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In vitro screening of bioactive extracts from *Abutilon indicum (L.)* Sweet leaves for anti-inflammatory and antitubercular activities

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ABSTRACT

Background: *Abutilon indicum (L.)* Sweet is an important medicinal plant widely used in traditional medicine for the treatment of inflammation, respiratory disorders, wounds, ulcers, and various infectious diseases. Despite its extensive ethnomedicinal use, comparative studies evaluating the anti-inflammatory and antitubercular potential of different solvent extracts of its leaves remain limited. **Objective:** To evaluate the phytochemical composition, *in vitro* anti-inflammatory activity, and antitubercular potential of various solvent extracts of *A. indicum*. **Materials and Methods:** Dried leaves of *A. indicum* were successively extracted using petroleum ether, benzene, chloroform, acetone, methanol, ethanol, and water in a Soxhlet apparatus. Extractive values and preliminary phytochemical profiles were determined using standard procedures. Anti-inflammatory activity was assessed by human red blood cell (HRBC) membrane stabilization and protein denaturation assays at concentrations ranging from 10-100 µg/mL. Antitubercular activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was evaluated using the Microplate Alamar Blue Assay (MABA). All experiments were performed in triplicate and results were expressed as mean ± SD. **Results:** The aqueous extract showed the highest extractive yield (27.5%), followed by methanol (7.835%). Preliminary phytochemical screening revealed the presence of flavonoids, phenolics, tannins, alkaloids, glycosides, steroids, and saponins in various solvent fractions. In the HRBC membrane stabilization assay, methanol, ethanol, and aqueous extracts exhibited the highest membrane protection, recording 68.69%, 65.47%, and 64.99%, respectively, at 100 µg/mL. Similar trends were observed in the protein denaturation assay, where methanol (77.6%), aqueous (75.91%), and ethanol (72.5%) extracts demonstrated the strongest inhibitory activity. Antitubercular screening revealed significant activity among the extracts, with benzene and acetone fractions exhibiting the lowest minimum inhibitory concentrations (MICs) of 1.6 and 3.12 µg/mL, respectively, followed by the aqueous extract (6.25 µg/mL). **Conclusion:** The results demonstrate that *A. indicum* possess significant anti-inflammatory and antitubercular activities, with biological activity varying according to the extraction solvent. Polar extracts exhibited superior anti-inflammatory effects, whereas benzene and acetone extracts showed the strongest antimycobacterial activity. These findings support the traditional medicinal use of *A. indicum* and warrant further phytochemical characterization, bioassay-guided isolation, and *in vivo* investigations to identify the active constituents responsible for the observed activities.

Keywords: *Abutilon indicum*, HRBC membrane stabilization, Protein denaturation assay, Anti-inflammatory activity, Antitubercular activity, Bioactive extracts.

INTRODUCTION

Medicinal plants continue to play an important role in drug discovery and healthcare owing to their rich diversity of bioactive secondary metabolites. A significant proportion of modern pharmaceuticals are either derived directly from natural products or developed from natural-product-inspired compounds, highlighting the continuing relevance of plants as sources of therapeutic agents [1,2]. The increasing prevalence of chronic inflammatory disorders and infectious diseases has renewed interest in medicinal plants as potential sources of safer and more effective bioactive compounds.

Plant extracts are therapeutically important because they contain diverse secondary metabolites capable of interacting with multiple biological targets. Such multi-component systems are particularly relevant in the management of complex diseases involving interconnected pathological pathways. Numerous studies have demonstrated that medicinal plant extracts possess antioxidant, anti-inflammatory, antimicrobial, and other pharmacological properties, supporting their continued importance in drug discovery and development [3-5]. The biological activities of plant extracts are often attributed to the combined action of various phytochemicals, which may act synergistically to produce therapeutic effects [6,7]. Consequently, medicinal plants continue to be investigated as potential sources of novel bioactive compounds and complementary therapeutic agents.

Inflammation is a complex biological response involved in the pathogenesis of numerous diseases, including arthritis, cardiovascular disorders, and neurodegenerative conditions [8]. Although conventional anti-inflammatory drugs are widely used, their prolonged administration is often associated with adverse effects. Consequently, there is growing interest in identifying plant-derived alternatives with anti-inflammatory potential. Recent evidence suggests that plant-derived extracts exert antibacterial effects through multiple mechanisms, including disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and induction of oxidative stress in bacterial cells [9]. *In vitro* assays such as human red blood cell (HRBC) membrane stabilization and protein denaturation inhibition are commonly employed as preliminary screening tools for evaluating anti-inflammatory activity [10,11].

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the leading infectious causes of mortality worldwide [12]. Despite the availability of effective chemotherapy, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, together with treatment-associated toxicity and poor patient compliance, continues to hamper global TB control efforts [13]. These challenges have prompted the search for novel antimycobacterial agents from natural sources, particularly medicinal plants traditionally used for respiratory and infectious ailments [14,15].

A. indicum Sweet, a member of the family Malvaceae, is a widely distributed medicinal shrub found throughout tropical and subtropical regions [16]. The plant has been extensively used in traditional systems of medicine for the treatment of inflammation, wounds, ulcers, fever, respiratory disorders, diabetes, and various other ailments [17,18]. Previous pharmacological investigations have reported antioxidant, anti-inflammatory, antimicrobial, antidiabetic, immunomodulatory, hepatoprotective, wound-healing, and anthelmintic activities from different parts of the plant [16,19-20]. Although several biological properties of *A. indicum* have been documented, information regarding the comparative anti-inflammatory and antitubercular potential of different solvent extracts of its leaves remains limited. Therefore, the present study was undertaken to evaluate the *in vitro* anti-inflammatory and antitubercular activities of different solvent extracts of *A. indicum* Sweet leaves using HRBC membrane stabilization assay, protein denaturation assay, and Microplate Alamar Blue Assay (MABA).

MATERIAL AND METHODS

Collection of plant material

Dried leaves of *A. indicum* Sweet (Figure 1) were gathered from Panapatti, Tamil Nadu (10.8765° N, 77.1003° E) and are authenticated by Dr. K Madhava Chetty, Assistant Professor in the Department of Botany at Sri Venkateswara University, Tirupati-5 17502, Andhra Pradesh, India. The collected leaves were thoroughly rinsed in running tap water, followed by distilled water. They were then shade-dried for 20 days, ground into a powder, and stored in containers for future use.

