

In vitro Anti-inflammatory and Antitubercular studies of various extracts of *Abutilon indicum* (L.) Sweet Leaves

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ABSTRACT

This study examines the anti-inflammatory and antitubercular effects of different extracts from *Abutilon indicum* (L.) Sweet a medicinal plant from the Malvaceae family. Sequential extraction with solvents ranging from non-polar to polar, including petroleum ether, benzene, chloroform, acetone, methanol, ethanol, and water. The anti-inflammatory activity was measured using human red blood cell membrane stabilization and protein denaturation tests. Antitubercular activity was tested against *Mycobacterium tuberculosis* H37Rv with the Microplate Alamar Blue Assay. The results showed that methanol, ethanol, and water extracts had the strongest anti-inflammatory effects, stabilizing membranes by about 65–69% and inhibiting protein denaturation by about 72–78% at 100 µg/mL. These results suggest that polar compounds like flavonoids, phenolics, and tannins are mainly responsible for the activity. Non-polar extracts had less effect. For antitubercular activity, benzene and acetone extracts had the lowest minimum inhibitory concentrations, ranging from 1.6 to 3.12 µg/mL, with the aqueous extract following at 6.25 µg/mL. Overall, the findings show that the type of solvent affects which bioactive compounds are present in *A. indicum* leaves. This supports the plant's traditional use and points to the potential for further isolating compounds with both anti-inflammatory and antimycobacterial properties.

Keywords: *Abutilon indicum*, Anti-inflammatory, Antitubercular, HRBC membrane stabilization, Protein denaturation, Microplate Alamar Blue Assay.

INTRODUCTION

Plant extracts have played a decisive role in modern therapeutics and continue to inform drug discovery, disease prevention, and adjunctive care. Historically and today, a substantial share of approved medicines is either a natural product, a natural product derivative, or a synthetic compound inspired by a natural scaffold, underscoring plants as a deep reservoir of bioactive chemistry with privileged, evolution-tested structures (e.g., complex polyphenols, terpenoids, alkaloids). Large-scale surveys of new chemical entities approved since the 1980s consistently show that natural products and their analogues contribute across oncology, infectious disease, cardiovascular, and neurologic indications, making them indispensable to the pharmacopeia^{1,2}

Beyond individual bioactive compounds, plant extracts are therapeutically significant because they contain many secondary metabolites that interact with multiple biological targets. This is especially relevant to complex, multifactorial diseases. Flavonoids, for example, have antioxidant, anti-inflammatory, cardiometabolic, neuroprotective, and chemopreventive activities. These effects are mediated through mechanisms such as redox modulation, NF-κB and MAPK signalling, endothelial function, and microbiome interactions³. Recent research has highlighted the diverse effects of flavonoids, ranging from vascular health to metabolic resilience, while also addressing challenges related to bioavailability and metabolism that impact their clinical application^{4,5}. Polyphenols show antibacterial, anti-inflammatory, and antioxidant properties, and mechanistic and preclinical studies are increasingly

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supported by human trials investigating their impact on inflammatory biomarkers and oxidative stress^{6,7}. These multi-target activities suggest that, when standardized and rigorously evaluated, plant-derived mixtures or fraction-enriched extracts can complement single-target drugs in prevention or as adjunctive therapies.

Inflammation is a key factor in various chronic illnesses, including arthritis, heart disease, and neurodegenerative conditions⁸. Plant extracts contain a wide array of anti-inflammatory compounds, including flavonoids, terpenoids, saponins, and alkaloids, which offer protective effects by influencing pathways like nuclear factor-kappa B (NF- κ B), cyclooxygenase (COX), lipoxygenase (LOX), and pro-inflammatory cytokines⁹. In vitro tests, such as human red blood cell (HRBC) membrane stabilization, protein denaturation inhibition, and enzyme assays for lipoxygenase and cyclooxygenase, serve as primary methods for evaluating the anti-inflammatory properties. The HRBC membrane stabilization test indicates the capability of extracts to preserve lysosomal membranes, thereby minimizing the release of inflammatory mediators¹⁰. Protein denaturation tests evaluate the ability to prevent protein denaturation, a common occurrence in inflammatory tissue damage¹¹. Research consistently demonstrates that plant extracts effectively diminish these inflammatory processes, with a safety profile comparable to that of standard medications like diclofenac and aspirin⁸. These results encourage further exploration of medicinal plants as potential sources of innovative, multi-faceted anti-inflammatory agents. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, continues to be one of the most lethal infectious diseases worldwide, resulting in over 1.2 million deaths each year and infecting around 1.7 to 2 billion people globally¹². Although several synthetic medications are available, including isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin, treatment encounters significant obstacles due to the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* strains¹³. Additionally, side effects like hepatotoxicity and prolonged treatment periods lead to decreased patient adherence. This situation underscores the pressing need for innovative, efficient, and safer

