RESEARCH PAPER

Extraction, Phytochemical Screening, Isolation, Characterization and Evaluation of Antioxidant and Anti-inflammatory Activities of Flavonoid Fraction from Biophytum sensitivum

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Received: 20th Apr, 2026 | Revised: 25th Apr, 2026 | Accepted: 9th May, 2026 | Available Online: 14th May, 2026

ABSTRACT

Biophytum sensitivum (L.) DC. is a medicinal herb extensively utilized in traditional medicine systems for addressing inflammation and disorders related to oxidative stress. This study was conducted to assess the phytochemical components, isolate flavonoid-rich fractions, and evaluate the antioxidant and anti-inflammatory properties of the ethanolic extract of Biophytum sensitivum. The dried plant material underwent extraction with ethanol via Soxhlet extraction and was subjected to preliminary phytochemical screening, which confirmed the presence of flavonoids, phenolics, tannins, saponins, alkaloids, and glycosides. Flavonoid-rich fractions were isolated through column chromatography and further characterized using TLC, UV-visible spectroscopy, FTIR, 1H NMR, and mass spectrometry.

The antioxidant activity was assessed using the DPPH free radical scavenging assay, where the extract demonstrated concentration-dependent activity with an 85.93% inhibition at 100 µg/mL and an IC_{50} value of 56.18 µg/mL, comparable to the standard ascorbic acid. The anti-inflammatory activity evaluated through the protein denaturation inhibition assay also showed significant concentration-dependent inhibition with an 81.93% inhibition at 100 µg/mL and an IC_{50} value of 62.87 µg/mL, comparable to diclofenac sodium. The biological activities observed may be attributed to the flavonoid and polyphenolic constituents identified during the phytochemical and spectroscopic characterization studies. These results indicate that Biophytum sensitivum has considerable antioxidant and anti-inflammatory potential, suggesting it may be a valuable natural source for developing therapeutic agents.

Keywords: Biophytum sensitivum, flavonoids, antioxidant activity, anti-inflammatory activity, DPPH assay, protein denaturation assay, phytochemical screening.

How to cite this article: Kamble K, Nhawkar S, Patade O, Irkar S, Jagdale V., Extraction, Phytochemical Screening, Isolation, Characterization and Evaluation of Antioxidant and Anti-inflammatory Activities of Flavonoid Fraction from Biophytum sensitivum. Int J Drug Deliv Technol. 2026;16(44s): 759-770; DOI: 10.25258/ijddt.16.44s.81

INTRODUCTION

Medicinal plants have historically played a significant role in healthcare systems worldwide due to their numerous pharmacological traits, therapeutic efficacy, and accessibility. Natural products continue to be vital sources for the discovery and development of novel therapeutic agents due to their rich phytochemical content and

comparatively lower adverse effects than synthetic drugs. Scientists have recently focused increasingly on medicinal plants as potential sources of bioactive compounds for the treatment and prevention of diseases linked to oxidative stress and inflammation. [1,2]

A little herbaceous medicinal plant, *Biophytum sensitivum* (L.) DC. belongs to the Oxalidaceae family and is found in tropical parts of Africa, Southeast Asia, India, and Sri Lanka. The plant, often referred to as "Mukkutti," is widely used in Ayurvedic and traditional medicine to treat a variety of conditions, including burns, wounds, asthma, diabetes, and inflammatory illnesses. *Biophytum sensitivum* contains significant bioactive substances such as flavonoids, biflavones, tannins, saponins, polysaccharides, and phenolic elements, according to several pharmacognostic and phytochemical studies. According to reports, the plant's biological activity is largely attributed to amentoflavone, a prominent biflavonoid that was isolated from the plant [3,4]

The wide range of pharmacological activity of *Biophytum sensitivum*, including its antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, and wound-healing properties, has drawn scientific attention. According to studies, this plant's polyphenolic and flavonoid content, which can efficiently scavenge free radicals and lessen oxidative stress, is primarily responsible for its antioxidant properties. Excessive generation of reactive oxygen species (ROS) leads to oxidative stress, which can harm proteins, DNA, and cellular membranes. This can lead to chronic illnesses including diabetes, cancer, and heart disease. Compared to synthetic antioxidants, natural antioxidants derived from medicinal plants are thought to be safer. [5,6]

