RESEARCH PAPER

Development and in vitro Evaluation of an Arnica montana and Chamomilla recutita Herbal Gel with Antioxidant and Anti-inflammatory Properties

Sharuniveda Sankaranarayanan[1] and Umamaheswari T N[1*]

[1]Post graduate Resident, Department of Oral Medicine, Radiology and Special Care Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS)
[1] Professor and Head, Department of Oral Medicine, Radiology and Special Care Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences(SIMATS)*

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT

Background: The development of plant-derived herbal formulations has gained increasing interest in medicine owing to their potential to address disease-related oxidative stress and inflammation. Arnica montana and Chamomilla recutita are medicinal plants with established traditional use and recognized pharmacological relevance for improving health outcomes.

Objective: This study aimed to develop and characterize a herbal gel formulation containing A. montana and C. recutita extracts, and to evaluate its physicochemical properties and in vitro biological performance in relation to health applications.

Methods: A gel formulation was prepared using carboxymethyl cellulose and carbopol as gelling agents. The formulation was evaluated for physicochemical parameters, including pH, viscosity, spreadability, and in vitro release behaviour. Biological activity was assessed using antioxidant assays (DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging) and anti-inflammatory protein denaturation assays, with standard controls for comparison.

Results: The developed herbal gel demonstrated favorable physicochemical properties, including skin-compatible pH, suitable rheological behaviour, and sustained release characteristics. The formulation exhibited significant antioxidant and anti-inflammatory activities comparable to standard controls, indicating potential benefit in reducing disease-associated oxidative mechanisms.

Conclusion: The developed herbal gel demonstrated favourable in vitro antioxidant and anti-inflammatory potential along with suitable physicochemical stability for topical application in medicine. These findings support its potential as a plant-based formulation for improving health outcomes and warrant further in vivo and clinical investigations.

Keywords: Arnica montana, Matricaria chamomilla ,Herbal medicine , Alternative medicine , medicinal plants

How to cite this article: Sankaranarayanan S, Umamaheswari TN, Development and in vitro Evaluation of an Arnica montana and Chamomilla recutita Herbal Gel with Antioxidant and Anti-inflammatory Properties. Int J Drug Deliv Technol. 2026;16(52s): 541-547. DOI: 10.25258/ijddt.16.52s.69

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Plant-based therapies are widely used globally for their affordability and minimal side effects. Arnica montana (AM), belonging to family Asteraceae, is traditionally used to alleviate inflammation and pain in Europe and North America [1-3]. Asteraceae-based medications are widely used in German primary care with no significant adverse effects [3-5]. Arnica montana L., also known as mountain snuff, wolf's bane, leopard's bane [6-8].

Chamomilla recutita (Matricaria chamomilla L.) is an aromatic herb native to Western Asia and Europe, extensively used in herbal infusions and cosmetics [9–11]. Its bioactivity arises from flavonoids (apigenin, quercetin, luteolin), terpenoids, and essential oils [12–15]. Chamomile acts as a COX-2 inhibitor and exhibits strong anti-inflammatory and antioxidant actions [13,16].

Given the therapeutic potential of both plants, this study aimed to formulate and evaluate an *Arnica–Chamomile* gel for its antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

Preparation of flower extracts and herbal gel formulations

Dried flowers of *Arnica montana* and *Chamomilla recutita*, both recognized medicinal plants, were used for preparation of the herbal extracts. Ten grams of dried *A. montana* flowers were extracted with 250 mL distilled water at 50 °C for 15–20 min and filtered. Similarly, 0.5 g

**Author for Correspondence: Umamaheswari T N*

of dried *C. recutita* flowers were extracted with 50 mL distilled water under identical conditions. The filtrates were combined and concentrated to 5 mL at 50 °C.

For preparation of the herbal gel base, carboxymethyl cellulose (CMC) and carbopol (5% w/v each) were dispersed in distilled water and homogenized. The concentrated combined extract (5 mL) was incorporated into 50 g of gel base to obtain the *A. montana–C. recutita* herbal gel formulation.