antituberculosis drugs derived from natural sources, including medicinal plants¹⁴. Medicinal plants, which are abundant in various bioactive phytochemicals such as alkaloids, terpenoids, flavonoids, phenolics, and coumarins, have been traditionally utilized in medicine to address respiratory illnesses, including TB. Their intricate chemical compositions allow for broad-spectrum antibacterial effects, which also reduce the likelihood of developing resistance^{12,15}. Furthermore, several plants exhibit immunomodulatory characteristics that enhance the host's defenses against *M. tuberculosis*, providing an additional beneficial effect to their antimicrobial properties¹³.

Abutilon indicum (L.) Sweet is a rapidly growing perennial shrub that belongs to the Malvaceae family and primarily flourishes in tropical and subtropical regions¹⁶. The plant possesses a variety of traditional applications that have been thoroughly documented within Indian medicinal practices and ethnobotanical studies, where its leaves, roots, bark, flowers, and seeds each serve unique therapeutic functions. Traditionally, the leaves have been utilized as topical treatments for wounds, ulcers, and inflammation and consumed orally for issues related to the respiratory system, diabetes, fever, and as diuretics^{17,18}. Historically, *A. indicum* is noted for its functions as an aphrodisiac, anti-inflammatory agent, laxative, demulcent, analgesic, and diuretic^{16,19}. This wide array of therapeutic properties corresponds with contemporary pharmacological findings, which indicate that various extracts possess antioxidant, anti-inflammatory, antidiabetic, antimicrobial, immunomodulatory, antipyretic, hepatoprotective, wound-healing, and anthelmintic properties^{16,19,20}.

MATERIALS & METHODS

1. Collection of plant material

Dried leaves of *Abutilon indicum* (L.) 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.



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

1.1. 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 situated in the central section of a Soxhlet extractor. Around 500 mL of solvent was added to the lower flask, which was then fitted with a reflux condenser. The solvent was heated to its boiling point, causing the generated vapours to travel through the side arm into the condenser, where they condensed and dripped onto the plant material. As the solvent circulated through the sample, the extract collected in the extractor body until it reached the siphon level, at which point the solvent-extract mixture was siphoned back into the lower flask. 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.

1.2. Anti-inflammatory study by HRBC membrane stabilization method²¹

A sample of fresh whole human blood (10ml) 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 minutes, and then cooling to room temperature). To 1mL of the HRBC suspension, equal volumes of *Abutilon indicum* leaves 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:

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

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

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

1.3. Anti-inflammatory activity by Protein Denaturation method²²

The reaction mixture (5ml) 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 *Abutilon indicum* leaves 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 minutes and subsequently heated at 70°C for 5

minutes. After cooling, the absorbance was measured at 660nm 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:

$$\text{Percentage of inhibition} = \frac{AC_{660} - AT_{660}}{AC_{660}} \times 100$$

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

1.4. Antitubercular studies using MAB Assay

The efficacy of the compounds against *M. tuberculosis* was assessed using the Microplate Alamar Blue Assay (MABA)²³. 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 hours. 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 pink²⁴.

RESULTS

1. Anti-inflammatory studies on various extracts of *Abutilon indicum* (L) Sweet leaves

The anti-inflammatory properties of *Abutilon indicum* were assessed through two complementary in vitro tests ie., 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 1, Figure 2). Among the extracts tested, methanol, ethanol, and water demonstrated the strongest activity, whereas petroleum ether, benzene, and chloroform exhibited relatively weaker effects.

