Pharmacogn. Res., 2026; 18(3):1006-1017.
https://www.phcogres.com

Original Article

Phytochemical Evaluation and Anti-inflammatory Efficacy of Phenolic and Flavonoid Constituents in *Euphorbia microphylla* B. Heyne ex Roth.

Nitin Balasaheb Lonikar[1], Manisha Sureshkumar Sutare[2,*], Amar Ramrao Mane[3], Nandkishor Bhimashankar Bavage[3]

[1]Department of Pharmaceutical Chemistry, Latur College of Pharmacy, Hasegaon, Latur, Maharashtra, INDIA.
[2]Department of Botany, Shri Yashwantrao Patil Science College, Solankur, Kolhapur, Maharashtra, INDIA.
[3]Department of Pharmacognosy, Latur College of Pharmacy, Hasegaon, Latur, Maharashtra, INDIA.

ABSTRACT

Background: Medicinal plants are natural sources of biochemical compounds with negligible side effects; *Euphorbia microphylla* is known for producing pharmacologically active compounds used in traditional medicine. **Materials and Methods:** Proximate analysis for organoleptic and physicochemical parameters was conducted alongside quantitative estimation of total phenolic and flavonoid contents of ethanolic leaf extracts., Three *in vitro* anti-inflammatory assays-protein denaturation inhibition, protease inhibition, and membrane stabilisation-were performed. **Results:** The research confirms the availability of phenolics and flavonoids in ample amounts. Phytochemicals quantified in the extract highlighted strong efficacy in the conducted assays. **Conclusion:** The findings suggest therapeutic potential and interest in formulating standard drugs, though further validation through *in vivo* experiments is needed.

Keywords: Ethanolic, Euphorbia *microphylla*, Flavonoid, Phenolics, Phytoconstituent.

Correspondence:
Dr. Manisha Sureshkumar Sutare
Department of Botany, Shri Yashwantrao Patil Science College, Solankur, Kolhapur, Maharashtra, INDIA.
Email: drmssutare@gmail.com

Received: 14-01-2026;
Revised: 09-02-2026;
Accepted: 27-04-2026.

INTRODUCTION

The use of medicinal plants for the treatment of various ailments has been deeply embedded in Indian culture since ancient times. For last many centuries, the well-established treatment systems like Ayurveda, Siddha and Unani are being practiced in India and since then these systems are playing important role in public healthcare. In these systems of treatment different parts of plants like roots, leaves, stems, seeds, flowers etc. are used to different types of healthcare issues.

At present, several Ayurvedic medicines derived and prepared from different plant formulations gained acceptance in modern medicine and reached to global market places (Patwardhan *et al*., 2005). Therefore, plants serve as vital raw materials in the development of pharmaceutical drugs. Though the synthetic drugs are effective in managing many diseases, they are often expensive and also comes with different side effects.

As of now, it is estimated that nearly 70,000 plant species have been used for medicinal purposes worldwide. As these plants contains, complex phytochemical constituents like alkaloids, flavonoids, tannins, terpenoids, and glycosides are the main reason behind such acceptance and use.

In India too more than 2,500 plant species have been recognized for its medicinal importance. Similarly, Sri Lanka and Nepal too have confirmed 1,400 and 700 species, respectively. Hence, these plants have plenty of bioactive compounds, however, the scientific research of many of which remain underexplored. Thus, the interest in natural remedies and alternative therapies is increasing the integration of traditional knowledge with modern pharmacological approaches opens up a promising avenue for drug discovery and healthcare discoveries. Among these, the genus *Euphorbia* is one of the largest and most pharmacologically potent, with over 2,000 species spread across various regions globally (Nabeelah *et al*., 2019). *Euphorbia microphylla* B. Heyne ex Roth, commonly known in some regions as Hayna, is a lesser-known but it is widely used species in traditional medicine. It belongs to the family Euphorbiaceae, characterized by the presence of milky latex, cyathium inflorescence, and a huge diversity of bioactive compounds. According to Althobaiti A.T., (2023) approximately 1,600 species of *Euphorbia* distributed globally. *Euphorbia* includes flavonoids, triterpenoids, alkanes,

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/pres.20260299

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

amino acids, and alkaloids, which possesses a range of pharmacological effects (Kamboj, 2000).

The *Euphorbia microphylla* is found primarily in dry and semi-dry regions, flourishing in poor soils and extreme climatic conditions. In traditional medicine system. it is commonly used for treating skin ailments, gastrointestinal issues, inflammation, and even tumours (Kgosiemang *et al*., 2025). Despite its regular use in folk remedies, scientific evaluation of its phytochemical composition, pharmacological efficacy, and toxicological safety is not explored on large scale.

Ethnobotanical and Traditional Uses

Traditionally, especially among indigenous and rural communities, *Euphorbia microphylla* (Hayna) has been used for different therapeutic applications like wound healing, Anti-inflammatory treatments, laxative and purgative effects, skin diseases, fever and treatment of gastrointestinal disorders.

Due to its irritant and caustic natures, the latex is used in diluted forms. As it contain repellent properties, in certain African and South Asian cultures, for and also in religious rituals. However, despite its popularity in folk medicine, scientific studies validating these uses are rare and thus underlines the need for pharmacological and toxicological assessments. The plant's latex, though toxic in high doses, is often diluted in small quantity and applied to treat warts, fungal infections, and even tumour in some folk traditions. On the other hand, in some cultures, the roots and leaves are used in decoctions or pastes which is often mixed with other herbs (Amtaghri *et al*., 2022),

Phytochemical Composition

The plant is rich in bioactive compounds like diterpenoids, flavonoids, alkaloids, tannins, steroids, saponins (Alabri *et al*., 2018).

Crude extract of *Euphorbia microphylla* identified several compounds with known anti-inflammatory and antimicrobial activities. These compounds are responsible for the plant's antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activities (Benjamaa, *et al*., 2024). Continued phytochemical screening is necessary to isolate and identify novel compounds with potential pharmaceutical value.

Pharmacological Activities

Preliminary investigations on pharmacological activities of *Euphorbia* suggest the various activities as anti-inflammatory by inhibiting of prostaglandin pathways (Das *et al*., 2022), antimicrobial i.e. Latex inhibits bacterial and fungal growth antioxidant because of flavonoids and polyphenols scavenge free radicals, and cytotoxicity because of some diterpenes exhibit selective cytotoxicity against cancer cells (Benjamaa *et al*., 2022).