Although continuous inflammation can result in chronic pathological illnesses like diabetes, cardiovascular problems, and arthritis, inflammation is a biological defence mechanism against toxic stimuli. Because they can inhibit inflammatory mediators and stabilise proteins, traditional medicinal herbs high in flavonoids and tannins are well known for their anti-inflammatory properties. *Biophytum sensitivum* has long been used to treat inflammatory conditions, and prior research has shown that it has strong anti-inflammatory and immunomodulatory properties that support its usage in ethnomedicine.[7]

To determine the therapeutic efficacy of *Biophytum sensitivum*, preliminary phytochemical screening and scientific validation of antioxidant and anti-inflammatory characteristics are crucial. Because it effectively extracts flavonoids, tannins, and phenolic chemicals, ethanol is frequently utilised as an extraction solvent. Reliable procedures for assessing antioxidant and anti-inflammatory activity are standard in vitro methods like the protein denaturation assay and the DPPH free radical scavenging assay. Thus, the current study was conducted to examine the phytochemical components and assess the ethanolic extract of *Biophytum sensitivum*'s antioxidant and anti-inflammatory capabilities Finding the bioactive components that cause pharmacological effects requires the isolation and characterisation of flavonoid-rich fractions from medicinal plants. UV, FTIR, NMR, and mass spectrometry are spectroscopic methods that offer structural details about separated phytoconstituents. [8]

MATERIALS AND METHODS

Plant Material Collection and Authentication

During the flowering season, the entire plant of *Biophytum sensitivum* (L.) DC. was gathered from its natural environment and verified by Prof. Sanjaykumar Magdum, Department of Botany, Arts & Commerce College, Kasegaon, Tal-Walwa, Dist-Sangli, Maharashtra, India, utilizing standard taxonomic features and floras. A voucher specimen (Voucher No. TSN 03) was submitted for future reference. Following collection, the plant material was meticulously washed with running water and subsequently with distilled water to eliminate dust and other contaminants. The cleaned plant material was shade-dried at room temperature for 10–15 days to maintain thermolabile phytoconstituents and then coarsely ground using a mechanical grinder. The powdered material was stored in airtight containers shielded from light and moisture until further extraction processes. [9,10,11]

Fig.no.1 ***Biophytum Sensitivum***

Preparation of Ethanolic Extract

Since ethanol is very good at extracting a variety of phytoconstituents, such as flavonoids, tannins, phenolics, glycosides, and other polar compounds, the dried and powdered entire plant material was treated to Soxhlet extraction using ethanol as the solvent. A thimble containing 50–100 g of powdered material was continually extracted with ethanol for 6–8 hours, or until the solvent in the syphon tube turned colourless, signifying exhaustive extraction. A rotating vacuum evaporator was used to filter and concentrate the extracted material under low pressure in order to eliminate surplus solvent. [12,13]

Fig no.2 Soxhlet Extraction

Preliminary Phytochemical Screening

A preliminary phytochemical screening was performed on the ethanolic extract of *Biophytum sensitivum* in order to detect the existence of significant secondary metabolites that are responsible for biological activities. Alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda test), tannins and phenolics (Ferric chloride test), saponins (Foam test), glycosides (Keller-Killiani test), carbohydrates (Molisch's test), and proteins (Biuret test) were all detected using standard qualitative methods. These phytochemical tests offer a first indication of bioactive substances that might support anti-inflammatory and antioxidant qualities. [14,15]

Isolation of Flavonoid Fraction:

Column chromatography was used to separate the flavonoid-rich fraction from the ethanolic extract of *Biophytum sensitivum*. Toluene was employed as the initial solvent system to load the column, and silica gel served as the stationary phase. After being carefully placed onto the column, about 20 g of ethanolic extract was adsorbed onto silica gel. Different ratios of toluene to ethyl acetate with a progressive rise in polarity were used for elution. Thin-layer chromatography (TLC) was used to collect and monitor each of the separated fractions. For additional investigation, fractions with comparable TLC characteristics were combined. [16,17]

Characterization of Isolated Fraction

Thin-Layer Chromatography

Toluene:ethyl acetate:formic acid (5:4:1) was used as the mobile phase for TLC analysis on silica gel 60 F254 plates. UV light at 254 nm and 366 nm was used to view the produced plates. The presence of flavonoid elements in the separated fraction was confirmed by distinct spots with distinctive fluorescence. [18]

UV-visible Spectroscopy Characterisation of Isolated Fraction

A UV-visible spectrophotometer operating in the 200–800 nm wavelength range was used to analyse the isolated flavonoid fraction. Flavonoid-type phytoconstituents were detected by characteristic absorption peaks about 245 and 275 nm. [19]

