminum chloride colorimetric method with quercetin as standard. 0.5 mL of extracts (1 mg/mL) were mixed with distilled water (1.5 mL) and 5% sodium nitrite (0.2 mL), and the resultant solutions were incubated for 2 min at room temperature (27±2° C). Subsequently, 10% aluminum chloride (0.2 mL) and sodium hydroxide 1N (0.6 mL) were added and mixed properly in every step. The samples were allowed to stand in the dark at 27± 2° C for 10 min, and the absorbance was noted at 510 nm using a UV-Visible spectrophotometer (Model: FM12038A2HSL, Motras Scientific). The total quantity of flavonoid in the samples was determined from the plotted calibration curve of quercetin (ranging from 10 to 50 µg/mL) and was expressed as mg Quercetin Equivalent (QE) flavonoid per g of extract[17].

2.5 Network Pharmacology

2.5.1 Data Collection and Target Prediction of Phytoconstituents

The phyto-constituents of both the plant leaf were collected from KNApSAcK family databases and IMPPAT databases[18,19]. KNApSAcK family databases are plant species metabolites databases for medicinal or edible plants[20]. IMPPAT databases are manually curated databases of Indian medicinal plants, their phytochemicals, and therapeutic uses[21]. The selection of the phytoconstituents was done depending on the presence of phytoconstituents in the leaf sections. And all the phytoconstituents were then screened for their drug-likeness using the Swiss ADME database, and if the Lipinski, Veber, Ghose, Muegge, and Egan had two or more than two "Yes", the phytoconstituents were selected for further study. The gene targets for the obtained phytoconstituents were predicted using Swiss Target Prediction by selecting *Homo sapiens* species and pasting SMILES of each phytoconstituent, and repeated targets were deleted[22-24].

2.5.2 Wound-related Target Genes

Genes associated with the wound healing process were collected from the Gene Cards database and the OMIM database using the keyword "wound healing"[25,26]. After screening all wound-related target data sets were combined and duplicates were deleted. Then Venn diagrams were obtained *via* using Venny2.1.0 to intersect potential targets of these compounds in the wound healing process[27,28].

2.5.3 Protein-protein Interaction (PPI) Network Construction and Core Target Screening

The string database version 12.0 was utilized for the PPI network construction study by selecting multiple proteins, limiting the study to Homo sapiens, and setting the minimum required interaction score to medium confidence at 0.400[29]. And the analysis was done to find out the number of nodes and edges involved in the PPI construction. The String file exported from STRING was imported into Cytoscape v3.10.2 software for visualization. The nodes were calculated, and the Top 10 nodes were ranked by degree. At last, the network topology analysis diagram was obtained from the software.

2.5.4 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analyses

For a better understanding of the mechanism and potential pathways involved in wound healing by both the plants phytoconstituents, Go and KEGG enrichment analysis was performed using online ShinyGO 0.80[30]. Three GO analyses, such as Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), as well as KEGG enrichment analysis, were performed by selecting species to humans and taking the top 10 genes obtained from Cytoscape[31].

2.6 Angiogenesis Activity

Angiogenesis activity of both the plant leaf extracts was determined using the CAM assay with slight modification. 21 numbers of freshly fertilized (3days old) chicken eggs were collected from a local hatchery, and the egg surfaces were disinfected with 70% ethanol, followed by drying. The eggs were incubated for 4 days at 37° C with 60-70% humidity in an incubator. Two groups were created: A control group (treated

with phosphate buffer saline) and a treatment group receiving various concentrations (such as 25, 50, and 75 µg/mL) of both the plant extracts individually. In a sterile environment, a small window (2 cm) as well as a tiny hole was carefully created in each egg to access the developing embryo without contamination. The window was then covered with parafilm. From the tiny hole at the end of the egg, 5-10 mL of albumen was removed and replaced with either Phosphate-Buffered Saline (PBS) for the control group or PBS containing the plant extracts for the treatment groups. All eggs were returned to the incubator under the same conditions for further development. On the 6th day of incubation, the eggs were observed visually and photographed to assess blood vessel development[32].

2.7 *In Vitro* Anti-inflammatory Studies

2.7.1 Albumin Denaturation Inhibition

Bovine Serum Albumin (BSA) was used as a source of protein in this experiment, and the assay mixture (0.5 mL) consisted of BSA aqueous solution (0.45 mL, 5% w/v) with 0.05 mL of each extract solution (such as CO and MM) or diclofenac sodium standard drug solution of varying concentrations ranging from 62.5µg/mL -500 µg/mL. To adjust the pH to 6.3 of the above mixture, 1 N Hydrochloric Acid (HCl) was utilised. The above solutions of CO, MM, and standard drug were then incubated at 37° C for 20 min, followed by heating at 57° C for 30 min and then cooled to room temperature. PBS (pH 6.4) of 2.5 mL was added to the assay mixture and mixed. The turbidity of samples was measured with a UV-vis spectrophotometer at a wavelength of 660 nm. For the control sample, distilled water (0.05 mL) was used instead of the extract solution or standard drug solution[33].