Table 1: HRBC Membrane stabilization Assay of various extracts of *Abutilon indicum* leaves

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



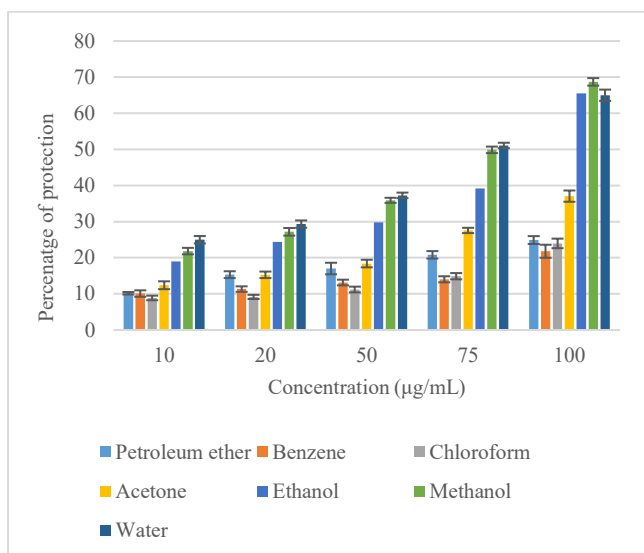


Figure 2: Anti-inflammatory activity of various extracts of *Abutilon indicum* leaves by HRBC membrane stabilization assa

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 2, Figure 3)

Table 2: Protein denaturation Assay of various extracts of *Abutilon indicum* leaves

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

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 $\mu\text{g/mL}$, 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%).

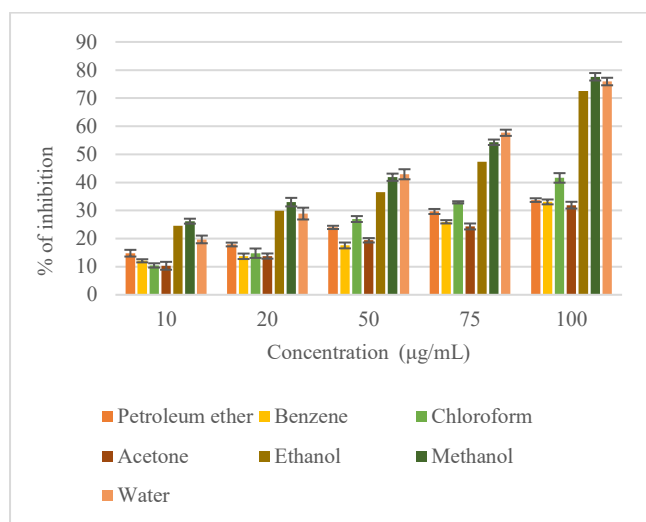


Figure 3: Anti-inflammatory activity of various extracts of *Abutilon indicum* leaves by Protein denaturation assay

At 75 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$, 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.

a. Antitubercular activities of *Abutilon indicum* leaves

Tests against *Mycobacterium tuberculosis* (H37Rv strain, ATCC 27294) showed that different solvent extracts from *Abutilon indicum* leaves varied greatly in how well they could stop bacterial growth. All extracts were effective at higher concentrations (Table 3, Figure 4), but each one started to lose effectiveness at different points that helped to identify the minimum inhibitory concentration (MIC) for each extract (Figure 5).

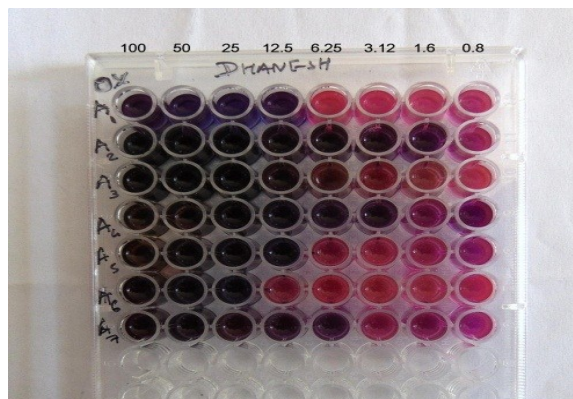
The benzene extract was the most powerful, staying effective down to 1.6 $\mu\text{g/mL}$, but stopped working at 0.8 $\mu\text{g/mL}$. This made it the top performer among all the extracts. Acetone extract remained effective until 3.12 $\mu\text{g/mL}$ and lost its effect at 1.6 $\mu\text{g/mL}$, also showing strong results. The water-based (aqueous) extract worked moderately well, being effective up to 6.25 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ and needing the highest amount to stop the bacteria from growing.