Infrared Fourier Transform Spectroscopy (FTIR)

To determine the functional groups included in the isolated fraction, FTIR spectroscopy was performed within the scanning range of 4000–400 cm^{-1}. The resulting spectra showed distinctive peaks that matched the carbonyl, aromatic, and hydroxyl functional groups connected to flavonoids. [20]

Spectroscopy of Nuclear Magnetic Resonance (NMR)

After being dissolved in a deuterated solvent, the purified isolated fraction was analysed using ^1H NMR and ^13C NMR. The acquired spectra verified the presence of flavonoid skeletal structures and aromatic proton signals. [21]

Mass Spectrometry.

Electrospray ionisation (ESI-MS) was used to analyse the separated fraction using mass spectrometry. The presence of flavonoid components in the separated fraction from the ethanolic extract of *Biophytum sensitivum* was confirmed by the molecular ion peak and fragmentation pattern. [22]

Antioxidant Activity

Method: DPPH (2,2-Diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

Procedure:

- The antioxidant activity of the ethanolic extract was assessed utilizing the DPPH

free radical scavenging method, which is widely recognized for evaluating the hydrogen donating or free radical scavenging capacity of plant extracts.

- A freshly prepared 0.1 mM DPPH solution in methanol served as the source of free radicals. Various concentrations of the extract (20, 40, 60, 80 and 100 µg/mL) were prepared. To each concentration, 1 mL of DPPH solution was added, and the final volume was adjusted with methanol. The reaction mixtures were vigorously shaken and incubated in the dark at room temperature for 30 minutes to ensure complete reaction.
- The absorbance of each sample was recorded at 517 nm using a UV-visible spectrophotometer against a blank. Ascorbic acid was employed as the standard antioxidant for comparison purposes. A reduction in the purple hue of DPPH indicated the presence of radical scavenging activity. [23,24]

Calculation

$$\% \text{ Radical Scavenging Activity} = \frac{A \text{ Control} - A \text{ Sample}}{A \text{ Control}} \times 100$$

Anti-inflammatory Activity

Method: Inhibition of Protein Denaturation

Procedure:

- The suppression of protein denaturation approach, which is predicated on the idea that denatured proteins might trigger inflammatory reactions, was used to assess the ethanolic extract's anti-inflammatory effectiveness.
- 0.2 mL of egg albumin, 2.8 mL of phosphate buffer saline (pH 6.4), and 2 mL of ethanolic extract at Various concentrations of the extract (20, 40, 60, 80 and 100 µg/mL) were prepared made up the reaction mixture (5 mL).
- After 15 minutes of incubation at 37°C, the mixtures were heated for five minutes at 70°C. A spectrophotometer was used to detect the absorbance at 660 nm after cooling.
- The common anti-inflammatory medication was diclofenac sodium.
- By contrasting test and control samples, the percentage inhibition of protein denaturation was determined. [25,26,27]

Calculation

$$\% \text{ Inhibition} = \frac{A \text{ Control} - A \text{ Sample}}{A \text{ Control}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical Screening

The ethanolic extract of *Biophytum sensitivum* contained a variety of bioactive components, including flavonoids, tannins, saponins, and alkaloids, according to the initial phytochemical screening. It is well recognised that these phytoconstituents have a major role in the pharmacological actions of therapeutic plants. [28]

Sr. No.	Phytoconstituents	Test Performed	Observation	Result
1.	Alkaloids	Mayer's Test / Dragendorff's Test	Creamy / Orange ppt	+++
2.	Flavonoids	Shinoda Test	Pink / Red coloration	+++
3.	Tannins	Ferric Chloride Test	Blue-black / Green	+++
4.	Phenolic Compounds	Ferric Chloride Test	Deep bluish-black	+++
5.	Saponins	Foam Test	Persistent froth	+
6.	Glycosides	Keller-Killiani Test	Brown ring formation	+
7.	Carbohydrates	Molisch's Test	Violet ring	+
8.	Proteins	Biuret Test	Violet coloration	-
9.	Steroids	Salkowski Test	No reddish-brown ring	-

Table no.1 Phytochemical Screening

Key:

(+++) Strongly present

(+) present

(-) Absent

Fig no. 3 Phytochemical Screening

Chromatographic Column Separation

The ethanolic extract of *Biophytum sensitivum* was subjected to column chromatography utilising silica gel as the stationary phase and ethyl acetate:toluene:formic acid (5:4:1) as the mobile phase. The effective separation of flavonoid-rich elements based on polarity differences was demonstrated by the appearance of a distinct yellow-colored band during elution. Thin layer chromatography (TLC) was used to further examine the elutes collected during the separation process. This led to the discovery of two fractions, F1 and F2. UV, FTIR, NMR, and mass spectroscopy were used to further characterise each fraction, which showed distinctive flavonoid profiles. [29,30]

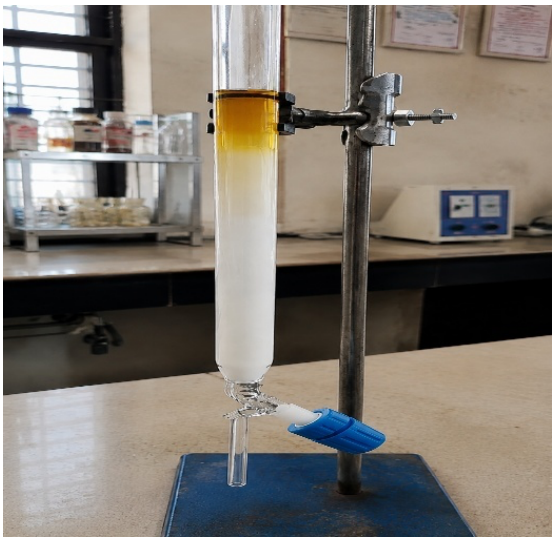

Fig no.4 Flavonoid-rich fraction extracted from *Biophytum sensitivum* ethanolic extract using column chromatography

Sr. No.	λmax (nm)	Absorbance	Interpretation
1	245 nm	3.92	Presence of aromatic conjugated system
2	275 nm	1.25	Characteristic flavonoid absorption peak

Sr. No.	Constituent	Mobile Phase	Ratio	Standard	Standard Rf Value	Sample Rf Value	Observation	Inference
1	Flavonoid	Toluene:Ethyl acetate:Formic acid	5:4:1	Quercetin	0.54	0.53	Yellow fluorescent spot observed under UV light	Flavonoids present

Thin-Layer Chromatography (TLC) [31,32]

The separated fraction from *Biophytum sensitivum* was subjected to thin layer chromatography (TLC) examination utilising silica gel as the stationary phase and Toluene: Ethyl acetate: Formic acid (5:4:1) as the mobile phase. Following chromatographic development, a clear fluorescent spot representing the flavonoid fraction was seen when the TLC plate was examined under UV light. The isolated sample's Rf value of 0.53 was discovered to be extremely near to the normal quercetin Rf value of 0.54. The existence of flavonoid ingredients in the isolated fraction was demonstrated by the similarity in Rf values between the standard and test sample. The ethanolic extract's flavonoid-rich phytoconstituents were successfully isolated, according to the TLC profile.

Table No.2: TLC Observation of Isolated Fraction

UV–Visible Spectroscopy [33,34]

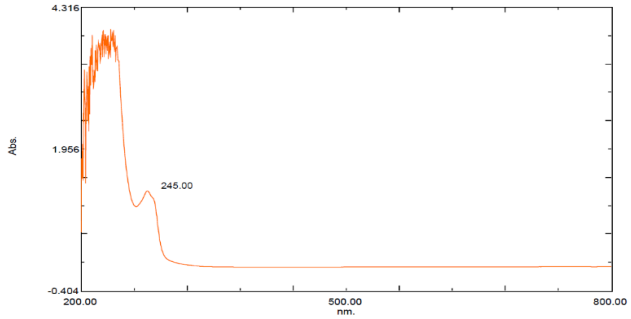

Fig no.5 Flavonoid Fraction UV-Visible Spectral Analysis

Table no.3 UV-Visible Spectral Analysis

The isolated fraction from *Biophytum sensitivum* exhibited distinctive absorption peaks at 245 and 275 nm in UV–visible spectroscopy examination, suggesting the presence of phytoconstituents of the flavonoid type. The UV absorption maxima are linked to π→π* electrical transitions of conjugated systems and aromatic rings that are frequently seen in flavonoid compounds. The resulting spectrum pattern verified that the isolated fraction included polyphenolic compounds

FTIR Spectroscopy [35]