Extraction procedure

Powdered plant material underwent a series of extractions using a Soxhlet extractor. This method involves extracting the active compounds present in the plant material through various solvents, ranging from non-polar to polar. The solvents employed included petroleum ether, benzene, chloroform, acetone, methanol, ethanol, and water. This extraction technique was applied for both pilot studies and large-scale extractions using the Soxhlet apparatus.

Approximately 200 g of powdered leaves were placed in a cellulose thimble and loaded into the main chamber of a Soxhlet extractor. Extraction was carried out using 500 mL of the respective solvent for 12 h at a temperature corresponding to the boiling point of each solvent. This continuous process of evaporation, condensation, and siphoning enabled thorough extraction, resulting in a progressively more concentrated extract in the lower flask. Once the process was complete, the lower flask was removed, the solvent was recovered through simple distillation, and the concentrated extract was weighed to determine the percentage yield. The plant residue in the thimble was then dried and underwent further extraction with benzene and other solvents of greater polarity to ensure a complete phytochemical extraction. Following extraction, the yield percentage was computed and documented. The resultant extracts were then analyzed through phytochemical screening to determine the presence of key bioactive components. The crude extracts obtained by successive extraction using different solvents are subjected to preliminary phytochemical screening by methods described by Kahandelwal (2007) [21].

Anti-inflammatory study by HRBC membrane stabilization method [22]

For the HRBC assay, leftover human blood samples scheduled for disposal were obtained from [Kallyani Medicare, Pappinisseri, Kannur - 670561]. The samples were completely anonymized and de-identified prior to use, and no personal information was accessible to the investigators. A sample of fresh whole human blood (10 mL) was collected and placed into heparinized centrifuge tubes. This blood sample was mixed with an equal volume of Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and 100 mL of distilled water) and then centrifuged with 0.85% isosaline (prepared by dissolving 8.5 g of NaCl in water, autoclaving at 121 °C for 15 min, and then cooling to room temperature). To 1 mL of the HRBC suspension, equal volumes of *A. indicum* extracts at five different concentrations (10, 20, 50, 75, 100 µg/mL) were introduced. All assay mixtures were incubated at 37 °C for half an hour and then centrifuged. The haemoglobin content in the supernatant was measured using a spectrophotometer set to 560 nm. Assay was analysed in triplicates for all extracts. The percentage of haemolysis was determined using the following formula:

$$\text{Percentage of hemolysis} = (\text{OD of the test solution} \div \text{OD of the control}) \times 100$$

Consequently, the percentage of protection can be calculated using the equation below:

$$\text{Percentage of protection} = 100 - \text{Hemolytic percentage}$$

Ethical Considerations

All study procedures were conducted in accordance with institutional biosafety regulations and established ethical guidelines governing the use of anonymized residual biological samples for research purposes. The study was approved under reference number BOT/HRBC/2024/01.

Anti-inflammatory activity by Protein Denaturation method [23]

The reaction mixture (5 mL) was composed of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS with pH 6.4), and 2 mL of varying concentrations (10, 20, 50,75, 100 µg/L) of the *A.*

indicum extracts. Test was conducted for all the extracts under study. An equal volume of double distilled water was used as the control. The mixtures were incubated at 37 °C in a BOD incubator for 15 min and subsequently heated at 70 °C for 5 min. After cooling, the absorbance was measured at 660 nm using the vehicle as a blank. All reactions are carried out in triplicates. The percentage of inhibition of protein denaturation was calculated using the following equation:

$$Percentage\ of\ inhibition = \frac{AC660 - AT660}{AC660} \times 100$$

Where AC represents the absorbance of the control solution and AT denotes the absorbance of the test sample.

Antitubercular studies using MAB Assay

The efficacy of the compounds against *M. tuberculosis* was assessed using the Microplate Alamar Blue Assay (MABA) [24]. *Mycobacterium tuberculosis* H37Rv (ATCC 27294) used in the Microplate Alamar Blue Assay (MABA) was obtained from Maratha Mandal Dental Institute, Belagavi (Belgaum), Karnataka, India. The antitubercular studies were carried out at the same institution under appropriate laboratory and biosafety conditions. This approach is non-toxic, uses a thermally stable reagent, and shows a significant correlation with both proportional and BACTEC radiometric methods.

To minimize medium evaporation in the test wells during incubation, 200 µL of sterile deionized water was placed into all the outer wells of a sterile 96-well plate. Each well of the 96-well plate received 100 µL of Middlebrook 7H9 broth and serial dilutions of the compounds were performed directly on the plate. The tested final drug concentrations ranged from 100 to 0.2 µg/mL. After covering the plates, they were sealed with parafilm and incubated at 37 °C for five days. Following this incubation, 25 µL of a freshly prepared solution consisting of equal parts Alamar Blue reagent and 10% Tween 80 was added to the plate, which was subsequently incubated for another 24 h. A blue coloration in the wells indicated no bacterial growth, while a pink coloration indicated the presence of growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the drug that inhibited the color change from blue to pink [25].

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Data were compiled and analyzed using Microsoft Excel 2019. The study was primarily designed to compare the relative biological activities of different solvent extracts of *A. indicum*. Therefore, the results were interpreted descriptively based on mean values and standard deviations.

RESULTS

The extraction of leaves from *A. indicum* using solvents of different polarities resulted in extracts with unique colors and varying percentage yields. The aqueous extract produced the highest yield (27.5% w/w) with a brown hue, indicating that water is a very effective solvent for obtaining polar phytochemicals. The methanol extract also demonstrated a relatively high yield (7.835% w/w), showcasing its ability to extract a wide range of bioactive compounds. In contrast, solvents such as acetone (0.605% w/w), ethanol (0.8% w/w), chloroform (1.23% w/w), and benzene (1.16% w/w) yielded comparatively low results, which suggests a limited solubility of leaf constituents in these solvents. The petroleum ether extract produced a moderate yield (2.39% w/w) with a greenish-brown tint, indicating the presence of nonpolar compounds like fats, waxes, and pigments.