2.7.2 Anti-proteinase Assay

A reaction mixture of 2 mL was prepared using 1 mL of trypsin solution (freshly made in 20 mM Tris-HCl buffer pH 7.4), and adding 1 mL of each extract solution (such as CO and MM) or diclofenac sodium samples of varying concentrations (62.5-500 µg/mL). Then the reaction mixture was incubated for 5 mins at 37° C, and again further incubated at the same temperature for an extra 20 minutes after the addition of 1 mL of 0.8% (w/v) casein to the reaction mixture. At the end of incubation, 2 mL of 70% perchloric acid was added to terminate the reaction. The reaction mixture was centrifuged, and the supernatant was collected, and the absorbance at 210 nm was measured. A buffer solution was used as the control.

2.7.3 Heat-induced Haemolysis

1 mL of 10% (v/v) red blood cell suspension was mixed with 1 mL of plant extract (62.5-500 µg/mL). The above mixture was heated for 30 minutes at a temperature of 56° C in a water bath and then cooled down at room temperature using running water. The absorbance of the supernatant was taken spectrophotometrically at 560 nm after centrifugation at 2500 rpm for 5 minutes. Diclofenac sodium was used as a standard[34].

2.8 Statistical Analysis

All the results are given in mean ± SD. Comparisons were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's method for comparisons. Statistically, the obtained data were considered significant if $P \leq 0.05$. All statistical analyses and graphs were plotted using GraphPad Prism software, version 5.00 (GraphPad Software, Inc. La Jolla, CA, USA).

3. Results and Discussion

3.1 TPC and TFC

TPC and TFC of the CO and MM were determined using GA and quercetin as standards (Figure 1). TPC and TFC of methanolic extracts of CO and MM are reported to be 83.95 ± 0.002 and 94.86 ± 0.025 mg GAE/g and 33.97 ± 0.033 and 47.94 ± 0.293 mg QE/g of crude extracts, respectively.

Both plant extracts showed the presence of secondary plant metabolites like flavonoids and phenols. Studies have revealed the role of flavonoids and phenolic compounds in wound healing[35]. Flavonoids are a great source of antioxidants and have various pharmacological activities, including anti-inflammatory and antimicrobial activities. Flavonoids are reported to inhibit bacterial growth by lysis and damaging cell walls[36,37]. Phenolic compounds are well-documented to be effective in healing. Kaltalioglu *et al.*, reported that the phenolic compounds reduce wound infection and acceleratethe healing process[38].

3.2 Network Pharmacology

From online KNApSAcK family databases and IMPPAT databases, the CO leaf contains 70 compounds, whereas MM contains 8 phytoconstituents, and all the phytoconstituents meet the criteria for drug-likeness. 570 targets were obtained from Swiss target prediction for the CO leaf phytoconstituents after combining and deleting duplicates, whereas, for MM phytoconstituents, 367 targets were obtained. For wounds, 6846 targets were obtained from the Gene cards and OMIM databases. At the end, there were 373 and 248 intersected targets between CO and wound, and MM and wound, respectively (Figure 2, A and B). From the Venn diagram, 373 and 248 potential targets were identified for CO and MM, respectively, and a potential PPI network was constructed for both the plant compounds (Figure 2, C and D).

The network consists of 373 and 248 nodes, and 6571 and 3656 edges for CO and MM, respectively. Proteins with larger nodes and intense colour are depicted as the most important proteins involved in wounds. At the end using Cytoscape, network topology analysis was performed and top 10 core network nodes were obtained (Figure 2, E and F). Pathway enrichment analyses for GO and KEGG were performed to determine the pathways involved in wound healing process when both the plant extracts were used individually and various plots for both the analyses were obtained[39]. Top

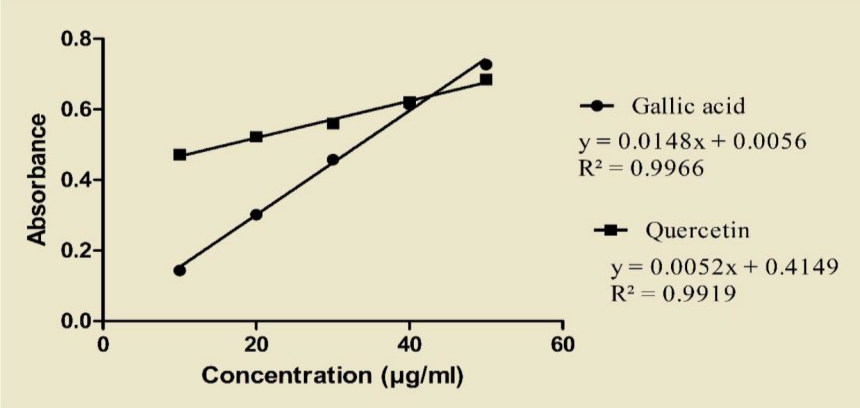


Figure 1. Graphical plot representing the standard curve of GA and quercetin for TPC and TFC.