These findings need to be continued with phytochemical screening necessary to isolate and identify novel compounds with potential pharmaceutical value. The study also need to validate through controlled *in vitro* and *in vivo* experiments to ascertain dosage safety, efficacy, and side effects (Figure 1).

MATERIALS AND METHODS

The collection of plant material, proximate analysis, extraction, yield calculation, pH determination, and phytochemical screening was conducted by following methods,

Collection of plant material

The plant leaves of *Euphorbia microphylla* were collected in accordance with local, national, and international guidelines and regulations for plant research the month of December 2024 from Sangli, MS and identified at Biocyte Research and Development Pvt. ltd, Sangali, Maharashtra. The species is not listed as endangered or protected, and no specific permits were required for its collection. Plant leaves were washed properly using distilled water, dried under shade and grinded to fine powder. The powder was sieved and a portion of the powder was used for proximate principle analysis and the other portion was subjected to solvent extraction.

Proximate principle analysis

The dried sample of *E. microphylla* was analysed for proximate principles like total Ash, Acid Insoluble Ash, Water-Soluble Extractive substances, Alcohol-Soluble Extractive substances, and pH (1% Solution). These parameters ensure the purity and stability of the drug, helping to detect adulteration and degradation (Park and Bell, 2004).

Extraction

Euphorbia microphylla leaves were collected, washed with distilled water, and dried in the shade at room temperature or in a hot air oven at 40-45°C for 7-10 days. The dried leaves were crushed to a rough powder and 30-50 g of powder was weighed for extraction. The powdered sample was placed in a Soxhlet apparatus thimble, and 250-300 mL of 70-95% ethanol was added to the round-bottom flask. The Soxhlet extractor was assembled, connected with a condenser, and heated to maintain the solvent at its boiling point (~78°C). Extraction was carried out for 6-8 hr or until the siphoning solvent became colourless, indicating complete extraction. After extraction, the solution was cooled, filtered through Whatman No. 1 filter paper to remove residues, and the filtrate was concentrated using a rotary evaporator under reduced pressure or evaporated over a water bath at 40-50°C. The concentrated extract was dried completely in a desiccator and stored in an airtight amber glass container at 4°C for further analysis. The yield of crude extract was typically 5-15% w/w of dried plant material.

Calculation of extractive yield

The weight of the dried crude ethanolic and hexanic extracts was recorded and the extractive yield was calculated as under Loss on Drying/ total ash/ Acid Insoluble Ash (Harborne, 1998).

(W1 is the weight of the empty crucible, W2 is the weight of the crucible plus sample before ashing/drying, and W3 is the weight of the crucible plus ash after incineration/drying.)

The Water-Soluble Extractive and Alcohol-Soluble Extractive (%) is then calculated using the formula:

$$\text{(Water or alcohol) Extractive (\%)} = \frac{\text{Weight of dried residue}}{\text{Weight of dried residue}} \times 100$$

pH determination

pH of a 1% w/v (weight/volume) aqueous solution of the sample, which helps assess its acidity or alkalinity by using pH meter.

Qualitative phytochemical screening

Qualitative tests were conducted on all extracts to identify the various phytoconstituents present. The tests performed and the reagents used as per the method given by Harborne A J (1998).

Quantitative phytochemical analysis

Phenols

To estimate the phenol content in a sample, the Folin-Ciocalteu method is used, which relies on the reduction of the Folin-Ciocalteu reagent by phenolic compounds to produce a blue complex measurable by spectrophotometry. The flavonoid content is calculated by plotting the absorbance values of the standards to generate a standard curve, from which the sample's flavonoid concentration is determined (Madhu *et al.*, 2016).

Flavonoid

To estimate the flavonoid content in a sample, the aluminum chloride colorimetric method is widely used, as it relies on the formation of a yellow complex between flavonoids and aluminum chloride that can be measured spectro-photometrically. The flavonoid content is calculated by plotting the absorbance values of the standards to generate a standard curve, from which the sample's flavonoid concentration is determined (Isaac *et al.*, 2024).

Statistical analysis

Results were expressed as mean±Standard Deviation (SD). The SD was calculated to assess the variability and precision of the experimental measurements.

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was conducted by three different methods i.e. protein denaturation inhibition assay (Dharmadeva *et al.*, 2018) protease inhibition assay (Sohemat *et al.*, 2023) and membrane stabilization assay (Chippada *et al.*, 2011).

Statistical Analysis

Data were analyzed using one-way ANOVA and expressed as Mean±SD of triplicate determinations. IC_{50} values were calculated by regression analysis, and $p<0.05$ was considered statistically significant.

RESULTS

Biochemical proximate principles in dried *E. microphylla* Organoleptic and Physicochemical Parameters

The evaluation of *Euphorbia microphylla* revealed typical organoleptic characteristics, with a greenish- brown colour, characteristic slightly pungent smell, coarse powder or dried plant material appearance, and a bitter, astringent taste. Physicochemical analysis showed acceptable parameters for herbal material, with a moisture content of 5.7%, total ash value of 5.4%, and acid insoluble ash of 0.5%, indicating low contamination with inorganic matter. In alcohol, the extractive values were significant followed by water. The pH value was observed as acidic. the findings confirm the identity, purity, and quality of the *Euphorbia microphylla* sample for further pharmacognostical or phytochemical investigations (Tables 1,2).

Figure 1: *Euphorbia microphylla* leaves powder.

Organoleptic Characters

Physicochemical Parameters

Qualitative phytochemical screening

To identifying the bioactive compounds in plant and also to assess its medicinal importance, phytochemical analysis is necessary. Such identification forms the basis for further targeted research and important compound isolation. The solvent which is used in this process determines the effectiveness of the phytochemical extraction. On the other hand, the qualitative tests confirms the presence or absence of specific phytochemicals. Therefore, in the present study, *Euphorbia microphylla* leaves tested positive for alkaloids, carbohydrates, glycosides, and saponins, suggesting potential pharmacological significance. However, it tested negative for proteins, amino acids, reducing sugars, flavonoids, cardiac glycosides, phenolic compounds, and tannins. These findings provide valuable insights for future studies on the therapeutic applications of *Euphorbia microphylla* (Table 3).