Preliminary Phytochemical analysis

The initial phytochemical evaluation of *A. indicum* leaf extracts showed a variety of secondary metabolites based on the solvent utilized (Table 1) Carbohydrates were found exclusively in polar solvents like acetone, methanol, and water, indicating their solubility in more polar environments. Alkaloids were primarily identified in chloroform, methanol, and ethanol extracts, highlighting their preference for moderately polar solvents. Glycosides were detected in both acetone and methanol extracts, with the modified Borntrager's test confirming their presence solely in acetone.

Steroids and flavonoids were consistently found in nearly all solvent extracts, suggesting that these compounds are broadly distributed in the plant and soluble in both polar and nonpolar solvents. Saponins were identified in methanol and aqueous extracts, aligning with their high polarity. Phenolic compounds and tannins were prominently present in methanol, ethanol, chloroform, and aqueous extracts, further indicating their affinity for polar solvents. Conversely, fixed oils and fats were primarily obtained using petroleum ether and benzene, which are nonpolar solvents.

Anti-inflammatory studies on various extracts of *A. indicum* Sweet leaves

The anti-inflammatory properties of *A. indicum* were assessed through two complementary *in vitro* assays, HRBC membrane stabilization and protein denaturation. Each method offers mechanistic insights, as the membrane stabilization approach models protection of lysosomes from lysis, while the inhibition of protein denaturation indicates how phytoconstituents can prevent changes in inflammatory proteins.

The HRBC membrane stabilization assay was performed to assess the anti-inflammatory properties of various solvent extracts of *A. indicum* at concentrations between 10 to 100 µg/mL. The findings revealed a distinct dose-dependent enhancement in membrane stabilization for all extracts, suggesting that the protective effect against hemolysis induced by hypotonic conditions intensifies with higher concentrations (Table 2, Figure 3). Among the extracts tested, methanol, ethanol, and water demonstrated the strongest activity, whereas petroleum ether, benzene, and chloroform exhibited relatively weaker effects.

At the minimum concentration of 10 µg/mL, stabilization values varied from 8.87% to 24.98%, with water showing the most robust activity, followed closely by methanol and ethanol. As the concentration was increased to 50 µg/mL, a significant rise in stabilization was observed, particularly in the polar extracts. Methanol achieved a stabilization of 35.90% and water reached 37.27%, while ethanol recorded 29.76%. In contrast, the benzene and chloroform extracts showed only slight progress, remaining at fairly low levels. By the time the concentration reached 75 µg/mL, the differences became more evident, with methanol (49.87%), water (51.09%), and ethanol (39.18%) displaying notably higher stabilization compared to acetone (27.56%) and the non-polar extracts, which still exhibited minimal activity.

The most significant effect was observed at 100 µg/mL, with methanol, ethanol, and water extracts achieving stabilization rates of 68.69%, 65.47%, and 64.99%, respectively, which clearly outperformed all other extracts. Acetone showed moderate efficacy at 37.06%, whereas petroleum ether (24.90%), benzene (21.80%), and chloroform (23.97%) were the least effective. This difference related to polarity highlights that the bioactive compounds contributing to membrane stabilization are more soluble in polar solvents, suggesting that phenolics, flavonoids, and tannins play a role in imparting anti-inflammatory properties. The results affirm that the potential for HRBC membrane stabilization is significantly dependent on both concentration and the specific extract used. Polar solvent extracts, particularly methanol, ethanol, and water, were the most effective in preventing hemolysis, indicating a high presence of pharmacologically active compounds. Acetone exhibited intermediate efficacy, while non-polar solvents, such as petroleum ether, benzene, and chloroform, contributed only minimally. These observations suggest that polar phytoconstituents are primarily responsible for the significant anti-inflammatory activity observed in this assay.

The protein denaturation assay was conducted and all extracts exhibited a concentration-dependent increase in inhibition, although the level of activity varied significantly depending on the solvent used (Table 3, Figure 4). At the lowest concentration of 10 µg/mL, the inhibition rates were relatively low among all extracts. Non-polar extracts such as petroleum ether (14.8%), benzene (12.09%), chloroform (10.45%), and acetone (10.32%) demonstrated minimal action. In contrast, polar extracts like ethanol (24.6%), methanol (26.2%), and water (19.7%) showed increased inhibition even at this lower concentration, suggesting a greater protective effect. This initial observation indicated that polar phytoconstituents play a crucial role in protein stabilization.

As the concentration increased, the activity steadily rose. At 50 µg/mL, methanol (41.84%) and water (42.9%) showed significantly greater inhibition than ethanol (36.56%) and all non-polar extracts. Interestingly, chloroform exhibited a moderate increase (26.96%), surpassing acetone (19.4%) and benzene (17.56%). Petroleum ether maintained a consistent but relatively weaker effect (23.98%).

At 75 µg/mL, the distinction between polar and non-polar extracts became more evident. Methanol and water displayed strong inhibition at 54.29% and 57.67%, respectively, while ethanol reached 47.34%. Chloroform (32.9%) and petroleum ether (29.65%) showed moderate stabilization, with benzene and acetone remaining at lower levels. The maximum activity was recorded at the highest concentration of 100 µg/mL, with methanol (77.6%), water (75.91%), and ethanol (72.5%) demonstrating significant inhibition of protein denaturation, comparable to standard anti-inflammatory agents. Chloroform also achieved a notable 41.6%, while petroleum ether (33.7%), benzene (33.06%), and acetone (31.9%) proved to be considerably less effective.

The findings clearly indicated that the anti-inflammatory activity, as measured by protein denaturation, was heavily influenced by solvent polarity. Methanolic, ethanolic, and aqueous extracts exhibited superior protein stabilization, likely due to the presence of polar phytoconstituents such as flavonoids, phenolics, and tannins. Acetone and chloroform showed moderate effects, while non-polar extracts like petroleum ether and benzene consistently demonstrated the weakest activity. This trend strongly suggests that polar bioactive compounds are primarily responsible for the anti-inflammatory potential of Abutilon indicum, as they can efficiently protect proteins from heat-induced denaturation

Antitubercular activities of *A. indicum*

Tests against *Mycobacterium tuberculosis* (H37Rv strain, ATCC 27294) showed that different solvent extracts from *A. indicum* varied greatly in how well they could stop bacterial growth. All extracts were effective at higher concentrations (Table 4, Figure 5), but each one started to lose effectiveness at different points that helped to identify the minimum inhibitory concentration (MIC) for each extract (Figure 7)

The benzene extract was the most powerful, staying effective down to 1.6 µg/mL, but stopped working at 0.8 µg/mL. This made it the top performer among all the extracts. Acetone extract remained effective until 3.12 µg/mL and lost its effect at 1.6 µg/mL, also showing strong results. The water-based (aqueous) extract worked moderately well, being effective up to 6.25 µg/mL before it lost potency.