Table 3: Anti-tubercular activity of various extracts of *Abutilon indicum* leaves

Sl. No.	Sample	100 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	12.5 $\mu\text{g/mL}$	6.25 $\mu\text{g/mL}$	3.12 $\mu\text{g/mL}$	1.6 $\mu\text{g/mL}$	0.8 $\mu\text{g/mL}$
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



A1 Petroleum ether A2 Benzene A3 Chloroform A4 Acetone A5 Methanol A6 Ethanol A7 Aqueous

Fig. 4 MABA study of *Abutilon indicum* (L) Sweet leaves various extracts

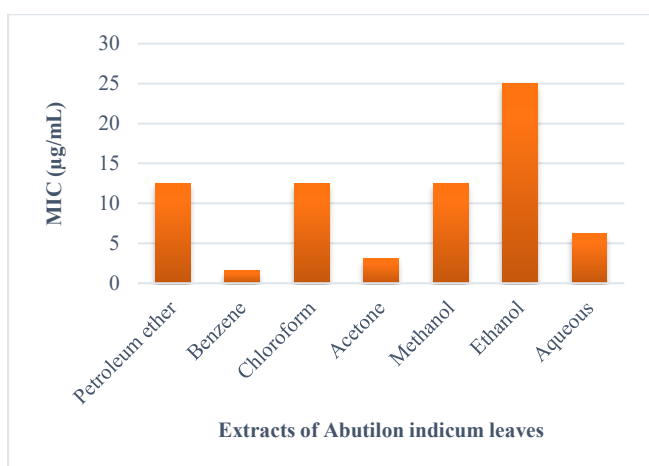


Figure 5: MIC value of MABA assay of various extracts *Abutilon indicum* leaves

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 *Abutilon indicum* leaves is found in the benzene and acetone fractions, with moderate activity in the aqueous extract, while the other solvents show comparatively weaker effects.

DISCUSSION

The assessment of anti-inflammatory activity of *Abutilon indicum* (L.) Sweet leaf extracts by employing two complementary in-vitro models: HRBC membrane stabilization and inhibition of heat-induced protein denaturation. These assays represent clinically relevant aspects of inflammation, namely the stabilization of erythrocyte and lysosomal membranes (which prevents the release of lytic enzymes from neutrophils) and the protection of serum proteins from denaturation, a process involved in inflammatory and arthritic diseases^{22,25}. The results indicated that polar phytochemicals, especially phenolics, flavonoids, and condensed tannins, are primarily responsible for the anti-inflammatory activity observed. This polarity v/s activity relationship is mechanistically plausible. Phenolics and flavonoids can intercalate at the aqueous interface of biological membranes to improve mechanical stability under osmotic stress and temperature changes, and their hydrogen-bonding or π -stacking capacities help to maintain native protein conformations against heat-induced unfolding effects that are captured by the two assays conducted^{22,25}. The HRBC assay results were in line with earlier demonstrations that *A. indicum* leaves stabilize erythrocyte membranes. It was reported that significant HRBC protection with *A. indicum* leaf extract establishes this plant as a credible candidate for membrane-stabilizing anti-inflammatory activity²⁶. The protein-denaturation results (strongest inhibition by methanol/ethanol/water extracts) are also consonant with other works, which showed that ethanolic leaf extract significantly inhibited bovine serum albumin denaturation and quantified a meaningful phenolic/flavonoid load in the same extract, an association consistent with present polarity-dependent efficacy²⁷. Besides these in-vitro studies, the present profile dovetails with in-vivo evidence, which demonstrated that whole-plant ethanolic extract of *A. indicum* significantly reduced carrageenan-induced paw edema in rats in a dose-dependent manner, with efficacy comparable to ibuprofen, supporting the translational relevance of



the in-vitro anti-inflammatory signals of the current study²⁸. The phytochemical context of *A. indicum* leaf ethanol extracts reported appreciable total phenolic and flavonoid content, alongside tannins and triterpenoids²⁹, which mirrors the constituents typically enriched by the most active (polar) fractions.