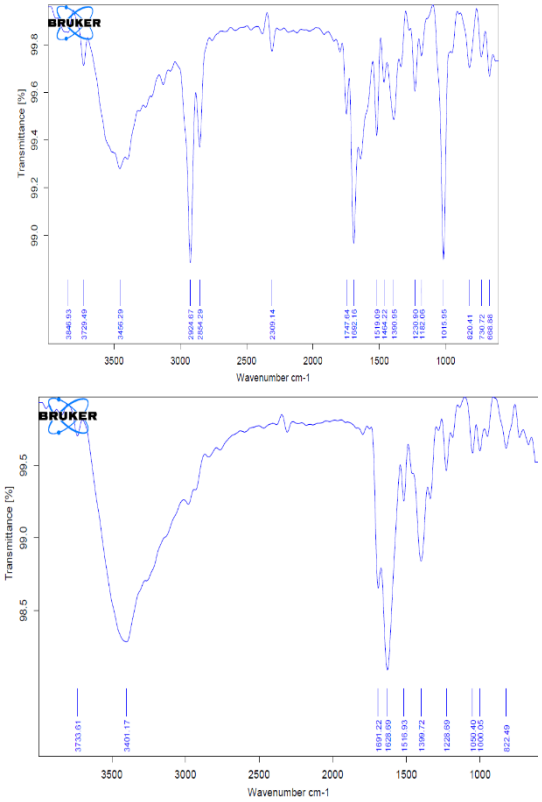

Fig no.6 FTIR study of *Biophytum sensitivum's* bioactive fraction F1.

FTIR study of *Biophytum sensitivum's* bioactive fraction F2

Peak Position (cm^{-1})	Functional Group	Interpretation
3456.29	O–H stretching	Hydroxyl group of phenols/flavonoids
1747.64	C=O stretching	Carbonyl group
1692.16	Conjugated carbonyl	Flavonoid nucleus
1519.09	Aromatic C=C stretching	Aromatic ring system
1464.22	C–H bending	Aromatic compounds
1230.90	C–O stretching	Phenolic compounds
1015.95	C–O–C stretching	Ether/alcohol functional group
820.41	Aromatic C–H bending	Substituted aromatic ring

Table no.4 FTIR Spectral Analysis of Fraction F1

Peak Position (cm^{-1})	Functional Group	Interpretation
3401.17	O–H stretching	Hydroxyl group of phenols/flavonoids
1691.22	C=O stretching	Conjugated carbonyl group
1628.69	C=C stretching	Aromatic flavonoid skeleton
1516.93	Aromatic C=C stretching	Aromatic ring system
1399.72	C–H bending	Aromatic compounds
1228.69	C–O stretching	Phenolic compounds
1050.40	C–O–C stretching	Ether/alcohol functional group
1000.05	C–O stretching	Secondary alcohol/phenolic group
822.49	Aromatic C–H bending	Substituted aromatic ring

Table no. 5 FTIR Spectral Analysis of Fraction F2

The existence of significant functional groups linked to flavonoids and polyphenolic substances was verified by the FTIR spectral analysis of the isolated fractions F1 and F2 from *Biophytum sensitivum*. Broad absorption bands about 3400–3456 cm^{-1} were seen in both fractions, indicating O–H stretching vibrations that corresponded to the hydroxyl groups of flavonoids and phenols. Strong peaks that appeared between 1691 and 1747 cm^{-1} were indicative of carbonyl (C=O) stretching vibrations, which may indicate the existence of conjugated ketones and flavonoid nuclei. Peaks around 1516–1519 cm^{-1} were found as aromatic C=C stretching vibrations, confirming aromatic ring structures characteristic of flavonoids. Additionally, C–O and C–O–C stretching vibrations of phenolic and ether functional groups were represented by peaks between 1230 cm^{-1} and 1000 cm^{-1}. The spectrum measurements showed that flavonoid and phenolic phytoconstituents were abundant in both the F1 and F2 fractions.

Mass Spectrometry [36,37]