Physicochemical properties of the herbal gel

The prepared herbal gel showed a pH of 5.5, indicating suitability for topical application. The viscosity of the formulation was recorded as 100 mg/mL in water.

The *A. montana–C. recutita* gel demonstrated higher spreadability (21 mm) than the control formulation (18 mm), suggesting ease of application and improved coverage over the affected area [Figure 1].

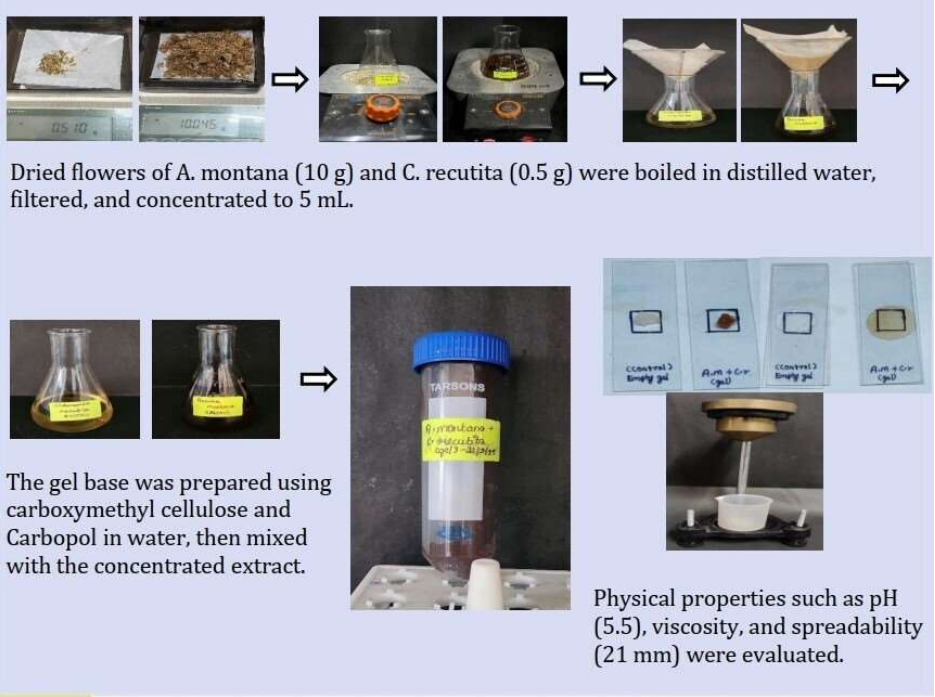

Figure 1: Preparation and physicochemical evaluation of the gel material

The gel demonstrated a time-dependent release profile, with lower extract concentrations (1–2%) showing higher cumulative release (~80–83% over 500 min) compared with higher concentrations. This may be attributed to increased viscosity and stronger polymer–extract interactions at higher concentrations, which can retard diffusion of the active constituents. These findings suggest that lower extract concentrations may provide more rapid release and improved availability of the herbal actives [Figure 2].

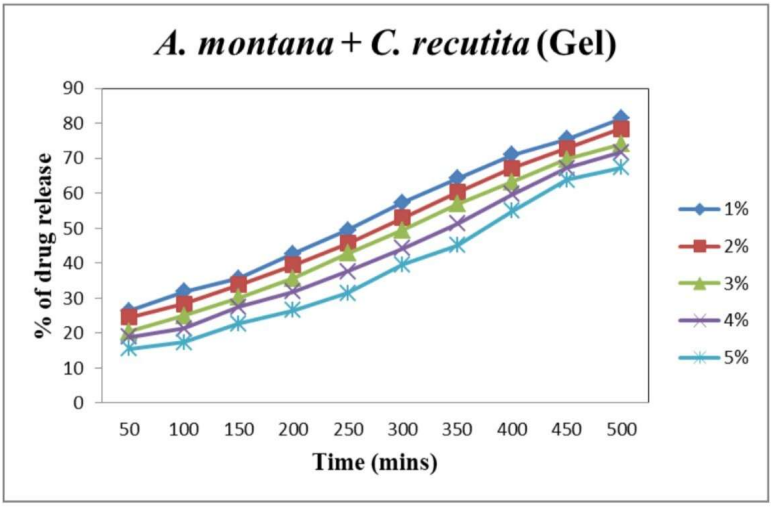

Figure 2: In vitro drug release profile of the gel material

Characterization of the herbal gel formulation

The developed herbal gel was evaluated to determine its physical quality, uniformity, and suitability for topical application. Rheological properties were assessed to determine viscosity and flow behaviour, which are important for ease of application and patient acceptability.