Quantitative phytochemical analysis

Phenolic compounds

During the study it was observed that the standard curve of gallic acid showed a linear increase in Optical Density (OD). Further, when concentration was increased, it confirms the reliability of the method for phenolic content estimation. The ethanolic extract of *Euphorbia microphylla* showed a mean OD of 0.93 at a concentration of 1000 µg/mL. Based on the standard curve, the total phenolic content of the extract was found to be 1.896 µg GA/g, equivalent to 0.1896 mg GA/g. The phenolic content is further expressed as mg GAE/g±SD, with a value of 0.1896 (Tables 4, 5 and Figure 2).

Flavonoid Content

The standard curve of quercetin demonstrated a clear linear increase in Optical Density (OD). While when the concentration of quercetin increased, it confirmed the method's suitability for flavonoid content analysis. For the ethanolic extract of *Euphorbia microphylla*, the mean OD at a concentration of 1000 µg/mL was 0.16. Based on the standard curve, the flavonoid content in the extract was calculated to be 260 µg Quercetin Equivalent (QE)/g, which is equivalent to 0.260 mg QE/g. These results shows that *Euphorbia microphylla* is a prominent source of flavonoids (Tables 6, 7 and Figure 3).

In vitro anti-inflammatory activity

Protein Denaturation Inhibition Assay

The ethanolic extract of *Euphorbia microphylla* showed significant protein denaturation inhibition and when the concentration increased, it leads to a higher percentage of inhibition. At a concentration of 200 µg/mL, the extract inhibited 43.34% of protein denaturation, and this effect increased gradually to 76.45% at 1000 µg/mL. The IC_{50} value for the ethanolic extract was found to be 365.05 µg/mL, which indicates its moderate inhibitory activity compared to the standard diclofenac sodium, i.e., IC_{50} of 77.14 µg/mL. These results suggest that *Euphorbia microphylla* possesses potential for protein denaturation inhibition (Table 8 and Figures 4,5).

Proteinase Inhibitory Activities

The ethanolic extract of *Euphorbia microphylla* demonstrated notable proteinase inhibitory activity and concentration-dependent increased inhibition. At 200 µg/mL, the extract exhibited 34.59% inhibition, which increased to 75.75% at 1000 µg/mL. The IC_{50} value of the extract was calculated to be 438.87 µg/mL, that indicated moderate inhibitory potency compared to the standard drug ibuprofen i.e. IC_{50} of 171.41 µg/mL. These findings suggest that *Euphorbia microphylla* holds potential anti-inflammatory properties through proteinase inhibition. Although its activity is lower than that of standard anti-inflammatory drugs (Table 9 and Figures 6-8).

Table 1: **Organoleptic Characters.**

Sl. No.	Sample	Parameter					
		Moisture content / Loss on soiling %	Total Ash value %	Acid insoluble ash	Alcohol soluble extractive	Water soluble extractive	pH
1.	*Euphorbia microphylla*	5.7	5.4	0.5	15	7.5	5.9

Table 2: **Physicochemical Parameters.**

Sl. No.	Sample code	Parameter			
		Colour	Smell	Appearance	Taste
1.	*Euphorbia microphylla*	Greenish- brown (typical of dried herb)	Characteristic, slightly pungent	Coarse powder / dried plant material (depending on form)	Bitter, astringent

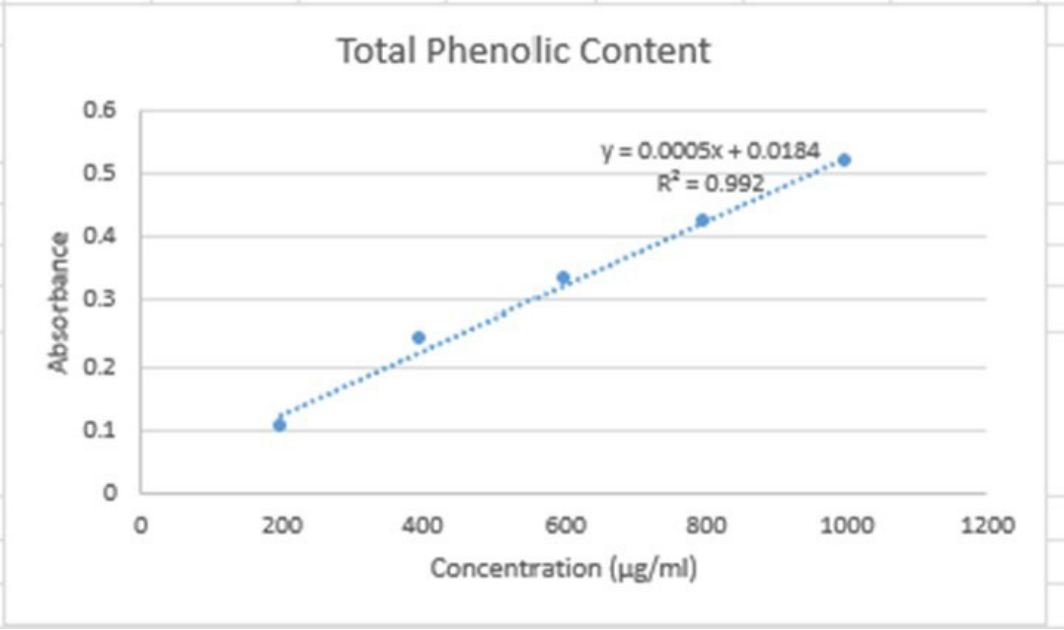

Figure 2: Estimation of Phenol Content.

Table 3: Qualitative phytochemical screening.

Sl. No.	Component	Test Performed	Confirmation Test
1	Carbohydrate	Molisch's test Test for starch	+VE +VE
2	Alkaloids	Wagner's test Hager's test	-VE ++VE
3	Reducing sugars	Benedict's test Fehling's test	+VE -VE
4	Glycosides	10% NaOH test Aqueous NaOH test	-VE +VE
5	Cardiac Glycosides	Baljet test Test for Cardenolides	-VE +VE
6	Proteins	Biuret test Millons Test	-VE +VE
7	Flavonoids	Lead acetate test Ferric chloride test	++VE ++VE
8	Phenolic compounds	Ferric chloride test Hot water test	++VE ++VE
9	Tannins	Braymer's test 10% NaOH test	-VE -VE
10	Saponins	Foam test Olive oil test	+VE +VE

+: Present; ++: Strongly present; -: Absent.