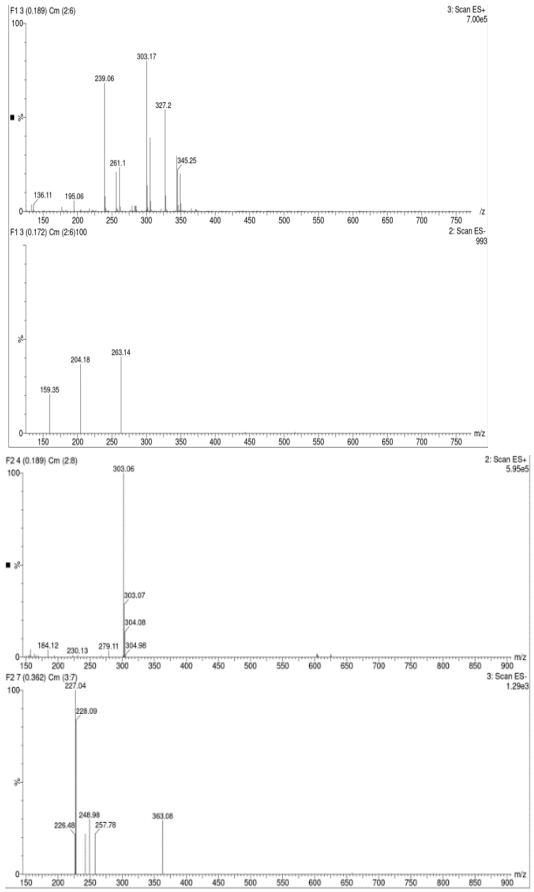

Fig no.7 Mass spectrometry study of *Biophytum sensitivum's* bioactive fraction F1 Mass spectrometry study of *Biophytum sensitivum's* bioactive fraction F2

Peak (m/z)	Interpretation
159.35	Phenolic fragment ion
196.86	Aromatic phytoconstituent
204.18	Polyphenolic fragment
239.06	Flavonoid-related fragment ion
261.10	Oxygenated aromatic compound
263.14	Phenolic metabolite
303.17	Flavonoid-type molecular ion
327.20	Oxygenated flavonoid derivative
345.25	Higher molecular weight phenolic compound

Table no. 6 Mass Spectrometry Analysis of Fraction F1

Peak (m/z)	Interpretation
184.22	Aromatic fragment ion
203.91	Polyphenolic fragment
226.48	Phenolic constituent
239.13	Flavonoid-related fragment ion
248.85	Oxygenated phytoconstituent
257.76	Aromatic metabolite
279.11	Flavonoid-associated compound
303.07	Flavonoid-type molecular ion
304.08	Flavonoid analogue
320.09	Oxygenated flavonoid derivative
363.08	Polyphenolic compound

Table no.7 Mass Spectrometry Analysis of Fraction F2

Several phytoconstituents with varying molecular masses were found in fractions F1 and F2 that were separated from *Biophytum sensitivum*, according to a mass spectrometry analysis. Flavonoid and polyphenolic substances were represented by significant molecular ion peaks in the spectra. Major ion peaks at m/z 303.17, 327.20, 261.10, and 239.06 were seen in fraction F1, suggesting the presence of flavonoid derivatives and oxygenated aromatic compounds. Additionally, fraction F2 showed distinctive molecular ion peaks at m/z 303.07, 304.08, 320.09, and 363.08, indicating the presence of polyphenolic ingredients and chemicals related to flavonoids. Both fractions' phytochemical richness and the existence of bioactive secondary metabolites were validated by the fragmentation pattern and molecular ion distribution.

NMR Spectroscopy[38,39]

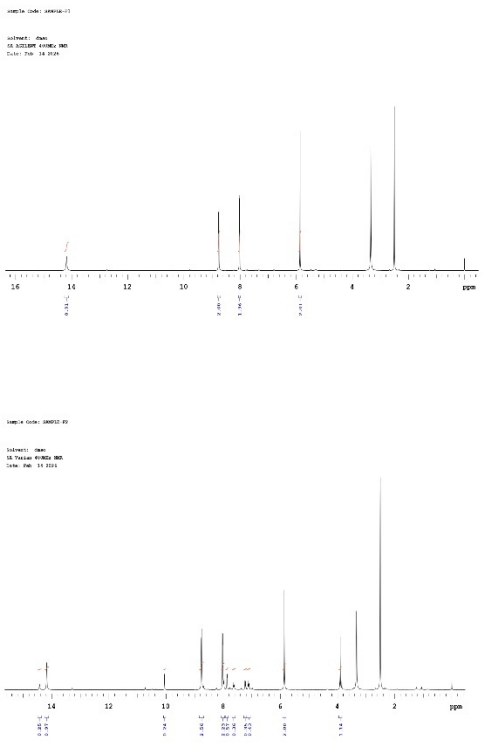

Figure no.8 1H NMR spectra of fractions F1 and F2 obtained at 400 MHz in DMSO-d6.