The formulation was visually examined for homogeneity, consistency, and uniform distribution of the incorporated herbal extracts. Stability studies were carried out under controlled conditions to assess changes in appearance, phase separation, and consistency during storage.

In addition, the gel was assessed for its ability to retain physical integrity during storage and application. These evaluations provide important information regarding the quality, reproducibility, stability, and suitability of the developed herbal gel formulation

Antioxidant activity

DPPH Radical Scavenging assay

A 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) stock solution in methanol was diluted to 20 µM for use. Different concentrations of Arnica–Chamomile gel (10–50 µg/mL) were mixed with 200 µL DPPH in a 96-well plate, incubated for 30 min in the dark, and read at 517 nm. Methanol served as blank and ascorbic acid (1 mg/mL) as positive control. The calculation of scavenging activity (%) was as follows:

% DPPH Scavenging Activity = [(A control – A sample) / A control] × 100

where A control is the absorbance of the DPPH without sample and A sample represents the absorbance of the test sample and ascorbic acid (1 mg/mL) served as positive control.

Hydrogen Peroxide (H_2O_2) Scavenging Assay

A 40 mM H_2O_2 solution was prepared in phosphate buffer (pH 7.4). Gel samples and ascorbic acid (standard) at 10–50 mg/mL were mixed with 0.6 mL H_2O_2 and incubated for 10 min at room temperature. Absorbance was recorded at 230 nm using spectrophotometer.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP reagent was prepared from acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in 40 mM HCl), and $FeCl_3 \cdot 6H_2O$ (20 mM) in a 10:1:1 ratio. The reaction mixture (3.6 mL reagent + 0.4 mL water + 80 µL sample) was incubated at 37°C for 10 min, and absorbance measured at 593 nm. Results were expressed as Fe^{2+} equivalents using $FeSO_4 \cdot 7H_2O$ (0.1–1.5 mM) as standard.

ABTS Assay

ABTS was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating in dark for 24 h at 4°C. The solution was diluted with 50% ethanol to an absorbance of 1.0 ± 0.02 at 734 nm. Test samples (20 µL) were mixed with 250 µL ABTS, incubated for 10 min in dark, and absorbance was read at 734 nm. Radical scavenging activity (%) was calculated as: I (%) = [(Abs0−Abs1)/Abs0] × 100, where Abs0 is the absorbance of the blank and Abs1 is the absorbance with the sample.

Nitric Oxide assay

Nitric oxide scavenging was assessed by the modified Griess Illosvoy method. Reaction mixture contained sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL), and varying concentrations of Arnica–Chamomile gel (10–160 µg) or rutin (standard). After incubation at 25°C for 150 min, 0.5 mL of the mixture was reacted with sulfanilic acid (0.33% in 20% acetic acid) and naphthylethylenediamine dihydrochloride (0.1% w/v). Absorbance was read at 540 nm after 30 min.

Anti-inflammatory activity

Bovine Serum Albumin (BSA) Denaturation Assay

Anti-inflammatory activity was evaluated by inhibition of heat-induced protein denaturation. Arnica–Chamomile gel (10–50 µg/mL, 0.05 mL) was mixed with 0.45 mL BSA, pH adjusted to 6.3, and incubated at 55°C for 30 min after standing 10 min at room temperature. DMSO served as control and diclofenac sodium as standard. Absorbance was recorded at 660 nm.

% inhibition= Absorbance of control- Absorbance of sample×100

Absorbance of control

Egg Albumin Denaturation Assay

Samples (10–50 µg/mL) were mixed with 2.8 mL phosphate buffer and 0.2 mL fresh egg albumin (pH 6.3), incubated for 10 min at room temperature and 30 min at 55°C. DMSO and diclofenac sodium were used as control and standard, respectively. Absorbance was measured at 660 nm, and % inhibition calculated as above.