Membrane Stabilization Assay

The ethanolic extract of *Euphorbia microphylla* demonstrated moderate membrane stabilization activity in a concentration-dependent manner. At 200 µg/mL, the extract showed 33.91% inhibition of hemolysis, which increased to 65.38% at 1000 µg/mL. The IC_{50} value of the extract was found to be 653.18 µg/mL, indicating comparatively lower potency than the standard drug, which exhibited an IC_{50} of 365.39 µg/mL. Although the membrane stabilizing effect of *Euphorbia microphylla* was weaker than that of the standard, the results

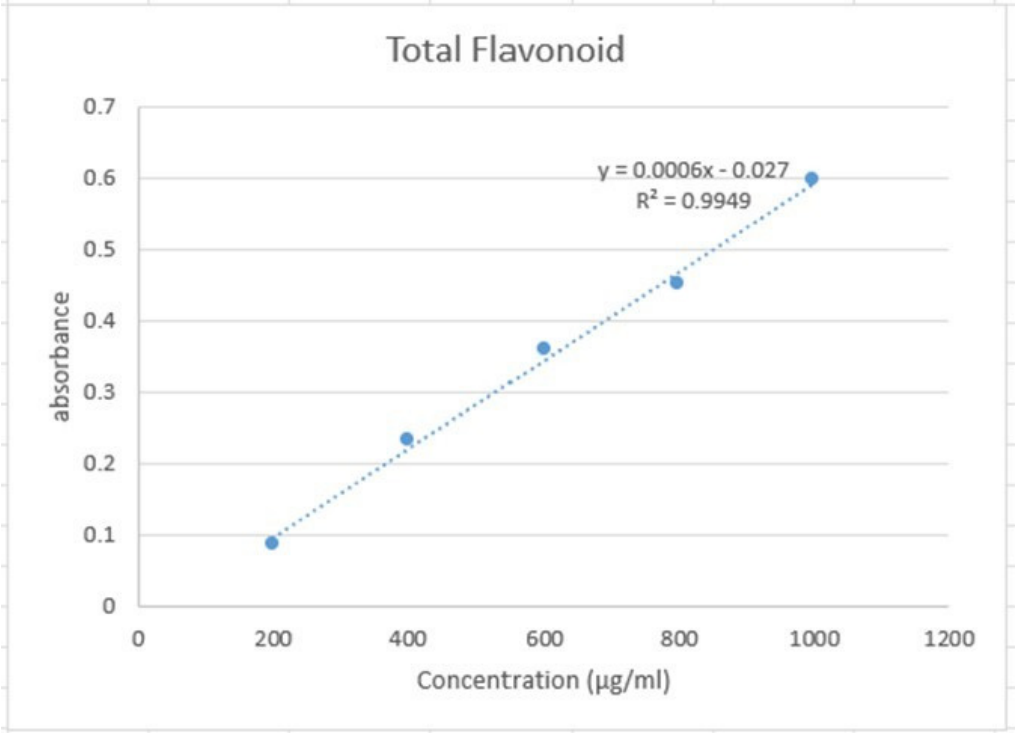

Figure 3: Estimate Flavonoid Content.

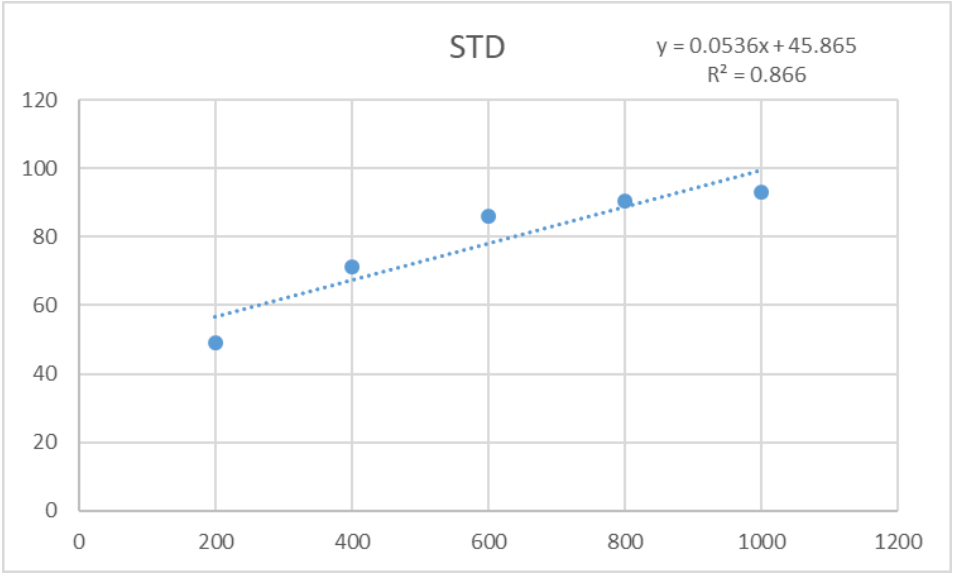

Figure 4: Effect of Ethanolic Standard Diclofenac Sodium by Protein Denaturation Inhibition assay.

suggest the extract still possesses potential anti-inflammatory properties by protecting red blood cell membranes against lysis under stress condition (Table 10 and Figures 9, 10).

Ethical Statement

This study did not involve human participants.

DISCUSSION

The qualitative phytochemical screening of the plant extract *E. microphylla* revealed the presence variety of secondary metabolites i.e., carbohydrates, reducing and non-reducing sugars, proteins, flavonoids, glycosides, saponins, steroids, tannins, phenolic compounds, alkaloids and triterpenoids. These earlier reports suggests that medicinal plants are biological factories for multiple classes of phytochemicals, which have potent biological activities (Peiris *et al.*, 2023). In the screening of phytochemicals, flavonoids, phenolic compounds and glycosides were found prominent. Flavonoids play key roles as antioxidants and antimicrobial agents. Plant-derived flavonoids are effective in membrane disruption of microbial cells and interfering in oxidative pathways. The studies on detection of glycosides and

saponins further supports the potential for pharmacological activity (Alhaithloul, 2023).

During the qualitative screening it is important to focus on reliability of qualitative phytochemical screening sample preparation, extraction solvent, test conditions and storage of material (Peiris *et al.*, 2023). In the present study, standard chemical tests i.e. Molisch, Benedict's, Seliwanoff's, Biuret, Xanthoproteic, foam test, Salkowski's, Keller-Killiani's, Wagner's etc. were used to screen the phytoconstituents. These tests gave rapid preliminary information. Further, chromatographic or spectrometric analyses (HPLC, GC-MS, NMR) is required to characterise and quantify each constituent (Kumar *et al.*, 2023).