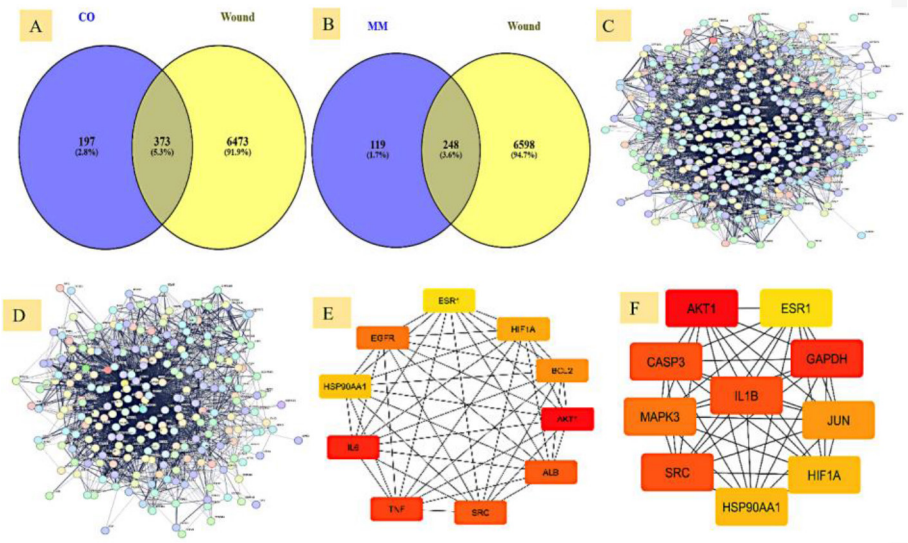


Figure 2. Representing network pharmacology **(A)** and **(B)** Intersected target between CO and wound, MM and wound respectively **(C)** and **(D)** STRING analysis indicating the PPI network CO and MM in wound treatment **(E)** and **(F)** Cytoscape analysis of the 10 key target networks involved in the effects of CO and MM in wound treatment.

10 Go terms were selected on the basis of significance level $p<0.05$, and the plots were obtained (Figure 3, A and H). The Go enrichment results suggested that CO and MM exert wound healing by regulating various biological processes, cellular components, and molecular functions like gland development, positive regulation of peptidyl-serine phosphorylation, gland morphogenesis, membrane region, estrogen receptor binding, phosphatase binding, regulation of nitric-oxide synthase activity, and cellular response to oxidative stress and chemical stress. The KEGG analyses revealed that the CO and MM help in the wound healing process *via* involvement of various pathways like the estrogen signalling pathway, the EGFR tyrosine kinase inhibitor resistance pathway, and the HIF-1 signalling pathway.

Due to the complex nature of medicinal plants and difficulties in the identification of proper targets and mechanisms of action, scientists have opted for network pharmacology as it helps in analysing the relation between the drug and the target (*i.e.*, gene) based on multi-compound and multi-target theory[40]. While choosing a drug for treating wounds, the 1st criterion of the compound is whether the compound can help in the proliferation of fibroblasts, keratinocytes cells, deposition in collagen, or have inhibitory actions on inflammation and oxidative stress[41]. The results obtained from the network pharmacology construction of both CO and MM revealed interaction between the phytoconstituents with various inflammatory genes like IL-1β and TNF-α, which are involved in the expression of inflammation, and their overexpression often leads to delayed wound healing[42,43]. However, anti-inflammatory genes like IL-6 expression help in faster wound healing and angiogenesis[44]. The KEGG enrichment analysis revealed the involvement of the HIF-1 signalling pathway in the angiogenesis stage of wound healing. Activation of HIF-1, results in enhanced delivery of oxygen and nutrients to the wound site, and results in upregulation of other genes such as VEGF and TGF-β, responsible for faster healing[45].

3.3 Angiogenesis Activity

Through the CAM assay, we have recorded the vessel's development by exposing it to plant extracts. In control groups, normal blood vessel development was observed with minimal secondary or tertiary vessels as compared to groups treated with CO and MM extracts after 48 hours, which is evidence of normal vessel growth. Whereas, A dose-dependent development of secondary and tertiary vessels from primary vessels was observed as compared to the control group after 48 hours of treatment with CO and MM extracts (Figure 4, A-G), which is evidence of the presence of an angiogenic agent.