On the other hand, petroleum ether, chloroform, and methanol extracts all showed the same pattern: they were effective down to 12.5 µg/mL but not at lower concentrations, making them less potent than the benzene, acetone, and aqueous extracts. Ethanol extract was the weakest, losing its effect at 25 µg/mL and needing the highest amount to stop the bacteria from growing.

The Microplate Alamar Blue Assay (MABA) depicted in the case of standard anti-tubercular drugs (Figure 6), Pyrazinamide, Ciprofloxacin, and Streptomycin against *Mycobacterium tuberculosis*. based on the Alamar Blue indicator's color shift from blue to pink, signalling bacterial viability. According to the results, Pyrazinamide and Ciprofloxacin maintained blue coloration (indicating no bacterial growth) up to 3.12 µg/mL, while Streptomycin was effective up to 6.25 µg/mL, establishing these as their respective MICs.

In comparison, the benzene extract of *A. indicum* was effective up to 1.6 µg/mL, which is lower than the MICs of the standard drugs, indicating a strong antimycobacterial effect. The acetone extract displayed efficacy up to 3.12 µg/mL, similar to Pyrazinamide and Ciprofloxacin, whereas the other extracts showed moderate to weak activity. These findings suggest that the bioactive compounds in *A. indicum*, particularly in its nonpolar and semipolar fractions, have significant inhibitory effects against *M. tuberculosis.* This supports its traditional use and highlights its potential as a natural source for new anti-tubercular drugs.

In summary, the overall potency ranking of the extracts is as follows: Benzene > Acetone > Aqueous > Petroleum Ether = Chloroform = Methanol > Ethanol. This ranking clearly indicates that the most effective anti-tubercular activity of *A. indicum* is found in the benzene and acetone fractions, with moderate activity in the aqueous extract, while the other solvents show comparatively weaker effects.

Figure 1: *A. indicum* (L.) Sweet

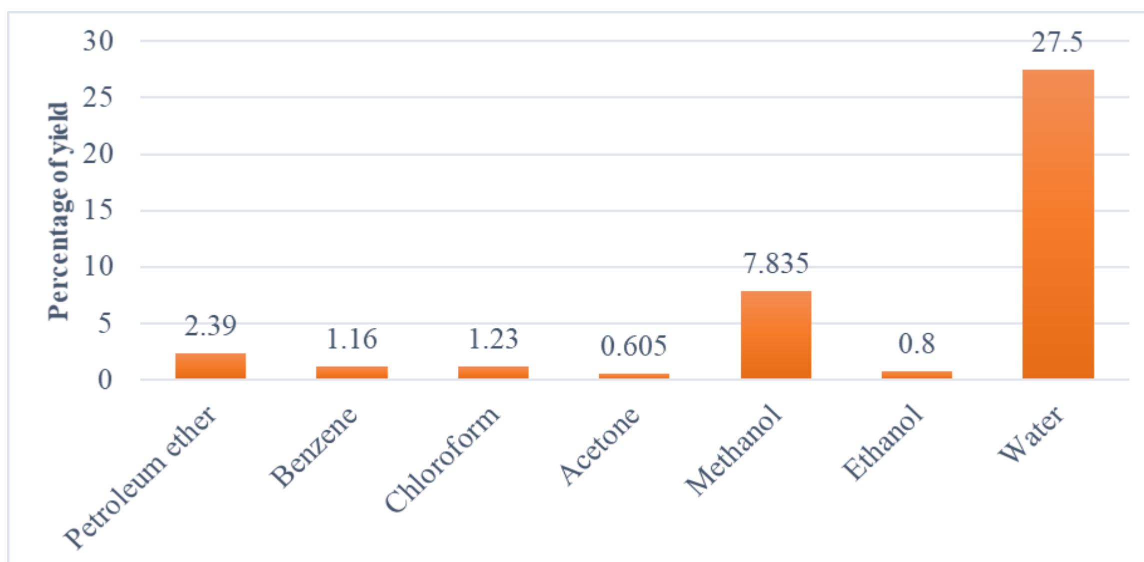

Figure 2: Extractive yield of *A. indicum (L.)* using different solvents

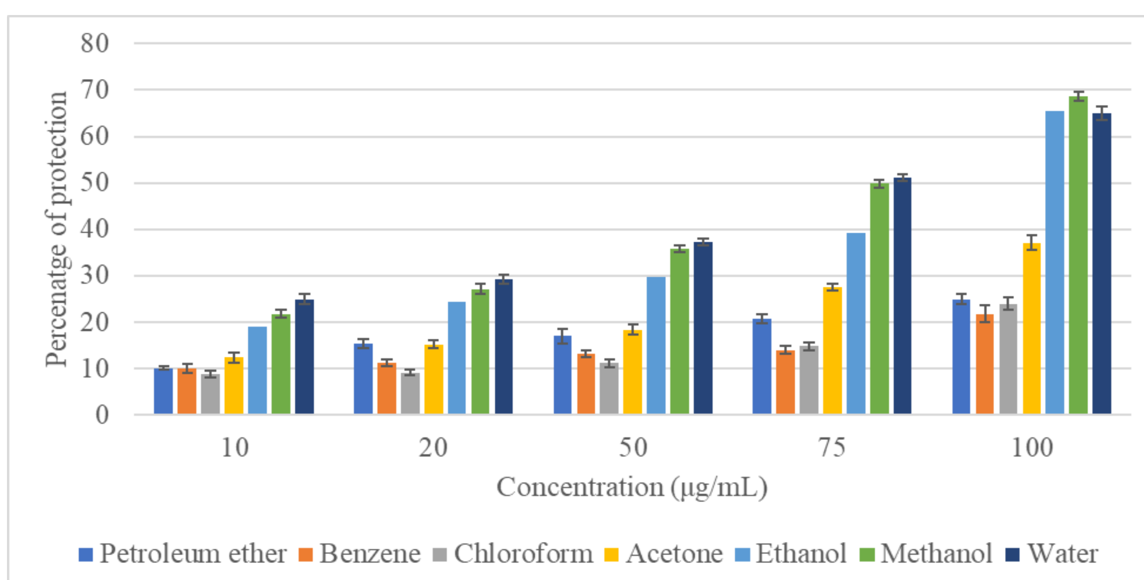

Figure 3: Anti-inflammatory activity of various extracts of *A. indicum (L.)* by HRBC membrane stabilization assay