The presence of active secondary metabolites suggests that the plant extract has potential to exhibit multi-faceted pharmacological effects E.g. antioxidant, antimicrobial, anti-inflammatory etc. A recent Ethiopian study found that flavonoids and phenols were dominant in medicinal plant extracts demonstrating antimicrobial efficacy (Dubale *et al.*, 2023).

While studying three types of anti-inflammatory assays, i.e. protein denaturation inhibition assay, protease inhibition assay and membrane stabilisation assay following points are discussed.

The protein denaturation inhibition assay evaluates the activity of plant extracts to prevent heat-induced unfolding of proteins like Bovine Serum Albumin (BSA) or egg albumin which is a key mechanism in inflammation where denatured proteins act as

Table 4: Standard curve of Gallic acid and Ethanolic Extract of *Euphorbia microphylla*.

Sl. No.	Gallic acid Concentration (µg/mL)	Gallic acid Concentration (mg/mL)	OD
1.	200	0.20	0.10
2.	400	0.40	0.24
3.	600	0.60	0.33
4.	800	0.80	0.42
5.	1000	0.1	0.52

Table 5: Ethanolic Extract of *Euphorbia microphylla*.

Sl. No.	Samples at Concentration (1000 µg/mL)	OD	Mean OD	Total phenolic contents (µg GA/g)	Total phen olic contents (mgGA/g) in±SD
1.	Ethanolic Extract of *Euphorbia microphylla*	0.97 0.93 0.91	0.93	1.896	0.1896

Table 6: Standard curve of Quercetin and Ethanolic Extract of *Euphorbia microphylla*.

Sl. No.	Quercetin Concentration (mg/mL)	Quercetin Concentration (µg/mL)	OD
1.	0.20	200	0.08
2.	0.40	400	0.23
3.	0.60	600	0.35
4.	0.80	800	0.45
5.	0.100	1000	0.59

Table 7: Ethanolic Extract of *Euphorbia microphylla*.

Sl. No.	Samples at Concentration (1000 µg/mL)	Absorbance (OD)	Mean (OD)	Flavonoid content (µg quercetin equivalent /g dry material)	Flavonoidcontent (mg quercetin equivalent /g dry material)
1.	Ethanolic Extract of *Euphorbia microphylla*	0.17 0.15 0.18	0.16	260 µg QE/g dry	0.260 mg QE/g

Table 8: Effect of Ethanolic Extract of *Euphorbia microphylla* by Protein Denaturation Inhibition assay.

Sl. No.	Sample	Conc.	O.D. (Triplicate)			Mean±SD	% inhibition	IC_{50}
			I	II	III			
1.	Control		0.98	0.99	0.96	0.97±0.015		
2.	Std Diclofenac Sodium	200	0.49	0.52	0.48	0.49±0.015	49.14	77.14
		400	0.29	0.28	0.27	0.28±0.01	71.33	
		600	0.15	0.14	0.12	0.13±0.015	86.00	
		800	0.11	0.09	0.08	0.09±0.015	90.44	
		1000	0.08	0.05	0.07	0.06±0.015	93.17	
3.	Ethanolic Extract of Euphorbia microphylla	200	0.57	0.55	0.54	0.55±0.01	43.34	365.05
		400	0.52	0.49	0.45	0.48±0.01	50.17	
		600	0.42	0.39	0.35	0.38±0.01	60.40	
		800	0.32	0.29	0.25	0.28±0.015	70.64	
		1000	0.24	0.22	0.23	0.23±0.015	76.45	

Table 9: Effect of Ethanolic Extract of *Euphorbia microphylla* by Proteinase Inhibitory Activities.

Sl. No.	Sample	Conc.	O.D. (Triplicate)			Mean±SD	% inhibition	IC_{50}
			I	II	III			
1	Control		0.896	0.889	0.892	0.892±0.003		
2	Std Ibuprofen	200	0.456	0.455	0.458	0.456±0.001	48.86	171.41
		400	0.345	0.348	0.348	0.347±0.001	61.11	
		600	0.225	0.228	0.219	0.224±0.004	74.89	
		800	0.198	0.199	0.197	0.198±0.001	77.81	
		1000	0.112	0.116	0.118	0.115±0.003	87.07	
3	Extract of *Euphorbia Microphylla*	200	0.589	0.584	0.578	0.583±0.01	34.59	438.87
		400	0.468	0.467	0.456	0.463±0.01	48.03	
		600	0.342	0.335	0.332	0.336±0.01	62.30	
		800	0.256	0.242	0.241	0.246±0.02	72.39	
		1000	0.213	0.223	0.213	0.216±0.01	75.75	

Table 10: Effect of Ethanolic Extract of *Euphorbia microphylla* by Membrane Stabilization Assay.

Sl. No.	Sample	Conc.	O.D. (Triplicate)			Mean±SD	% inhibition	IC_{50}
			I	II	III			
1	Control		0.98	0.92	0.96	0.95±0.03		
2	Std	200	0.56	0.58	0.59	0.57±0.015	39.51	365.39
		400	0.48	0.44	0.42	0.44±0.03	53.14	
		600	0.37	0.32	0.34	0.34±0.02	63.98	
		800	0.29	0.27	0.25	0.27±0.02	71.67	
		1000	0.22	0.19	0.18	0.19±0.02	79.370	
3	Extract of *Euphorbia microphylla*	200	0.62	0.63	0.64	0.6357±0.02	33.91	653.18
		400	0.58	0.59	0.55	0.5757±0.01	39.86	
		600	0.53	0.51	0.49	0.5157±0.02	46.50	
		800	0.42	0.45	0.44	0.4357±0.03	54.19	
		1000	0.32	0.3	0.37	0.3357±0.01	65.38	