Fraction	δ (ppm)	Interpretation
F1	14.18	Phenolic –OH proton
F1	8.77–8.01	Aromatic protons
F1	5.86	Olefinic proton
F2	14.18	Phenolic –OH proton
F2	10.05	Aldehydic/phenolic proton
F2	8.75–7.09	Aromatic protons

Table no.8 Interpretation of 1H NMR spectral peaks of fractions F1 and F2

The presence of aromatic and hydroxyl-containing phytoconstituents was shown by the 1H NMR spectra of fractions F1 and F2 obtained in DMSO-d6 at 400 MHz. The existence of flavonoids or polyphenolic chemicals was suggested by the distinctive signal that both fractions displayed at δ 14.18 ppm, which corresponds to highly hydrogen-bonded phenolic –OH groups. Signals at δ 5.86 ppm showed olefinic or oxygenated methine protons, while multiple aromatic proton signals between δ 8.77–7.09 ppm indicated conjugated aromatic systems. Methoxy or other oxygenated functional groups were suggested by peaks in the δ 3.90–3.33 ppm range.

Compared to fraction F1, fraction F2 showed much more aromatic proton signals, suggesting increased chemical complexity and the potential existence of additional phenolic compounds. Overall, the spectrum analysis verified the existence of bioactive secondary metabolites that could support the isolated fractions' anti-inflammatory and antioxidant properties.

Antioxidant Activity[40,41]

- In the DPPH test, the ethanolic extract of *Biophytum sensitivum* demonstrated strong concentration-dependent antioxidant activity. The percentage inhibition was similar to that of ordinary ascorbic acid and rose with concentration.

Sr. no	Sample Code	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean	% Inhibition	IC_{50} (µg/ml)
1	**Control**	-	1.92	1.92	1.92	1.92	-	-
2	**Standard (Ascorbic Acid)**	20	1.46	1.48	1.44	1.46	23.95 %	58.92
		40	1.28	1.25	1.27	1.26	34.37 %	
		60	0.95	0.91	0.93	0.93	51.56 %	
		80	0.78	0.80	0.76	0.78	59.37 %	
		100	0.31	0.29	0.30	0.30	84.37 %	
3	**Isolated Flavonoid Fraction**	20	1.47	1.43	1.45	1.45	24.47 %	56.18
		40	1.18	1.15	1.17	1.16	39.58 %	
		60	0.90	0.87	0.89	0.88	54.16 %	
		80	0.61	0.58	0.60	0.59	69.27 %	
		100	0.	0.	0.	0.27	85.93 %	

			28	26	27			

Table no 9: Antioxidant activity of isolated flavonoid fraction by DPPH assay

Anti-Inflammatory Activity [42,43]

Using the protein denaturation assay, the ethanolic extract of *Biophytum sensitivum* demonstrated significant anti-inflammatory efficacy in a concentration-dependent manner. Comparable to normal diclofenac sodium, the percentage inhibition increased with increasing concentration, indicating efficient protein stabilisation against denaturation.

Sr. no	Sample Code	Concentration (µg/ml)	Test 1	Test 2	Test 3	Mean	% Inhibition	IC_{50} (µg/ml)
1	**Control**	-	1.555	1.555	1.555	1.55	-	-
2	**Standard (Diclofenac Sodium)**	20	1.36	1.34	1.35	1.35	12.90 %	69.95
		40	1.14	1.10	1.12	1.12	27.74 %	
		60	0.88	0.85	0.86	0.86	44.51 %	
		80	0.64	0.61	0.63	0.62	60.00 %	
		100	0.20	0.18	0.19	0.19	87.74 %	
3	**Isolated Flavonoid Fraction**	20	1.24	1.22	1.23	1.23	20.64 %	62.87
		40	1.03	1.00	1.02	1.01	34.83 %	
		60	0.80	0.77	0.79	0.78	49.67 %	
		80	0.56	0.53	0.55	0.54	65.16 %	
		100	0.30	0.27	0.28	0.28	81.93 %	

Table no. 10: Anti-inflammatory activity of isolated flavonoid fraction by protein denaturation assay.