Looking outside *A. indicum*, HRBC membrane stabilization and protein-denaturation assays consistently indicate that polar extracts are superior. For example, *Artemisia nilagirica* leaf extract produced high HRBC protection ($\approx 75\%$ at 200 $\mu\text{g/mL}$), and the authors emphasized that HRBC stabilization implies lysosomal membrane stabilization, precisely the mechanistic rationale adopted here³⁰. The final solvent extracts of methanol/water/ethanol achieve approximately 65–69% stabilization at just 100 $\mu\text{g/mL}$, which compares favourably on a concentration-normalized basis, acknowledging differences in plant matrices and extraction conditions³⁰. More broadly, the membrane-stabilizing mechanism captured by HRBC is well described as a legitimate anti-inflammatory pathway that links erythrocyte membrane stabilization to anti-inflammatory action by curtailing lysosomal enzyme release²⁵, which is often invoked across by plants (e.g., *Clerodendrum inerme*) when HRBC protection is observed³⁰.

The concordance between both in-vitro models and existing literature indicates that *A. indicum*'s anti-inflammatory effects are at least partly due to (i) membrane-stabilizing compounds that prevent hypotonic or thermal lysis and (ii) protein-protective agents that inhibit denaturation and aggregation, both features typical of polyphenol-rich extracts. Recent phytochemical analyses of *A. indicum* confirm the presence of flavonoids, phenolic acids, and tannins in ethanol and methanol fractions²⁹, consistent with the observed solvent-polarity effects. On a broader biological level, these compounds frequently act by downregulating pro-inflammatory mediators (such as COX-2 and NF- κ B) and scavenging reactive species, in turn reducing protein denaturation and membrane damage; though these downstream effects were not directly measured, they align with the protective outcomes observed in both assays and the in vivo carrageenan model²⁸. Employing two distinct in-vitro assays enhanced internal validity and minimized the

likelihood that the observed activity was due to an artifact of a single assay. Nonetheless, both HRBC and protein-denaturation models serve as proxies and do not identify specific molecular targets or pharmacokinetic properties. Future studies should (i) fractionate the active polar extracts to identify the specific phenolic or tannin subclasses responsible, (ii) investigate enzyme targets (such as COX-1, COX-2, and 5-LOX) and cytokine responses in cell-based assays, and (iii) verify anti-inflammatory effects in established rodent models, including dose–response and mechanistic biomarker analyses—directly extending the promising in-vivo findings for *A. indicum*²⁸.

The microplate Alamar Blue assay (MABA) used in the present work is a validated, rapid, and highly correlated alternative to radiometric BACTEC for determining MICs of compounds against *Mycobacterium tuberculosis* (including the virulent H37Rv strain) and closely related mycobacteria, supporting the reliability of the MIC values reported here³¹. In the foundational comparison, visual and fluorometric MABA MICs agreed within ≤ 1 dilution of BACTEC for most agents tested against H37Rv/H37Ra, and subsequent clinical isolate work confirmed high concordance and operational robustness, establishing MABA as a standard for high-throughput antimycobacterial screening²³. Against this methodological backdrop, the present findings in which benzene and acetone fractions exhibited the lowest MICs (1.6–3.12 $\mu\text{g/mL}$), followed by the aqueous fraction (6.25 $\mu\text{g/mL}$) and then petroleum ether, chloroform, and methanol (12.5 $\mu\text{g/mL}$), with ethanol found least active. These results indicated that unusually strong activity relative to much of the published record on *Abutilon indicum*. Several peer-reviewed sources reported a very weak activity for certain solvent systems at concentrations of 100–200 $\mu\text{g/mL}$ or below. For example, *A. indicum* leaf and seed extracts was screened³² against *M. tuberculosis* H37Ra and *M. bovis* using MABA and found diethyl ether, acetone, and ethyl acetate leaf extracts (and diethyl ether/acetone seed extracts) inactive at 100 $\mu\text{g/mL}$, contrasting with the sub-10 $\mu\text{g/mL}$ MICs observed here for benzene/acetone fractions. Likewise, a work surveying Indian habitat plants against H37Rv reported *A. indicum* activity only at concentrations of ≥ 200 $\mu\text{g/mL}$, far above the



present MICs³³. Such differences between studies are common when working with crude plant extracts. Variations usually arise because of differences in the type of plant used, where and when it was collected, how the extraction was done, and which solvents were used to draw out certain active compounds. Results can also be affected by using different bacterial strains or by changes in lab procedures, like how much bacteria used, how long the test runs, or how results are measured. All these factors can influence how potent a plant extract appears in tests against mycobacteria^{31,32,33}.