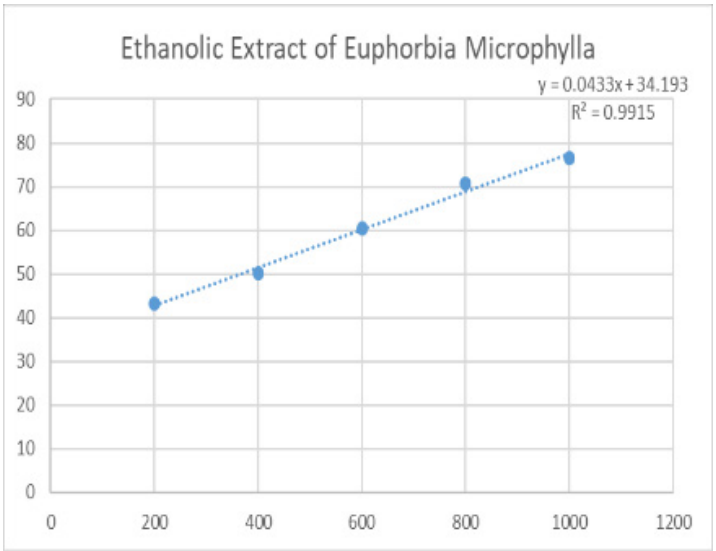

Figure 5: Effect of ethanolic extract of *Euphorbia microphylla* by Protein Denaturation Inhibition assay.

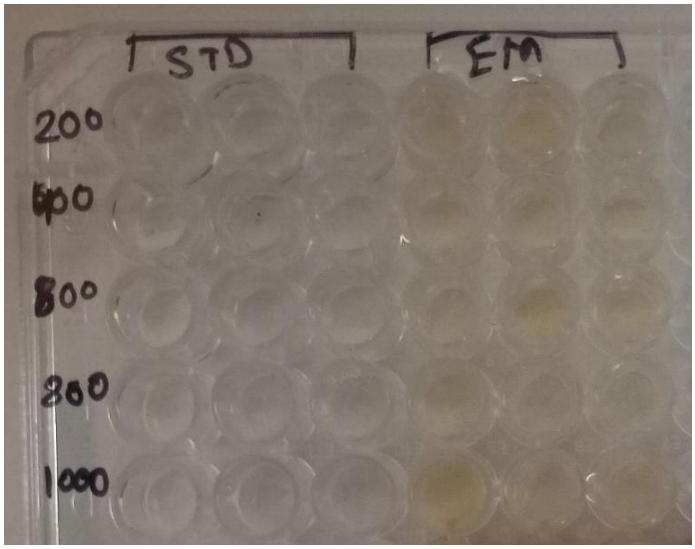

Figure 6: Effect of Ethanolic Extract of *Euphorbia microphylla* by Proteinase Inhibitory Activities.

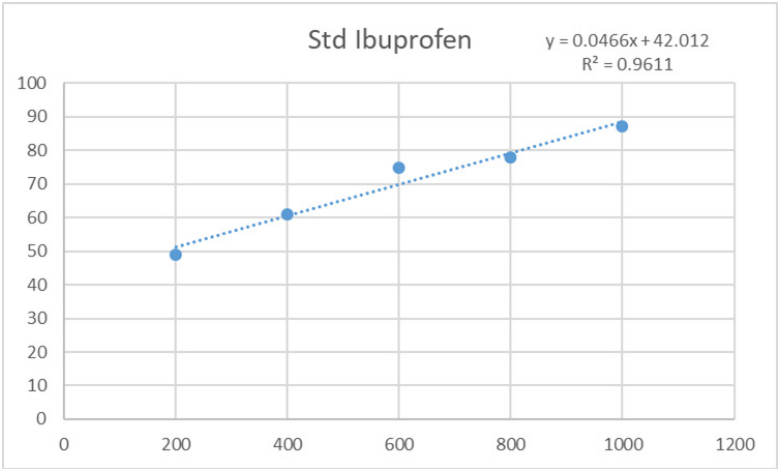

Figure 7: Effect of Ibuprofen (STD) by Proteinase Inhibitory Activity.

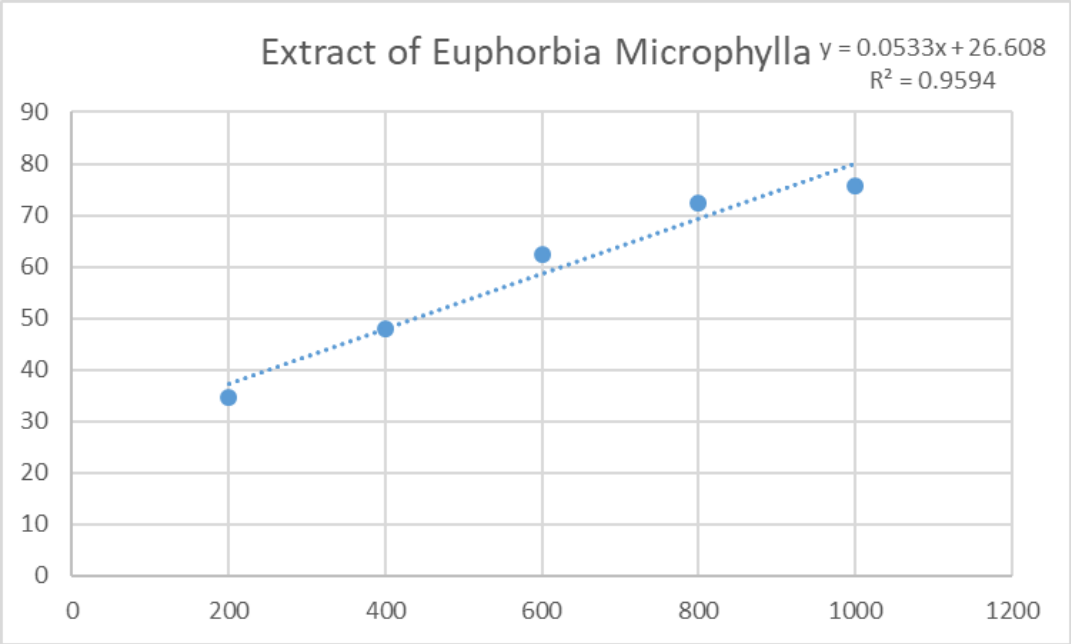

Figure 8: Effect of Ethanolic Extract of *Euphorbia microphylla* by Proteinase Inhibitory Activities.