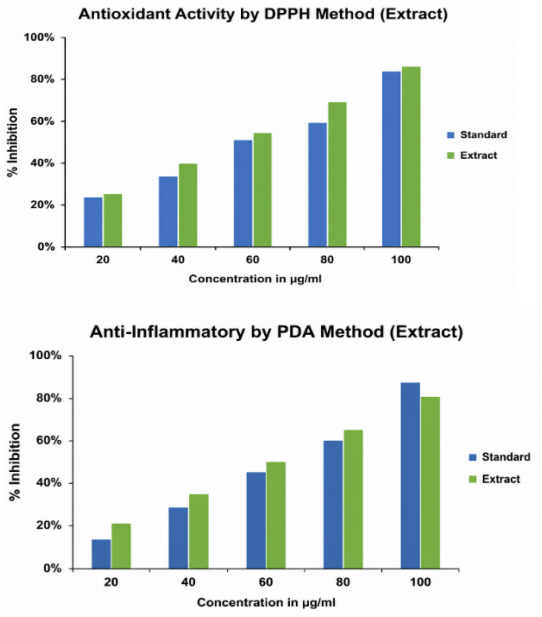

Fig.no.9 % Inhibition of DPPH Radical
Fig.no.10 % Inhibition of Protein Denaturation

DISCUSSION

According to the current study, the ethanolic extract of *Biophytum sensitivum* has significant anti-

inflammatory and antioxidant qualities. This is probably because it contains a variety of phytoconstituents that were found during the initial phytochemical analysis, such as flavonoids, tannins, phenolic compounds, alkaloids, saponins, and glycosides. The medicinal and pharmacological relevance of these secondary metabolites is acknowledged. Additionally, flavonoid-rich fractions (F1 and F2) were obtained by column chromatographic extraction of the ethanolic extract. These fractions were further examined using TLC, UV-visible spectroscopy, FTIR, NMR, and mass spectrometry. The presence of aromatic, hydroxyl, carbonyl, and conjugated functional groups which are typical of flavonoid and polyphenolic compounds was verified by spectroscopic investigations. The DPPH free radical scavenging assay, which measures antioxidant activity, showed a concentration-dependent rise in percentage inhibition. Comparable to the standard ascorbic acid, the ethanolic extract showed 24.47%, 39.58%, 54.16%, 69.27%, and 85.93% inhibition at doses of 20, 40, 60, 80, and 100 µg/mL, respectively. The extract's IC^{50} value of 56.18 µg/mL indicated a potent ability to scavenge free radicals. The presence of flavonoids and phenolic chemicals, which can donate hydrogen atoms or electrons to neutralise free radicals and lessen oxidative stress, may be responsible for the antioxidant capacity. Similarly, concentration-dependent suppression was seen in the anti-inflammatory activity assessed using the inhibition of protein denaturation technique. At doses between 20 and 100 µg/mL, the extract showed inhibitions of 20.64%, 34.83%, 49.67%, 65.16%, and 81.93%. When compared to the standard, diclofenac sodium, the extract's IC_{20} value was found to be 62.87 µg/mL, suggesting substantial anti-inflammatory potential. Flavonoids, tannins, and phenolic compounds which are known to stabilise proteins and suppress inflammatory mediator may be responsible for the reported activity. The results of this study offer scientific support for the traditional usage of *Biophytum sensitivum* to treat inflammation and diseases linked to oxidative stress. The identification and isolation of fractions rich in flavonoids provide additional evidence that polyphenolic components play a part in the biological activities that have been reported. The study suggests that the plant may be a natural source for the production of therapeutic chemicals with antioxidant and anti-inflammatory qualities. To ascertain its therapeutic efficacy and safety, however, additional research is required, including mechanistic studies, in vivo evaluations, and the structural clarification and purification of active ingredients. [44-48]

CONCLUSION

The present study indicates that the ethanolic extract of *Biophytum sensitivum* is rich in significant phytochemical constituents, including flavonoids, tannins, phenolic compounds, alkaloids, saponins, and glycosides, which are likely responsible for its observed biological activities. Preliminary phytochemical analysis confirmed the presence of these important secondary metabolites, thereby emphasizing the medicinal importance of the plant.The extract demonstrated considerable antioxidant activity in the DPPH free radical scavenging assay and exhibited significant anti-inflammatory properties through the inhibition of protein denaturation, both in a concentration-dependent manner. These findings imply that *Biophytum sensitivum* possesses a promising ability for free radical scavenging and protein stabilization, which may contribute to the prevention and management of conditions associated with oxidative stress and inflammation. The results provide scientific support for the traditional use of *Biophytum sensitivum* in herbal medicine and suggest that the plant could serve as a valuable natural resource for the development of antioxidant and anti-inflammatory therapeutic agents. However, further advanced research is necessary, involving the isolation, purification, and structural characterization of active components, as well as comprehensive mechanistic studies and in vivo pharmacological evaluations, to thoroughly establish its therapeutic efficacy and safety profile.

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