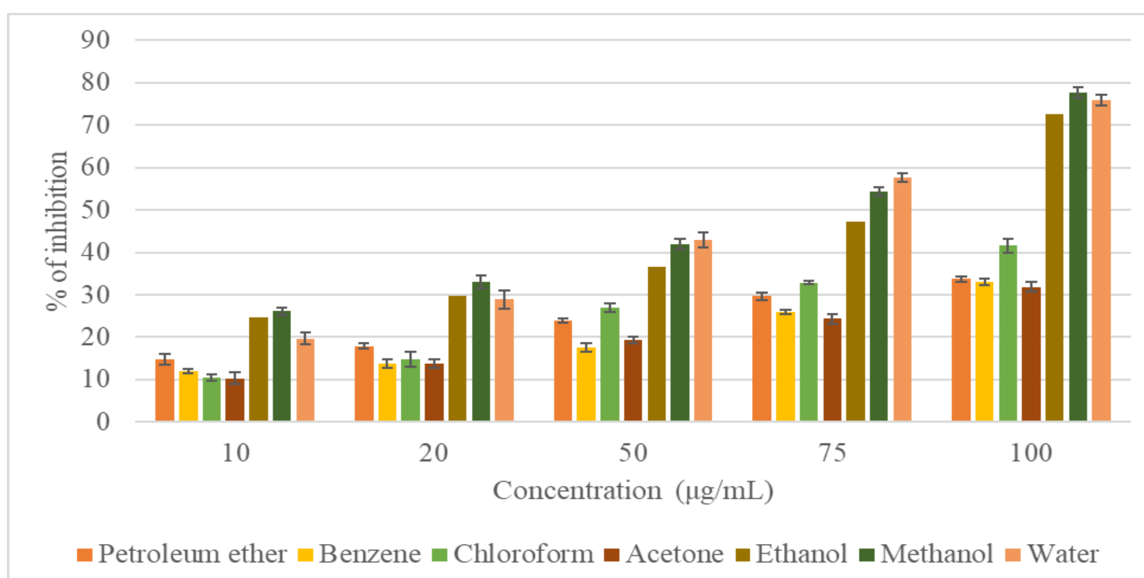

Figure 4: Anti-inflammatory activity of various extracts of *A. indicum (L.)* by Protein denaturation assay

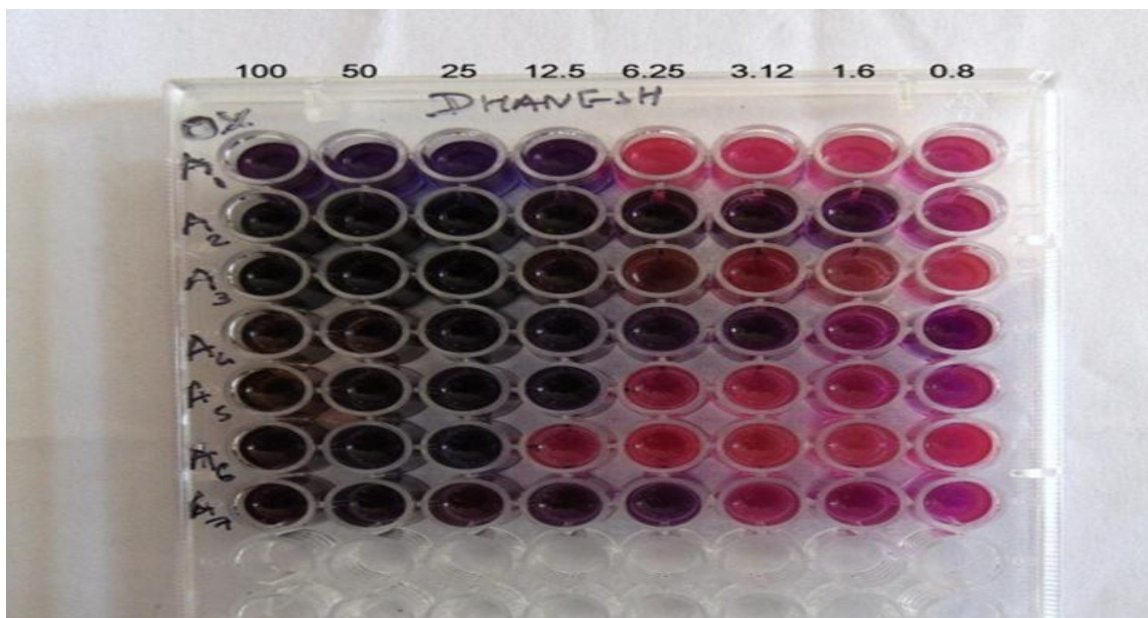

Figure 5: MABA study of *A. indicum* (L) Sweet leaves various extracts
A1Petroleum ether A2 Benzene A3 Chloroform A4 Acetone A5 Methanol A6 Ethanol A7Aqueous