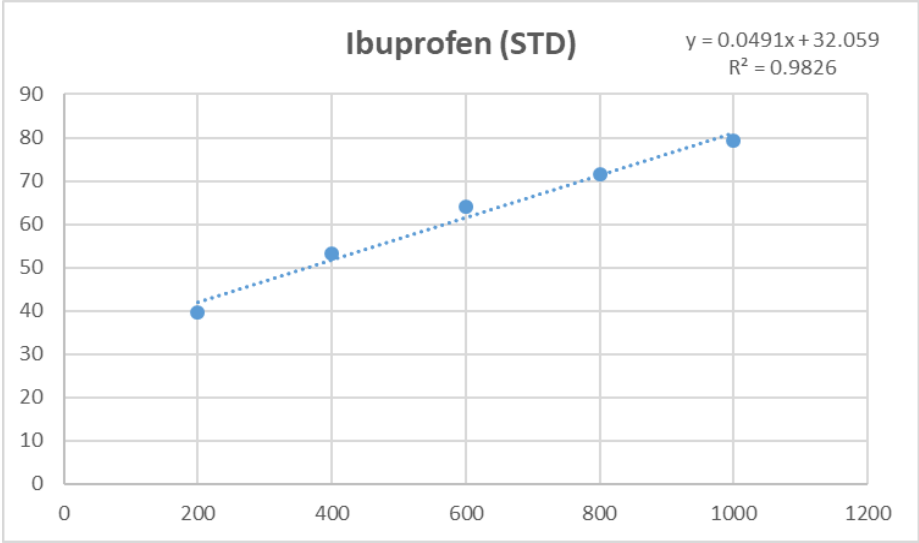

Figure 9: Effect of Ibuprofen by Membrane Stabilization Assay.

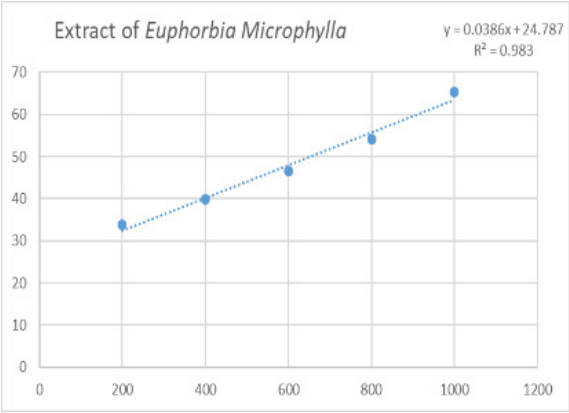

Figure 10: Effect of ethanolic extract *E. microphylla* by membrane stabilization assay.

autoantigens in conditions resembling with rheumatoid arthritis. Ethanolic extracts from plants i.e. *Euphorbia microphylla*, viz. rich in phenolics and flavonoids, likely inhibit this process in a concentration-dependent manner, as compared to standards like diclofenac sodium. Similar studies were conducted on *Ficus racemosa* bark where inhibition increased with concentration up to 1000 µg/mL (Dharmadeva *et al.*, 2018), confirming the method's reliability for screening anti-inflammatory potential in leaf extracts.

Protease inhibition assay measures suppression of enzymes in degradation of proteins like trypsin during inflammation. It also reduces the tissue damage and migration of immune cell. Hence, based on this principle, the study was performed on plant extracts that showed significant inhibition with IC_{50} values close with diclofenac sodium (93 µg/mL). This confirms that polyphenolics acts as natural protease inhibitors. During study of ethanolic extract of *Euphorbia microphylla*, this supports anti-inflammatory efficacy (Sohemat *et al.*, 2023), in which aqueous extracts demonstrated dose-dependent protection. In membrane stabilization assay protection of Human Red Blood Cell (HRBC) membranes from hypotonic lysis, mimicking lysosomal membrane stability in inflamed tissues is assessed. Studiy conducted on extracts of *Centella asiatica* showed up to 94.97% stabilization at 2000 µg/mL, due to flavonoids preventing lipid peroxidation (Chippada *et al.*, 2011). In *Euphorbia microphylla,* high phenolic content shows similar results validating for ethanolic leaf extracts.

CONCLUSION

The detailed evaluation of *Euphorbia microphylla* leaves confirms it's pharmacological potential. Similar to a typical herbal material, *Euphorbia* too shows the organoleptic characteristics i.e., greenish-brown colour, pungent smell, and bitter, astringent taste. Physicochemical parameters such as low moisture content (5.7%), total ash (5.4%), and acid-insoluble ash (0.5%) indicate the material's quality and suitability for medicinal use.

On the other hand, phytochemical analysis confirms the presence of alkaloids, carbohydrates, glycosides, and saponins, suggesting bioactive compounds with potential therapeutic effects. Notably, the extract was negative for proteins, amino acids, reducing sugars, flavonoids, cardiac glycosides, phenolic compounds, and tannins that confirms the plant's explicit bioactive components.

The quantification of phenolic content using a gallic acid standard curve confirms a total phenolic content of 1.896 µg GA/g, which ultimately indicates a moderate level of phenolic compounds in the extract. Similarly, the flavonoid content, determined using a quercetin standard curve, was 260 µg QE/g, that makes *Euphorbia microphylla* as a significant source of flavonoids.

By focusing protein denaturation, protease release, and membrane stabilisation, the three *in vitro* methods of anti-inflammatory activity assay together supports the findings *Euphorbia microphylla* leaf ethanolic extract as core inflammatory pathways. Phytochemicals such as flavonoids and phenolics, which are previously quantified in the extract, highlights on its efficacy, suggesting its therapeutic potential and further interest in formulating standard drugs.

ACKNOWLEDGEMENT

The authors sincerely thank the management and staff of Latur College of Pharmacy, Hasegaon, and Shri Yashwantrao Patil Science College, Solankur, for providing the necessary facilities and support to carry out this research work.

ABBREVIATIONS

MS: Maharashtra State; **E. microphylla:** *Euphorbia microphylla*; **BSA:** bovine serum albumin; **GA:** Gallic acid; **QE:** Quercetin equivalent; **OD:** Optical density; **IC_{50}:** Half maximal inhibitory concentration; **HRBC:** Human red blood cell; **HPLC:** High-performance liquid chromatography; **GC-MS:** Gas chromatography-mass spectrometry; NMR: Nuclear magnetic resonance; **STD:** Standard; **SD:** Standard deviation; **-:** Absent; **+:** Present; **++:** Strongly present.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research work does not receive any financial support or sponsorship.