Membrane Stabilization Assay

Human RBCs were used to assess membrane stabilization against hypotonicity-induced haemolysis. Cells suspended in PBS (pH 7.4) were incubated with Arnica–Chamomile gel (10–50 µg/mL) in 50 mM Tris-HCl buffer. Haemolysis was quantified spectrophotometrically, and results expressed as % inhibition of haemolysis relative to control.

RESULTS

The antioxidant activity of *A. montana* + *C. recutita* gel was evaluated using DPPH, H_2O_2, FRAP, ABTS, and nitric oxide radical scavenging assays [18] across concentrations of 10–50 µg/mL.

In the DPPH assay, gel showed inhibition of 58.23%, 71.52%, 78.46%, 82.11%, 87.85%, compared to the standard values of 66.25%, 78.52%, 85.63%, 88.68%, 93.15%, respectively. In H_2O_2 assay, gel exhibited scavenging activity of 45.8%, 51.6%, 62.1%, 73.7%, and 83.9%, while the standard showed 51.1%, 56.9%, 66.1%, 78.8%, and 89.9%.

For FRAP assay, antioxidant activity of gel was 65.47%, 70.64%, 74.11%, 78.31%, 84.68%, compared to the standard (72.98%, 76.84%, 81.31%, 85.84%, 90.89%). In the ABTS assay, the gel showed inhibition of 64.13%, 69.72%, 76.38%, 81.79%, 85.44%, while the standard demonstrated 70.56%, 75.68%, 82.43%, 86.57%, 91.39%.

Lastly, in the nitric oxide scavenging assay, gel exhibited activity of 64.15%, 71.39%, 75.48%, 80.65%, and 84.76%, compared to the standard values of 72.43%, 77.94%, 80.37%, 84.28%, and 88.67% [Figure 3]

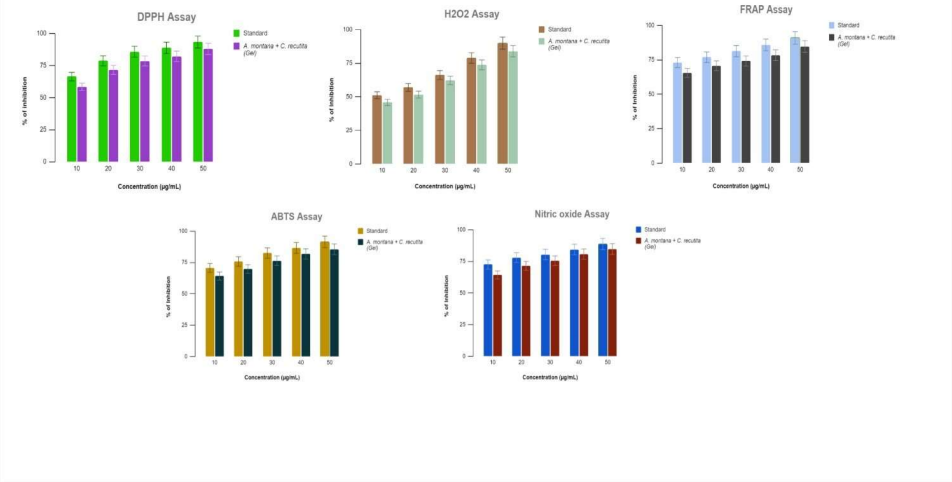

Figure 3: Antioxidant activity of the gel material

These results confirm that *A. montana* + *C. recutita* gel demonstrated strong antioxidant activity in every assay, matching the standard, underscoring its promise as a natural antioxidant formulation.

Using BSA, EA, MSA assays [19] denaturation assays at doses ranging from 10 to 50 µg/mL, the anti-inflammatory effectiveness of the A. montana + C. recutita gel was evaluated, and the outcomes were compared with those of standard drug.

In BSA assay, gel showed percentage inhibition values of 43%, 54%, 67%, 72%, 78%, while the standard achieved 47%, 60%, 72%, 78%, and 84%, respectively.