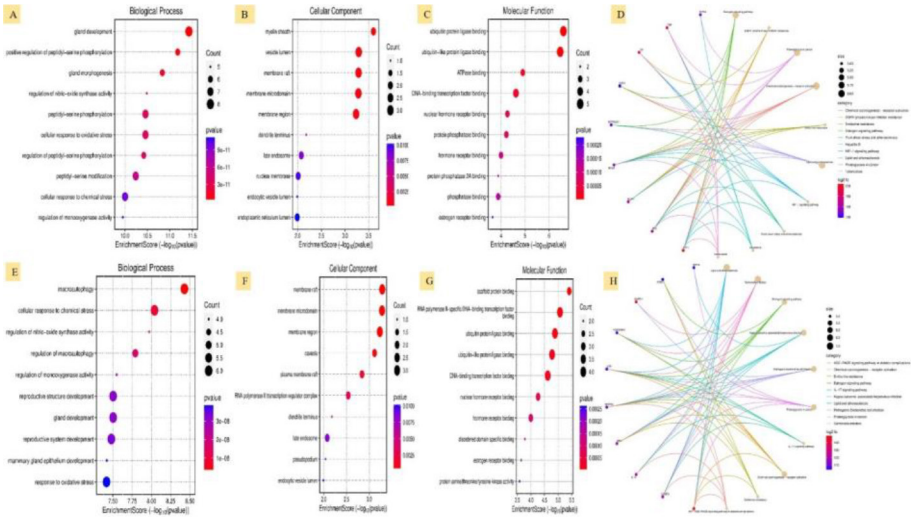


Figure 3. Enrichment analysis of CO and MM in the treatment of wounds. The bubble diagrams show the terms in the (A and E) biological process **(B)** and **(F)** cellular component and **(C)** and **(G)** Molecular function categories in the GO analysis of 373 and 248 overlapping genes for the treatment of wounds with CO and MM respectively **(D)** and **(H)** The relations between the top 10 KEGG pathways and the key targets of CO and MM respectively.

Angiogenesis is a process of generating new blood vessels. This process helps in wound healing by supplying nutrients and oxygen to the wound tissue microenvironment[46,47].

3.4 *In Vitro* Anti-inflammatory Studies

Dose-dependent anti-inflammatory activities were observed in albumin denaturation, anti-proteinase assay, and heat-induced haemolysis inhibition assay (Figure 5, A-D).

IC_{50} values were determined using GraphPad Prism software, version 5.00 (GraphPad Software, Inc., La Jolla, CA, USA), and the values are given in tabulated form (Tables 1-3) for albumin denaturation, anti-proteinase assay, and heat-induced haemolysis inhibition assay, respectively. From the observation, it has been noticed that higher extract concentration gives a higher percentage of inhibition, which is comparable to the standard drug. Albumin denaturation, proteinase, and heat-induced haemolysis are the major causes of inflammation, which results in tissue damage at the site of injury. The inflammatory phase is a crucial phase during the healing process; however, prolonged inflammation may result in scarring. Therefore, plants with anti-inflammatory agents help in balancing this phase[48,49].

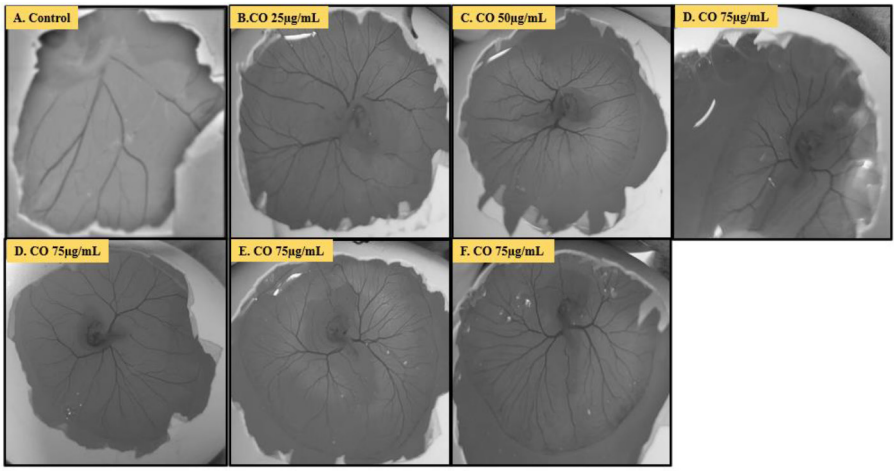


Figure 4. Photographic images of CAM assay of a control group and treatment group of CO and MM extracts at different doses (25, 50, and 75 µg/mL).