AUTHOR CONTRIBUTIONS

Dr Nitin B. Lonikar: Conceptualisation, design of the study, experimental studies, data acquisition, data analysis, interpretation of results, and manuscript preparation. **Dr. Manisha S. Sutare:** conceptualisation, literature search, manuscript preparation, scientific editing, and final manuscript review. **Dr. Amar R. Mane:** Experimental design, Investigations, data acquisition, data analysis, interpretation of results, and manuscript editing. **Dr. Nandkishor B. Bavage:** Experimental studies, analytical procedures, data validation, statistical analysis, interpretation of results, and manuscript review.

SUMMARY

In the present study, the phytochemical composition *Euphorbia microphylla* B. Heyne ex Roth were studied and effect of its ethanolic extract against inflammation was also studied. The initial qualitative phytochemical analysis confirmed the presence of major secondary metabolites while quantitative analysis validated considerable levels of phenolic and flavonoid constituents. Through *in vitro* protein denaturation assay, the

anti-inflammatory efficiency of the extract was evaluated in which the extract showed concentration reliant inhibition when compared to the standard diclofenac sodium. The statistical analysis conducted in triplicates which supports the reproducibility and reliability findings. In conclusion, the results indicates that *Euphorbia microphylla* is a promising source of bioactive phenolic and flavonoid compounds which can contribute to its observed anti-inflammatory activity.

REFERENCES

Alabri, Z. K., Hussain, J., Mabood, F., Rehman, N. U., Ali, L., Al-Harrasi, A., Hamaed, A., Khan, A. L., Rizvi, T. S., Jabeen, F., Khan, A., Naureen, Z., and Farooq, S. (2018). Fluorescence spectroscopy-partial least square regression method for the quantification of quercetin in *Euphorbia masirahensis*. Measurement, 121, 355-359. https://doi.org/10.1016/j.measurement.2018.02.036

Alhaithloul, H. A. S. (2023). Phytochemical screening of some medicinal plants in Al Jouf, KSA. Open Journal of Ecology, 13, 61-79. https://doi.org/10.4236/oje.2023.132006

Althobaiti, A. T. (2023). Taxonomic studies on family Euphorbiaceae based on some morphological, biochemical and molecular characteristics. Journal of Advanced Zoology, 44.

Amtaghri, S., Akdad, M., Slaoui, M., and Eddouks, M. (2022). Traditional uses, pharmacological, and phytochemical studies of Euphorbia: A review. Current Topics in Medicinal Chemistry, 22(19), 1553-1570.

Arthur, I. A. (2024). Phytochemical analysis of medicinal plants: Methods, bioactive compounds, and applications.

Benjamaa, R., Elbouny, H., Errati, H., Moujanni, A., Kaushik, N., Gupta, R., *et al.* (2024). Comparative evaluation of antioxidant activity, total phenolic content, anti-inflammatory, and antibacterial potential of Euphorbia-derived functional products. Frontiers in Pharmacology, 15, 1345340.

Benjamaa, R., Moujanni, A., Kaushik, N., Choi, E. H., Essamadi, A. K., and Kaushik, N. K. (2022). Euphorbia species latex: A comprehensive review on phytochemistry and biological activities. Frontiers in Plant Science, 13, 1008881.

Chippada, S. C., Volluri, S. S., Bammidi, S. R., and Vangalapati, M. (2011). *In vitro* anti-inflammatory activity of methanolic extract of Centella asiatica by HRBC membrane stabilisation. Rasayan Journal of Chemistry, 4(2), 457-460.

Das, K., Asdaq, S. M. B., Khan, M. S., Amrutha, S., Alamri, A., Alhomrani, M., *et al.* (2022). Phytochemical investigation and evaluation of *in vitro* anti-inflammatory activity of *Euphorbia hirta* ethanol leaf and root extracts: A comparative study. Journal of King Saud University - Science, 34(7), 102261.

Dharmadeva, S., Galgamuwa, L. S., Prasadinie, C., and Kumarasinghe, N. (2018). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayu, 39(4), 239-242. https://doi.org/10.4103/ayu.AYU_27_18

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., and Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. Journal of Experimental Pharmacology, 15, 51-62.

Harborne, A. J. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Springer.

Kamboj, V. P. (2000). Herbal medicine. Current Science, 78(1), 35-39.

Kgosiemang, I. K. R., Lefojane, R., Adegoke, A. M., Ogunyemi, O., Mashele, S. S., and Sekhoacha, M. P. (2025). Pharmacological significance, medicinal use, and toxicity of extracted and isolated compounds from Euphorbia species found in southern Africa: A review. Plants, 14(3), 469.

Kumar, A., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., and Oz, F. (2023). Major phytochemicals: Recent advances in health benefits and extraction methods. Molecules, 28(2), 887. https://doi.org/10.3390/molecules28020887

Madhu, M., Sailaja, V., Satyadev, T. N. V. S. S., and Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species using various organic solvents. Journal of Pharmacognosy and Phytochemistry, 5(2), 25-29.

Nabeelah, B. S., Fawzi, M. M., Gokhan, Z., Rajesh, J., Nadeem, N., and R. R. K. (2019). Ethnopharmacology, phytochemistry, and global distribution of mangroves: A comprehensive review. Marine Drugs, 17(4), 231.

Park, Y. W., and Bell, L. N. (2004). Determination of moisture and ash contents of foods. Food Science and Technology, 138(1), 55.

Patwardhan, B., Warude, D., Pushpangadan, P., and Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: A comparative overview. Evidence-Based Complementary and Alternative Medicine, 2(4), 465-473.

Peiris, D. S. H., Fernando, D. T. K., Senadeera, S. P. N. N., and Ranaweera, C. B. (2023). Phytochemical screening for medicinal plants: Guide for extraction methods. Asian Plant Research Journal, 11(4), 13-34. https://doi.org/10.9734/aprj/2023/v11i4216

Sohemat, A. A., Atrooz, O. M., and Farah, H. S. (2023). Evaluation of the anti-inflammatory, antioxidant, and protease inhibitory activity of the crude methanol extract of *Portulaca oleracea*. Tropical Journal of Natural Product Research, 7(2).

Cite this article: Lonikar NB, Sutare MS, Mane AR, Bavage NB. Phytochemical evaluation and anti-inflammatory efficacy of phenolic and flavonoid constituents in *Euphorbia microphylla* B. Heyne ex Roth. Pharmacog Res. 2026;18(3):1006-17.