For EA assay, the gel demonstrated inhibition of 48%, 57%, 63%, 68%, 77%, compared to standard values of 55%, 64%, 69%, 72%, and 81%.

In MSA assay, inhibition by the gel was 52%, 63%, 72%, 78%, 82%, while standard recorded 58%, 70%, 77%, 82%, 89%, respectively [Figure 4]

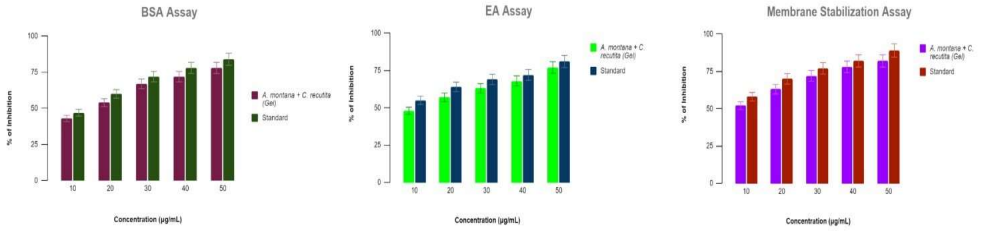

Figure 4: Anti-inflammatory activity of the gel material

The combined results of the three assays demonstrate that the *A. montana* + *C. recutita* gel has significant anti-inflammatory properties that vary with concentration. Furthermore, its effectiveness is comparable with that of the standard gel, indicating its promise as a natural therapeutic agent for the control of inflammation.

DISCUSSION

The present study reports the development and in vitro evaluation of a herbal gel formulation containing *Arnica*

montana and *Chamomilla recutita* flower extracts, with emphasis on its antioxidant and anti-inflammatory properties. The results from multiple validated assays demonstrate that the formulation possesses significant bioactivity, supporting its potential as a therapeutic topical preparation.

The formulation was evaluated for anti-inflammatory activity using **various in-vitro assays.** In all methods, the gel exhibited concentration-dependent activity, with DPPH radical scavenging values ranging from 58.23% to 87.85% (10–50 µg/mL). These values were comparable to those obtained with ascorbic acid (66.25%–93.15%), indicating strong free radical neutralizing capacity.

This activity may be attributed to the synergistic effects of bioactive compounds from both extracts. *A. montana* contains sesquiterpene lactones and flavonoids, while *C. recutita* is rich in apigenin, quercetin, and bisabolol—all known for their potent antioxidant properties [13,15]. The enhanced performance in FRAP and ABTS assays further confirms the gel's reducing ability and radical quenching efficiency, consistent with earlier studies on plant-based flavonoid and terpenoids formulations capable of stabilizing reactive oxygen species (ROS) [5,16].

The formulation was evaluated for anti-inflammatory activity using **various in-vitro assays.**At 50 µg/mL, the gel's BSA assay inhibition was 78%, which is very close to diclofenac sodium's activity of 84%. Comparable inhibitory patterns were noted in the membrane stabilization and EA assays, demonstrating the reliability of its anti-inflammatory activity in various assay systems.

The inhibition of protein denaturation, a key inflammatory marker, suggests that the formulation may modulate inflammatory pathways. Literature reports indicate that A. montana reduces the production of pro-inflammatory cytokines and inhibits NF-κB activation to regulate inflammation [17], whereas *C. recutita* attenuates COX-2 activity and prostaglandin synthesis, primarily through apigenin derivatives [13]. The observed effects in the current study may result from a combination of lysosomal membrane stabilization, inhibition of inflammatory enzyme activity, and free radical scavenging, thereby mitigating oxidative stress–mediated inflammation [20-22].

The physicochemical parameters of the gel further support its suitability for topical use. The pH (5.5) is compatible with skin physiology, minimizing irritation risk. The measured spreadability (21 mm) and viscosity ensures ease of application and uniform coverage over the target site. In vitro drug release studies indicated that gels containing 1–2% extract achieved ~83% release within 500 min, potentially due to reduced polymer–drug interactions and lower viscosity, which favour enhanced diffusion. These findings underscore importance of optimizing extract concentration within the gel matrix to balance stability, bioavailability, and therapeutic performance [23,24].