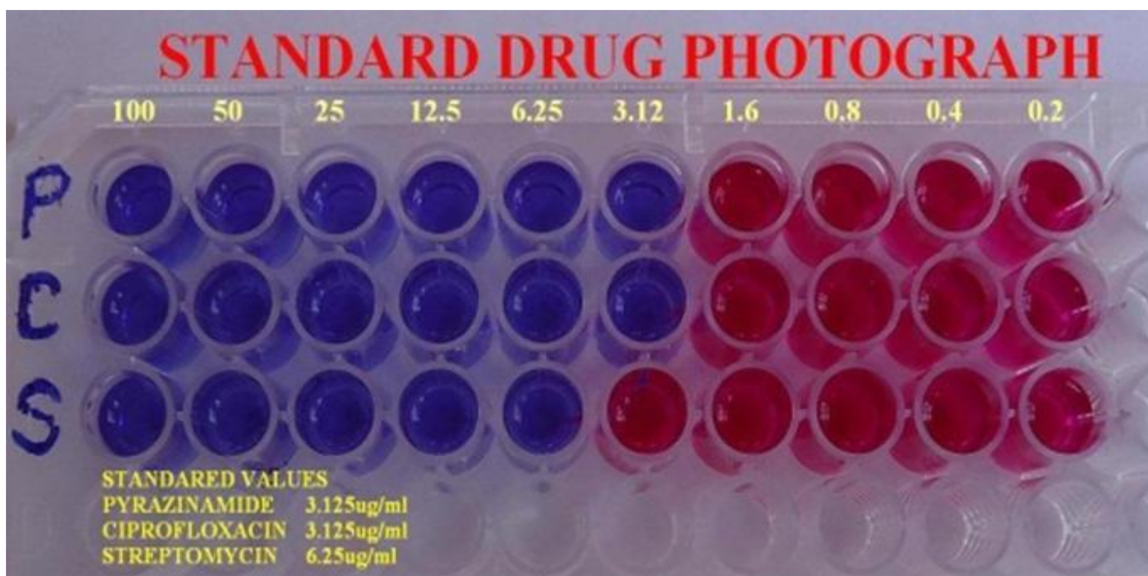

Figure 6: MABA study of standard drugs

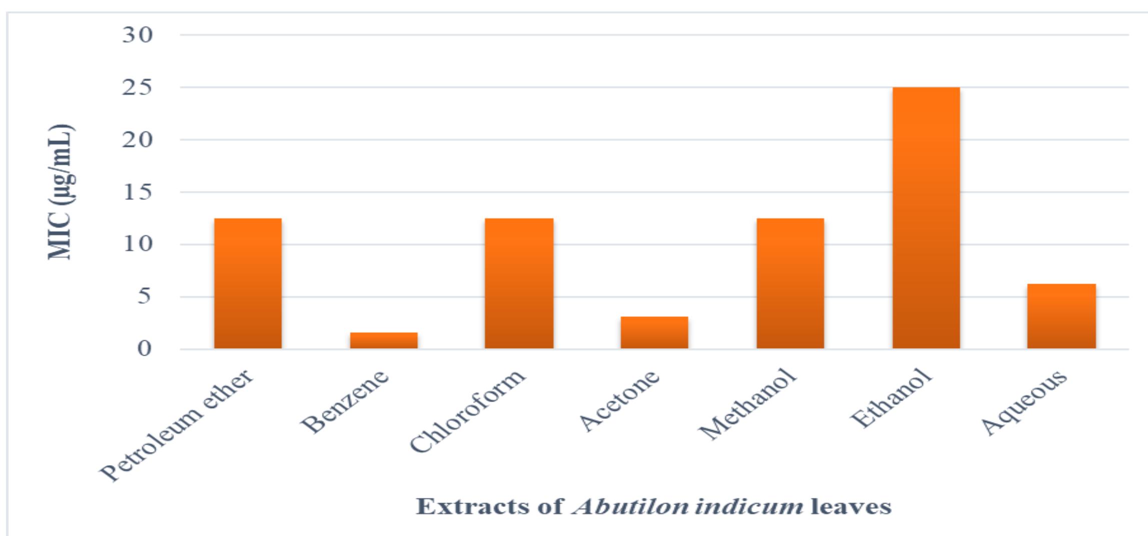

Figure 7: MIC value of MABA assay of various extracts *A. indicum (L)*

Table 1: Phytochemical screening of *A. indicum (L.)* various extracts

S. No	Test	Phytochemical test	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Ethanol	Aqueous
1	Alkaloids	Mayer's test	-	-	+	-	+	+	-
		Wagner's test	-	-	+	-	+	-	-
		Dragondroff's test	-	-	+	-	+	+	-
		Hager's test	-	-	+	-	+	-	-
2	Glycosides	Legal's test	-	-	-	+	+	-	-
		Keller killiani test	-	-	-	+	+	-	-
		Borntrager's test	-	-	-	-	-	-	-
		Modified borntragers test	-	-	-	+	-	-	-
3	Steroids	Libermann burchards test	+	+	+	+	+	+	+
		Salkowski test	+	+	+	+	+	+	+
3	Saponins	Foam test	-	-	-	-	+	-	+
4	Flavonoids	Shinoda test	+	+	+	+	+	+	+
		alkaline reagent test	+	+	+	+	+	-	-
5	Phenolic and Tannins	Ferric chloride test	-	-	+	-	+	+	+
		Lead acetate test	-	-	-	-	+	-	-
		Gelatin test	-	-	-	-	+	-	-
6	Resins	Acetone water test	+	+	-	-	-	-	-

Table 2: HRBC Membrane stabilization Assay of various extracts of *A. indicum (L)*

Concentration (µg/mL)	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Methanol	Water
10	10.17±0.34	10.09±0.89	8.87±0.64	12.39±1.09	18.96±1.2	21.84±0.9	24.98±1.03
20	15.32±0.94	11.33±0.76	9.16±0.57	15.28±0.9	24.36±0.78	27.18±1.06	29.32±1.01
50	17.02±1.6	13.15±0.78	11.21±0.8	18.4±1.05	29.76±1.09	35.9±0.7	37.27±0.76
75	20.81±1.03	14.03±0.84	14.89±0.87	27.56±0.73	39.18±1.6	49.87±0.9	51.09±0.75
100	24.9±1.09	21.8±1.8	23.97±1.32	37.06±1.56	65.47±1.3	68.69±1.04	64.99±1.56

Table 3: Protein denaturation Assay of various extracts of *A. indicum (L)*

Concentration (µg/mL)	Petroleum ether	Benzene	Chloroform	Acetone	Ethanol	Methanol	Water
10	14.8±1.2	12.09±0.56	10.45±0.76	10.32±1.4	24.6±1.45	26.2±0.88	19.7±1.37
20	17.9±0.67	13.7±0.98	14.8±1.67	13.78±0.93	29.87±1.67	32.98±1.52	28.9±2.1
50	23.98±0.58	17.56±1.04	26.96±1.05	19.4±0.78	36.56±0.93	41.84±1.29	42.9±1.79
75	29.65±0.9	25.98±0.59	32.9±0.39	24.32±1.06	47.34±1.3	54.29±0.97	57.67±1.1
100	33.7±0.65	33.06±0.87	41.6±1.7	31.9±1.2	72.5±2.06	77.6±1.33	75.91±1.34