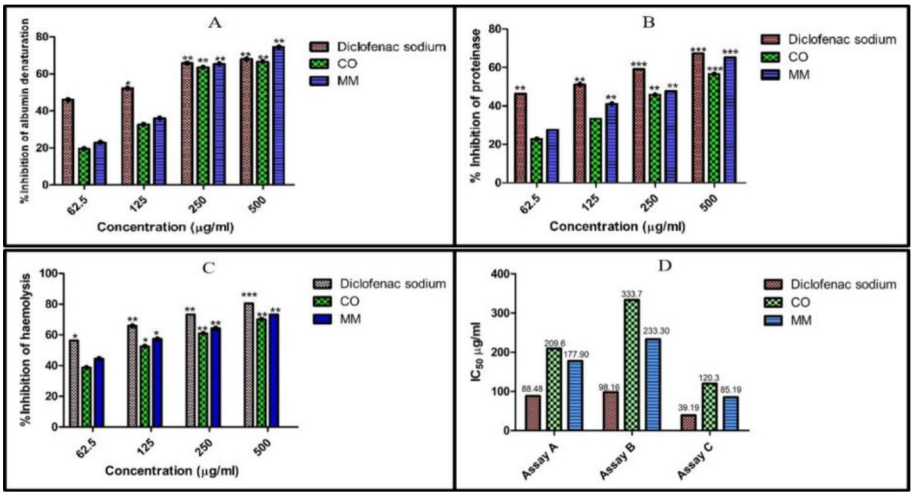


Figure 5. Graphical representation of *in vitro* inflammatory inhibition **(A)** Inhibition of albumin denaturation **(B)** Proteinase inhibition **(C)** Haemolysis inhibition **(D)** IC_{50} of all three assay studies.

Table 1. Effect of diclofenac sodium, CO, and MM leaf extract on inhibition of albumin denaturation

Treatments	Concentration (µg/ml)	Absorbance	*P*-value	% Inhibition	IC_{50} (µg/ml)
Control	-	0.703 ± 0.252		-	-
Standard	62.5	0.380±0.066	>0.05	46.003	88.48
	125	0.336±0.068*	<0.05	52.243	
	250	0.241±0.054**	<0.01	65.771	
	500	0.225±0.066**	<0.01	68.065	
CO	62.5	0.566±0.214	>0.05	19.477	209.6
	125	0.473±0.041	>0.05	32.707	
	250	0.256±0.138**	<0.01	63.537	
	500	0.235±0.095**	<0.01	66.529	
MM	62.5	0.542±0.223	>0.05	22.930	177.9
	125	0.450±0.158	>0.05	35.946	
	250	0.244±0.156**	<0.01	65.334	
	500	0.179±0.052**	<0.01	74.552	

Values represented in the results are mean ± SD of 3 replicates; experimental group was compared with control; ***: significant at $p \leq 0.001$, **: significant at $p \leq 0.01$ and *: significant at $p \leq 0.05$.

Table 2. Effect of diclofenac sodium, CO, and MM leaf extract on proteinase inhibition action

Treatments	Concentration (µg/ml)	Absorbance	*P*-value	% Inhibition	IC_{50} (µg/ml)
Control	-	0.234±0.06820		-	-
Standard	62.5	0.126±0.026**	<0.01	46.368	98.16
	125	0.114±0.040**	<0.01	51.168	
	250	0.096±0.007***	<0.001	59.145	
	500	0.076±0.005***	<0.001	67.422	
CO	62.5	0.181±0.035	>0.05	22.707	333.7
	125	0.156±0.007	>0.05	33.276	
	250	0.127±0.055**	<0.01	45.741	
	500	0.102±0.013***	<0.001	56.538	
MM	62.5	0.169±0.019	>0.05	27.578	233.3
	125	0.138±0.026**	<0.01	41.083	
	250	0.122±0.017**	<0.01	47.707	
	500	0.081±0.024***	<0.001	65.242	

Values represented in the results are mean ± SD of 3 replicates; experimental group was compared with control; ***: significant at $p \leq 0.001$, **: significant at $p \leq 0.01$ and *: significant at $p \leq 0.05$.

The *in-vitro* anti-inflammatory and angiogenesis study confirms and supports computational results obtained from network pharmacology. However, our study was limited to network pharmacology and *in-vitro* evaluation due to lack of computational tools and the unavailability of *in-vivo* models.