Limitations and Future scope of the study

The A. montana + C. recutita gel demonstrated potent anti-inflammatory and antioxidant properties in vitro, however these results are limited to laboratory settings. The absence of in vivo experiments restricts understanding of its pharmacokinetics, systemic absorption, metabolism, long-term safety. Additionally, variability in extract composition due to plant source, seasonal factors, or extraction methods—may affect reproducibility. Additionally, only short-term stability was assessed; long-term stability and shelf life remain unestablished.

Subsequent assessments should include in vivo evaluations to confirm efficacy, safety, tolerability in animal models, followed by randomized clinical trials to validate therapeutic potential in humans. Investigating molecular mechanisms underlying the observed activities could further clarify its mode of action. Optimization of formulation parameters, improved skin permeation, and inclusion of stability enhancers are recommended to enhance overall effectiveness. Furthermore, extending investigations to assess antimicrobial and wound-healing properties may broaden clinical applications.

CONCLUSION

The *A. montana* + *C. recutita* herbal gel demonstrated significant concentration-dependent antioxidant and anti-inflammatory activities in vitro, with responses comparable to standard reference controls. Favourable physicochemical characteristics, including skin-compatible pH, good spreadability, and sustained release behaviour, support its suitability as a topical herbal formulation. These findings indicate the potential therapeutic value of combining *Arnica montana* and *Chamomilla recutita* in a gel-based delivery system. Further in vivo studies and clinical investigations are required to confirm its efficacy, safety, and long-term stability. Future optimization of the formulation and evaluation of additional properties such as antimicrobial and wound-healing effects may further broaden its clinical relevance

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1) Kriplani P, Guarve K, Baghael US. Arnica montana L.–a plant of healing. J.Pharm. Pharmacol.2017;69:925-45.

2) Mohanta YK, Mishra AK, Nongbet A, Chakrabartty I, Mahanta S, Sarma B, Panda J, Panda SK. Potential use of the Asteraceae family as a cure for diabetes: A review of ethno pharmacology to modern day drug and nutraceuticals developments. Front. pharmacol. 2023; 14:1153600.

3) Ela MA, El-Lakany A, Hijazi MA. General introduction on family Asteracea. InPhytochemicals-a global perspective of their role in nutrition and health 2012. IntechOpen.

4) Jeschke E, Ostermann T, Lüke C, Tabali M, Kröz M, Bockelbrink A, Witt CM, Willich SN, Matthes H. Remedies containing Asteraceae extracts: a prospective observational study of prescribing patterns and adverse drug reactions in German primary care. Drug Saf. 2009; 32:691-706.

5) Lawrence WT. Plastic surgery educational foundation DATA committee. Arnica Plast Reconstr Surg. 2003; 112:1164-6.

6) Sebai H, Jabri MA, Souli A, Rtibi K, Selmi S, Tebourbi O, El-Benna J, Sakly M. Antidiarrheal and antioxidant activities of chamomile (Matricaria recutita L.) decoction extract in rats. J. Ethnopharmacol. 2014; 152:327-32

7) Abeysiri GR, Dharmadasa RM, Abeysinghe DC, Samarasinghe K. Screening of phytochemical, physico-chemical and bioactivity of different parts of Acmella oleraceae Murr. (Asteraceae), a natural remedy for toothache. Ind.Crop.Prod.2013 ;50:852-6.

8) Singh O, Khanam Z, Misra N, Srivastava MK. Chamomile (Matricaria chamomilla L.): an overview. Phcog Rev. 2011; 5:82.

9) El Mihyaoui A, Esteves da Silva JC, Charfi S, Candela Castillo ME, Lamarti A, Arnao MB. Chamomile (Matricaria chamomilla L.): a review of ethnomedicinal use, phytochemistry and pharmacological uses. Life. 2022; 12:479.

10) Lim TK. Edible medicinal and non-medicinal plants. Dordrecht, The Netherlands: Springer; 2012.