Table 4: Anti-tubercular activity of various extracts of *A. indicum (L)*

Sl. No.	Sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
1	Petroleum ether	S	S	S	S	R	R	R	R
2	Benzene	S	S	S	S	S	S	S	R
3	Chloroform	S	S	S	S	R	R	R	R
4	Acetone	S	S	S	S	S	S	R	R
5	Methanol	S	S	S	S	R	R	R	R
6	Ethanol	S	S	S	R	R	R	R	R
7	Aqueous	S	S	S	S	S	R	R	R

DISCUSSION

The extractive value analysis revealed marked differences among the solvents used, with the aqueous extract showing the highest yield (27.5%), followed by methanol (7.835%). Similar observations have been reported for *Abutilon indicum*, where polar solvents were found to extract higher quantities of phytoconstituents than non-polar solvents [30]. The higher yields obtained with water and methanol suggest the abundance of polar constituents in the leaves. Preliminary phytochemical screening demonstrated the presence of flavonoids, phenolics, tannins, alkaloids, glycosides, steroids, and saponins in various extracts. Previous phytochemical investigations on *A. indicum (L.)* have also reported similar classes of secondary metabolites [16,30]. The predominance of phenolics, tannins, and flavonoids in methanol, ethanol, and aqueous extracts may partly explain their superior anti-inflammatory activity. However, since only qualitative phytochemical screening was performed, further chromatographic and quantitative analyses are required to identify the specific compounds responsible for the observed biological activities.

In the anti-inflammatory assays, methanol, ethanol, and aqueous extracts consistently exhibited superior activity compared with the non-polar fractions. These extracts produced the highest protection against hemolysis in the HRBC membrane stabilization assay and the greatest inhibition of heat-induced protein denaturation. Membrane stabilization is considered an indicator of the ability of test substances to stabilize lysosomal membranes and thereby limit the release of inflammatory mediators, while inhibition of protein denaturation

reflects protection against one of the processes associated with inflammatory tissue injury [23,26].

The anti-inflammatory activity observed in the present study is consistent with earlier reports on *A. indicum*. Rajurkar *et al* [27] demonstrated significant HRBC membrane stabilization by leaf extracts of the plant, while Packialakshmi and Vairaprajaa [28] reported notable inhibition of protein denaturation. Furthermore, *in vivo* investigations have shown that extracts of *A. indicum* can suppress carrageenan-induced paw edema, supporting the anti-inflammatory potential of this species [29]. The superior activity of the methanol, ethanol, and aqueous extracts observed in the present study may be associated with the higher occurrence of phenolics, tannins, flavonoids, and saponins in these fractions as revealed by the phytochemical screening. However, qualitative phytochemical tests alone cannot establish which specific compounds are responsible for the observed activity, and further chromatographic characterization and bioassay-guided fractionation are required.

The antitubercular screening revealed marked differences among the solvent extracts. Benzene and acetone extracts exhibited the lowest MIC values, followed by the aqueous extract, whereas petroleum ether, chloroform, methanol, and ethanol extracts displayed comparatively weaker activity. The Microplate Alamar Blue Assay (MABA) employed in this study is a recognized method for antimycobacterial screening and has been shown to correlate well with standard susceptibility testing procedures [24,31].

The inclusion of standard drugs in the assay permitted comparative evaluation of the extracts. Under the experimental conditions employed, the benzene extract exhibited an MIC of 1.6 µg/mL, while the acetone extract showed an MIC of 3.12 µg/mL. These values were comparable to those obtained for the reference drugs pyrazinamide and ciprofloxacin and lower than that observed for streptomycin. Although these findings indicate substantial antimycobacterial activity, they should be interpreted cautiously because crude plant extracts generally exhibit lower potency than purified antimicrobial agents. Confirmation using independent susceptibility assays and bioassay-guided isolation studies is therefore warranted. The strong activity observed in the benzene and acetone fractions may indicate enrichment of bioactive compounds with intermediate polarity. Qualitative phytochemical screening confirmed the presence of flavonoids and steroids in these fractions, while previous studies have reported various flavonoids and related secondary metabolites from *A. indicum* [32-34].

The present investigation did not include standard anti-inflammatory drugs in the HRBC membrane stabilization and protein denaturation assays because the study was primarily designed to compare the anti-inflammatory efficacy of different solvent extracts of *A. indicum*. Therefore, direct comparison of the extracts with established anti-inflammatory drugs was beyond the scope of the present work.

The findings demonstrate that *A. indicum* possesses promising anti-inflammatory and antitubercular properties. The anti-inflammatory activity was most pronounced in the methanol, ethanol, and aqueous extracts, whereas benzene and acetone extracts exhibited the strongest antimycobacterial activity. These observations support further investigation of *A. indicum* as a potential source of bioactive compounds for the development of novel therapeutic agents.

CONCLUSION

This study found that *A. indicum* Sweet leaves contain bioactive compounds with strong anti-inflammatory and antitubercular effects in laboratory tests. The results show that the type of solvent used matters, as polar solvents like methanol, ethanol, and water gave the best results in tests for stabilizing red blood cell membranes and preventing protein denaturation. These effects are probably due to the high amounts of flavonoids, phenolics, and tannins in the extracts, which help protect cells and proteins. The Microplate Alamar Blue Assay also showed that benzene and acetone extracts, and to a lesser extent, the water extract, had strong activity against tuberculosis bacteria. This suggests that certain plant compounds can get through the bacteria's fatty cell wall. The clear link between solvent choice, plant chemicals, and biological activity highlights the need for careful extraction methods in plant research. *A. indicum* shows promise as a source of both anti-inflammatory and antitubercular agents. Future research should focus on separating and identifying the active compounds, testing them in living systems, and understanding their mechanisms to support drug development.

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Author Contributions

Dhanesh P. V. carried out the anti-inflammatory and antitubercular studies with the assistance of experts in the respective laboratories and was involved in data acquisition. Ajithkumar T. G. conceived the study, analyzed and interpreted the results, compiled the manuscript, and substantially contributed to the introduction, methodology, and discussion sections, particularly those related to the anti-inflammatory investigations. S. Kamboja examined the antitubercular study results, assisted in data interpretation, and contributed to the analysis of the findings. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available and will be made available upon request.

Use of AI in Drafting of Manuscript

The authors declare that they have not used any generative AI/AI-assisted technologies in the writing of this manuscript.