Table 3. Effect of diclofenac sodium, CO, and MM leaf extract on inhibition of heat-induced haemolysis

Treatments	Concentration (µg/ml)	Absorbance	*P*-value	% Inhibition	IC_{50} (µg/ml)
Control	-	0.364±0.211		-	-
Standard	62.5	0.159±0.028*	<0.05	56.281	39.19
	125	0.124±0.053**	<0.01	65.886	
	250	0.098±0.011**	<0.01	73.210	
	500	0.071±0.023***	<0.001	80.471	
CO	62.5	0.223±0.048	>0.05	38.675	120.3
	125	0.173±0.049*	<0.05	52.573	
	250	0.142±0.052**	<0.01	60.932	
	500	0.109±0.042**	<0.01	70.116	
MM	62.5	0.202±0.048	>0.05	44.544	85.19
	125	0.155±0.072*	<0.05	57.343	
	250	0.130±0.054**	<0.01	64.293	
	500	0.098±0.018**	<0.01	73.201	

Values represented in the results are mean ± SD of 3 replicates; experimental group was compared with control; ***: significant at $p \leq 0.001$, **: significant at $p \leq 0.01$ and *: significant at $p \leq 0.05$.

4. Conclusion

A significant amount of TPC and TFC in CO and MM extracts shows bioactivity of the plants, especially in wound healing, as TPC and TFC content have already been proven to be an effective helping hand in the healing process. An integrative network pharmacology study revealed CO and MM extracts potency in wound healing *via* various signalling pathways, like the estrogen signalling pathway, EGFR tyrosine kinase inhibitor resistance pathway, and HIF-1 signalling pathways to reduce inflammation and induce angiogenesis. Finally, an *in vitro* anti-inflammatory study also revealed the capability of the plant's extract to suppress protein denaturation during the study. Therefore, our study concludes that the extracts of CO and MM have the potential for faster wound healing, and further study may be warranted to unleash their action in an *in vivo* study model and also in the molecular expression of different wound healing markers.

5. Acknowledgements

We acknowledge Director Dr. Sanjay Dinkar Sawant, RIPANS, Aizawl, Mizoram, India, for giving us the necessary facilities to perform the study.

6. Author Contribution

Debbarma A, Lalhriatpuii TC, contributed in designing the study. Debbarma A, performed the study, analysed the report, and wrote the manuscript. Roy PK, reviewed and edited the draft manuscript. Das S, helped with the report analysis. Lalhlenmawia H, helped in reviewing the manuscript. All authors have read and approved the final version of the manuscript.

7. References

1. Chhabra S, Chhabra N, Kaur A, Gupta N. Wound healing concepts in clinical practice of OMFS. J Maxillofac Oral Surg. 2017; 16(4):403-423. PMID: 29038623 PMCID: PMC5628060. https://doi.org/10.1007/s12663-016-0880-z
2. Walker M. Human skin through the ages. Int J Pharm. 2022; 622:121850. PMID: 35623487. https://doi.org/10.1016/j.ijpharm.2022.121850
3. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. J Int Med Res. 2009; 37(5):1528-1542. PMID: 19930861. https://doi.org/10.1177/147323000903700531
4. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing. J Chin Med Assoc. 2018; 81(2):94-101. PMID: 29169897. https://doi.org/10.1016/j.jcma.2017.11.002
5. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies

for skin wound care and regeneration. Pharmaceutics. 2020; 12(8):735. PMID: 32764269. PMCID: PMC7463929. https://doi.org/10.3390/pharmaceutics12080735
6. Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. Open Biol. 2020;10(9):200223. PMID: 32993416. PMCID: PMC7536089. https://doi.org/10.1098/rsob.200223
7. Okur ME, Karantas ID, Senyigit Z, Ustundag Okur N, Siafaka PI. Recent trends on wound management: New therapeutic choices based on polymeric carriers. Asian J Pharm Sci. 2020; 15(6):661-684. https://doi.org/10.1016/j.ajps.2019.11.008 PMID: 33363624. PMCID: PMC7750807.
8. Yan R, Wang Y, Li W, Sun J. Promotion of chronic wound healing by plant-derived active ingredients and research progress and potential of plant polysaccharide hydrogels. Chin Herb Med. 2024; 17(1):70-83. PMID: 39949811. PMCID: PMC11814255. https://doi.org/10.1016/j.chmed.2024.11.005
9. Majumdar A, Sangole P. Alternative approaches to wound healing. Wound Healing - New Insights into Ancient Challenges. InTech. 2016. https://doi.org/10.5772/63636
10. Fahimi S, Mortazavi SA, Abdollahi M, Hajimehdipoor H. Formulation of a traditionally used polyherbal product for burn healing and HPTLC fingerprinting of its phenolic contents. Iran J Pharm Res. 2016; 15(1):95-105. PMID: 27610150. PMCID: PMC4986119.
11. Nagoba B, Davane M. Studies on wound healing potential of topical herbal formulations- do we need to strengthen study protocol?. J Ayurveda Integr Med. 2019; 10(4):316-318. PMID: 31685309. PMCID: PMC6938859. https://doi.org/10.1016/j.jaim.2019.09.002
12. Dubey S, Dixit AK. Preclinical evidence of polyherbal formulations on wound healing: A systematic review on research trends and perspectives. J Ayurveda Integr Med. 2023; 14(2):100688. PMID: 36841194. PMCID: PMC9988554. https://doi.org/10.1016/j.jaim.2023.100688
13. Vitale S, Colanero S, Placidi M, Di Emidio G, Tatone C, Amicarelli F, D'Alessandro AM. Phytochemistry and biological activity of medicinal plants in wound healing: An overview of current research. Molecules. 2022; 27(11):3566. PMID: 35684503. PMCID: PMC9182061. https://doi.org/10.3390/molecules27113566
14. Sawmliana M. The book of Mizoram plants (includes wild animals, birds, *etc*.). 2nd Edition. Springer. 2013.
15. Chandran U, Mehendale N, Patil S, Chaguturu R, Patwardhan B. Network pharmacology. Innovative Approaches in Drug Discovery. 2017: pp. 127-164. https://doi.org/10.1016/B978-0-12-801814-9.00005-2
16. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. Sci World J. 2020. PMID: 32256249. PMCID: PMC7102453. https://doi.org/10.1155/2020/8780704
17. Ojha S, Raj A, Roy A, Roy S. Extraction of total phenolics, flavonoids and tannins from *Paederia foetida* L. leaves and their relation with antioxidant activity. Phcog J. 2018; 10(3):541-547. https://www.phcogj.com/article/594
18. KNApSAcK family databases (http://www.knapsackfamily.com/knapsack_core/top.php, accessed on 12th September 2024)
19. IMPPAT databases (https://cb.imsc.res.in/imppat/, accessed on 12th September 2024).
20. Afendi FM, Okada T, Yamazaki M, Morita AH, Nakamura Y, Nakamura K, *et al*. KNApSAcK family databases: Integrated metabolite-plant species databases for multifaceted plant research. Plant Cell Physiol. 2012; 53(2):e1. PMID: 22123792. https://doi.org/10.1093/pcp/pcr165
21. Mohanraj K, Karthikeyan BS, Ananth RPV, Chand RB, Aparna SR, Mangalapandi P, Samal A. IMPPAT: A curated database of Indian medicinal plants, phytochemistry and therapeutics. Sci Rep. 2018; 8(1):4329. PMID: 29531263. PMCID: PMC5847565. https://doi.org/10.1038/s41598-018-22631-z
22. The Swiss ADME database (http://www.swissadme.ch/index.php, accessed on 12th September 2024).
23. The Swiss Target Prediction (http://www.swisstargetprediction.ch/ , accessed on 12th September 2024).
24. Qiu F, Fan S, Diao Y, Liu J, Li B, Li K, Zhang W. The mechanism of *Chebulae fructus* Immaturus promote diabetic wound healing based on network pharmacology and experimental verification. J Ethnopharmacol. 2024; 322:117579. PMID: 38104882. https://doi.org/10.1016/j.jep.2023.117579
25. Gene Cards database (https://www.genecards.org/, accessed on 12th September 2024).
26. OMIM database (https://www.omim.org/ , accessed on 20th April 2025).
27. Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/ , accessed on 20th April 2025).
28. El-dien RTM, Maher SA, Hisham M, Saber EA, Khedr AI, Fouad MA, *et al*. Network pharmacology, molecular docking study, and *in vivo* validation of the wound healing activity of the Red Sea soft coral *Paralemnalia thyrsoides* (Ehrenberg 1834) ethanolic extract and bioactive metabolites. Beni-Suef Univ J Basic Appl Sci. 2024; 13(1):54. https://doi.org/10.1186/s43088-024-00512-x
29. String database version 12.0 (https://cn.string-db.org/ , accessed on 13th September 2024).
30. ShinyGO 0.80 (http://bioinformatics.sdstate.edu/go, accessed on 13th September 2024).
31. Ding Y, Xie C, Feng S, Yuan Z, Wang W, Liu M, *et al*. Mechanism of Wenyang Shengji ointment in treating diabetic wounds based on network pharmacology and animal experiments. DCM. 2024; 7(1):79-89. https://doi.org/10.1016/j.dcmed.2024.04.009
32. Talekar YP, Apte KG, Paygude SV, Tondare PR, Parab PB. Studies on wound healing potential of polyherbal formulation using *in vitro* and *in vivo* assays. J Ayurveda

Integr Med. 2017; 8(2):73-81. PMID: 28601354. PMCID: PMC5497006. https://doi.org/10.1016/j.jaim.2016.11.007