11) Guimarães R, Barros L, Dueñas M, Calhelha RC, Carvalho AM, Santos-Buelga C, Queiroz MJ, Ferreira IC. Infusion and decoction of wild German chamomile: Bioactivity and characterization of organic acids and phenolic compounds. Food Chem. 2013; 136:947-54.

12) Ross SM. Chamomile: a spoonful of medicine. Holist. Nurs. Pract. 2008; 22:56-7.

13) Srivastava JK, Pandey M, Gupta S. Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity. Life Sci. 2009; 85:663-9.

14) Shrikhande BK, Goupale DC. Development and evaluation of anti-inflammatory oleogels of Bosewellia serrata (gugul) and Curcuma longa (turmeric). Indian Drugs. 2001; 38:613-6.

15) McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.). Phytotherapy Research: PRENAP. 2006; 20:519-30.

16) Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. J. Funct.Foods. 2015; 18:820-97.

17) Knuesel O, Weber M, Suter A. Arnica montana gel in osteoarthritis of the knee: an open, multicenter clinical trial. Adv Ther. 2002; 19:209-18.

18) Dathan PC, Nallaswamy D, Rajeshkumar S, Joseph S, Ismail S, Rashid N. Evaluation of Anti-inflammatory, Antioxidant and Antimicrobial Activity of Pomegranate Peel Extract: An In-vitro Study. JCDR.2024 ;18(6).

19) Mahalingam M, Pillai DS, Shanmugam RK. Assessment of Anti-Inflammatory and Cytotoxic Property of Herbal Gel Containing Saffron, Withania Somnifera, Dry Ginger, and Tulsi Against Recurrent Aphthous Stomatitis: An in Vitro Study.JIAOMR. 2024; 36:278-82.

20) Oyedapo OO, Famurewa AJ. Antiprotease and membrane stabilizing activities of extracts of Fagara zanthoxyloides, Olax subscorpioides and Tetrapleura tetraptera. Int. J. Pharmacogn. 1995; 33:65-9.

21) Ameena M, Arumugham M, Ramalingam K, Rajeshkumar S, Shanmugam R. Evaluation of the anti-inflammatory, antimicrobial, antioxidant, and cytotoxic effects of chitosan thiocolchicoside-lauric acid nanogel. Cureus. 2023 ;15.

22) Balajee V, Kumar L, Kumar R, Shanmugam R. Antioxidant and anti-inflammatory properties of the two varieties of Musa acuminata: An in vitro study. Cureus. 2023 28;15.

23) Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharm. J. 2012; 20:63-7.

24) Ramya G, Varghese SS, Rajeshkumar S. Anti-inflammatory and antioxidant activity of Stevia and peppermint herbal formulation. J Pharm Res Int. 2021; 33:10.

Table 1: Represents summary of Antioxidant activity of Arnica Chamomile gel

Concentration (µg/mL)	DPPH* (%)		H_2O_2 † (%)		FRAP ‡ (%)		ABTS § (%)		NO ‖ (%)	
	GEL	STANDARD	GEL	STANDARD	GEL	STANDARD	GEL	STANDARD	GEL	STANDARD
10	58.23	66.25	45.8	51.1	65.47	72.98	64.13	70.56	64.15	72.43
20	71.52	78.52	51.6	56.9	70.64	76.84	69.72	75.68	71.39	77.43
30	78.46	85.63	62.1	66.1	74.11	81.31	76.38	82.43	75.48	80.37

40	82.11	88.68	73.7	78.8	78.31	85.84	81.79	86.57	80.65	84.28
50	87.85	93.15	83.9	89.9	84.68	90.89	85.44	91.39	84.76	88.67

Table 2: Represents summary of Anti-inflammatory activity of Arnica Chamomile gel

Concentration (µg/mL)	BSA ¶ (%)		EA** (%)		MSA†† (%)	
	GEL	STANDARD	GEL	STANDARD	GEL	STANDARD
10	43	47	48	55	52	58
20	54	60	57	64	63	70
30	67	72	63	69	72	77
40	72	78	68	72	78	82
50	78	84	77	81	82	89