Conflict of interest

The authors declared no conflict of interest.

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REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod*. 2020;83(3):770-803.
2. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. *Biotechnol Adv*. 2021; 20(3):200-16.
3. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *Nutrients*. 2016;8(12):1-23.
4. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, *et al*. Flavonoids: a bioactive compound

from medicinal plants and its therapeutic applications. *Biointerface Res Appl Chem*. 2022;12(4):5561-81.

5. Duarte M, Pedrosa SS, Khusial PR, Madureira AR. The biological potential and health-benefits of flavonoids. *Chem Biol Interact*. 2025;388:110509.
6. Sun S, Liu Z, Lin M, Gao N, Wang X. Polyphenols in health and food processing: antibacterial, anti-inflammatory and antioxidant actions. *Food Sci Hum Wellness*. 2024;13:100-14.
7. Singh A, Holvoet S, Mercenier A, Guyonnet D. Interaction of polyphenols as antioxidant and anti-inflammatory agents: clinical and experimental evidence. *Nutrients*. 2020;12(6):1583.
8. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2017;9(6):7204-18.
9. Zouine N, El Ghachtouli N, El Abed S, Ibnsouda Koraichi S. A comprehensive review on medicinal plant extracts as antibacterial agents: factors, mechanism insights and future prospects. *Sci Afr*. 2024;26:e02395.
10. Sharma S, Kota K, Ragavendhra P. HRBC membrane stabilization as a study tool to explore the anti-inflammatory activity of Allium cepa Linn.- relevance for 3R. *J Adv Med Dent Sci Res*. 2018;6(6):29-34.
11. Hasan MM, Islam ME, Hossain MS, Akter M, Rahman MAA, Kazi M, *et al*. Unveiling the therapeutic potential: evaluation of anti-inflammatory and antineoplastic activity of Magnolia champaca Linn's stem bark isolate through molecular docking insights. *Heliyon*. 2023;10(1):e22972.
12. Patil P, Joshi S, Prajapati AK. A review on plants possesses anti-tubercular activity. *J Prev Med Holist Health*. 2023;9(2):50-6.
13. Gupta VK, Kumar MM, Bisht D, Kaushik A. Plants in our combating strategies against Mycobacterium tuberculosis: progress made and obstacles met. *Pharm Biol*. 2017;55(1):1536-44.
14. Martolia J, Soni H, Tandel FB. *In vitro* anti-tubercular activity and physicochemical standardization of selected medicinal plant extracts. *Indian J Pharm Sci*. 2021;83(2):230-7.
15. Polak Ż, Kapka-Skrzypczak L. Anti-tubercular substances produced by plants. *J Pre-Clin Clin Res*. 2017;11(2):147-52.
16. Rajeshwari S, Sevarkodiyone SP. Medicinal properties of Abutilon indicum. *Open J Plant Sci*. 2018;3(1):11-6.
17. Swaliha CK, Sarmila S, Manoj Kumar M, Praveen Kumar S, Satheesh P, Surendra Kumar M. Pharmacological properties of *A. indicum (L.)* Linn: an overview. *Bull Environ Pharmacol Life Sci*. 2023;12(2):114-7.
18. Bolleddu R, Venkatesh S, Narasimhaji CV, Hazra J. Pharmacognostical and phytochemical studies of Atibala (*A. indicum (L.)*. Sweet) fruit. *Ayu*. 2021;42(3):138-42.
19. Dharuman A, Muthusamy P, Radha R, Rubikaa G. Review on Abutilon indicum-an update. *Int J Pharm Pharm Res*. 2023;26(4):95-113.
20. Pavithra P, Kavimani S, Pavazhaviji P, Hemalatha A, Snega R. A review on phytochemical screening and pharmacological activities of fruit of Indian mallow. *Indian J Pharm Pharmacol*. 2023;10(4):265-71.
21. Khandelwal KR. Practical Pharmacognosy. 18th ed. *Pune: Nirali Prakashan*; 2007. p. 149-56.
22. Gandhidasan R, Thamaraichelvan A, Baburaj S. Anti-inflammatory action of Lannea coromandelica by HRBC membrane stabilization. *Fitoterapia*. 1991;62(1):81-3.
23. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol*. 1968;20(3):169-73.
24. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, *et al*. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *J Clin Microbiol*. 1998;36(2):362-6.
25. Maria CSL, Marcus VN, Alessandra CP, Ferreira ML, Rasnisb B. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. *Arkivoc*. 2007;(15):181-91.
26. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *Daru J Pharm Sci*. 2012;20(1):76;1-7.
27. Rajurkar R, Jain R, Matake N, Aswar P, Khadbadi SS. Anti-inflammatory action of *A. indicum (L.)* Sweet leaves by HRBC membrane stabilization. *Res J Pharm Technol*. 2009;2(2):415-6.
28. Packialakshmi B, Vairaprajaa V. Anti-inflammatory and antioxidant potential of *A. indicum (L): in vitro* study. *J Pharm Sci Innov*. 2019;8(4):142-6.
29. Tripathi P, Chauhan NS, Patel JR. Anti-inflammatory activity of *A. indicum (L)* extract. *Nat Prod Res*. 2012;26(17):1659-61.
30. Sunil M, Vedavijaya T, Sree PK, Babu Sayana S. Phytochemical analysis and antioxidant evaluation of the ethanolic extract of the leaves of Abutilon indicum. *Cureus*. 2023;15(10):e47703.
31. Parameswari P, Devika R, Vijayaraghavan P. *In vitro* anti-inflammatory and antimicrobial potential of leaf extract from *Artemisia nilagirica* (Clarke) Pamp. *Saudi J Biol Sci*. 2019;26(3):460-3.
32. Lavanya R. Screening of *A. indicum (L.)*. leaf and seed extracts for antitubercular activity. World J Pharm Res. 2017;6(4):772-6.
33. Matławska I, Sikorska M. Flavonoid compounds in the flowers of *A. indicum (L.)* Sweet (Malvaceae). *Acta Pol Pharm*. 2002;59(3):227-9.
34. Matławska I, Sikorska M, El-Sayed NH, Budzianowski J, Hołderna-Kędzia E, Mabry TJ. Bioactive flavone sulfates of *A. indicum (L.)*. *Nat Prod Commun*. 2007;2(10):1003-8.

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