33. Hasan AUH. Evaluation of *in vitro* and *in vivo* therapeutic efficacy of *Ribes alpestre* Decne in Rheumatoid arthritis. Braz J Pharm Sci. 2019; 55. https://doi.org/10.1590/s2175-97902019000217832
34. Agarwal H, Shanmugam VK. Anti-inflammatory activity screening of *Kalanchoe pinnata* methanol extract and its validation using a computational simulation approach. Inform Med Unlocked. 2019; 14:6-14. https://doi.org/10.1016/j.imu.2019.01.002
35. Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem. 2014; 2(5):69-73.
36. Royani A, Hanafi M, Lotulung PDN, Julistiono H, Dinoto A, Manaf A. Analysis of the antibacterial activity and the total phenolic and flavonoid contents of the *Moringa oleifera* leaf extract as an antimicrobial agent against *Pseudomonas aeruginosa*. Scientifica. 2023. PMID: 37811131. PMCID: PMC10560120. https://doi.org/10.1155/2023/5782063
37. Zulkefli N, Zahari CNC, Sayuti NH, *et al.* Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. Int J Mol Sci. 2023; 24(5):4607. PMID: 36902038. PMCID: PMC10003005. https://doi.org/10.3390/ijms24054607
38. Kaltalioglu K, Balabanli B, Cevher SC. Phenolic, antioxidant, antimicrobial, and *in-vivo* wound healing properties of *Potentilla erecta* L. root extract in diabetic rats. Iran J Pharm Res. 2020; 19(4):264-274. PMID: 33841541. PMCID: PMC8019876. https://doi.org/10.22037/ijpr.2019.112411.13742
39. Bioinformatics (https://www.bioinformatics.com.cn/en, accessed on 16th September 2024).
40. Kim TH, Yu GR, Kim H, *et al.* Network pharmacological analysis of a new herbal combination targeting hyperlipidemia and efficacy validation *in vitro*. Curr Issues Mol Biol. 2023; 45(2):1314-1332. PMID: 36826031. PMCID: PMC9955970. https://doi.org/10.3390/cimb45020086
41. Boakye YD, Agyare C, Ayande GP, *et al.* Assessment of wound-healing properties of medicinal plants: The case of *Phyllanthus muellerianus*. Front Pharmacol. 2018; 9:945. PMID: 30186175. PMCID: PMC6111538. https://doi.org/10.3389/fphar.2018.00945
42. Shen S, Miskolci V, Dewey CN, *et al.* Infection induced inflammation impairs wound healing through IL-1β signaling. iScience. 2024; 27(4):109532. PMID: 38577110. PMCID: PMC10993181. https://doi.org/10.1016/j.isci.2024.109532
43. Ashcroft GS, Jeong MJ, Ashworth JJ, *et al.* Tumor necrosis factor-alpha (TNF-α) is a therapeutic target for impaired cutaneous wound healing. Wound Repair Regen. 2012; 20(1):38-49. PMID: 22151742. PMCID: PMC3287056. https://doi.org/10.1111/j.1524-475X.2011.00748.x
44. Lin ZQ, Kondo T, Ishida Y, *et al.* Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. J Leukoc Biol. 2003; 73(6):713-721. PMID: 12773503. https://doi.org/10.1189/jlb.0802397
45. Hong WX, Hu MS, Esquivel M, *et al.* The role of hypoxia-inducible factor in wound healing. Adv Wound Care. 2014; 3(5):390-399. PMID: 24804159. PMCID: PMC4005494. https://doi.org/10.1089/wound.2013.0520
46. Hassan A, Elebeedy D, Matar ER, *et al.* Investigation of angiogenesis and wound healing potential mechanisms of zinc oxide nanorods. Front Pharmacol. 2021; 12:661217. PMID: 34721007. PMCID: PMC8552110. https://doi.org/10.3389/fphar.2021.661217
47. Shi Z, Yao C, Shui Y, *et al.* Research progress on the mechanism of angiogenesis in wound repair and regeneration. Front Physiol. 2023; 14:1284981. PMID: 38089479. PMCID: PMC10711283. https://doi.org/10.3389/fphys.2023.1284981
48. Ezzat SM, Ezzat MI, Okba MM, Menze ET, Naim ABA. The hidden mechanism beyond ginger (*Zingiber officinale* Rosc.) potent *in vivo* and *in vitro* anti-inflammatory activity. J Ethnopharmacol. 2018; 214:113-123. https://doi.org/10.1016/j.jep.2017.12.019 PMid:29253614
49. Shukla SK, Sharma AK, Gupta V, Yashavarddhan MH. Pharmacological control of inflammation in wound healing. J Tissue Viability. 2019; 28(4):218-222. https://doi.org/10.1016/j.jtv.2019.09.002 PMID: 31542301