When compared to the phytochemical knowledge of *A. indicum*, the solvent-ranked MICs are chemically plausible. *Abutilon indicum* is rich in flavonoids such as luteolin, apigenin, chrysoeriol, quercetin glycosides and also contains phenolic acids and other polar to semi-polar metabolites³⁴. These constituents are variably distributed across plant parts and are solvent-extractable according to polarity³⁵. The benzene and acetone fractions likely concentrated semi-polar lipophilic flavonoids and related phenolic derivatives that traverse the lipid-rich mycobacterial cell envelope more effectively than highly polar compounds, aligning with structure-activity observations that certain flavonoid aglycones and moderately lipophilic phenolics display superior antimycobacterial action³⁶. At the same time, the efficacy of the aqueous fraction (MIC 6.25 µg/mL) suggests that more polar phenolics and glycosides may also contribute, potentially through redox-active mechanisms, enzyme targeting, or membrane perturbation once they gain access as previously described for phenolics/flavonoids against *M. tuberculosis* and nontuberculous mycobacteria³⁷. Research widely agrees that flavonoids and similar phenolic compounds have natural antimycobacterial properties. Studies and reviews have shown that these compounds can fight *M. tuberculosis* in several ways: they can block cell-wall formation, damage cell membranes, inhibit key bacterial enzymes (like DNA gyrase and topoisomerase), affect how bacteria pump out drugs (efflux modulation), and even disrupt biofilm formation. Compounds like quercetin, rutin, naringenin, luteolin, and apigenin are often mentioned for their strong effects³⁸. It was also observed that quercetin and rutin can stop the growth of H37Rv and other mycobacteria, highlighting the value of

flavonoid-rich extracts³⁹. Computer modelling and targeted studies also suggested that flavonoids can bind to important mycobacterial enzymes, hence blocking bacterial growth⁴⁰. Altogether, this explains why benzene and acetone extracts, both rich in flavonoids as well as, to a lesser degree, the water and alcohol extracts, were effective at low MICs in the MABA tests. Conversely, the relatively weak performance of some non-polar fractions is also consistent with prior reports. A non-polar “antitubercular” fraction of *A. indicum* that yielded sterols and triterpenes (e.g., β -sitosterol, stigmasterol, β -amyrin palmitate) showed insignificant activity when the isolated compounds were tested (MIC > 128 µg/mL), implying that abundant lipophiles alone are unlikely to explain strong antimycobacterial effects⁴¹. This helps reconcile the current ranking, where petroleum ether and (for some reports) chloroform extracts were less active than benzene/acetone, and suggests that semi-polar constituents, rather than ubiquitous neutral sterols, dominate the observed bioactivity⁴¹. It is worth noting that broader plant-based anti-TB screenings from Southeast Asia and India typically report crude extract MICs ranging from tens to hundreds of µg/mL, with only a few botanicals achieving MICs in the low double digits. *A. indicum* occupies varying positions in such compilations, sometimes showing only modest activity or none at the tested concentrations^{33,42}. This makes the current findings of sub-10 µg/mL MICs particularly significant and, for research purposes, highlights the need to carefully consider factors such as sample provenance and processing methods. Standardized collection (including plant part and phenological stage), verified identity, and consistent extraction or fractionation can greatly influence the levels of desired secondary metabolites. Additionally, environmental and seasonal variables are known to affect phytochemical composition, while the choice and polarity of solvents strongly impact the range and yield of phenolics and flavonoids extracted^{43,44}. Therefore, the observed potency likely results from both chemical enrichment (with benzene/acetone enhancing the extraction of semi-polar flavonoid and phenolic aglycones) and increased biological accessibility through the mycobacterial cell envelope. Although differences from previous studies probably arise from chemotypic and methodological



variability, future research should explicitly account for and report these factors.

Finally, since MABA endpoints can vary slightly between strains (H37Rv versus H37Ra) and laboratories, it is advisable to include suitable first-line drug controls, document inoculum and media parameters, to verify key MIC values using an alternative method such as broth microdilution or MGIT to ensure translational relevance⁴⁵. Even with these caveats, the present solvent-dependent activity profile maps convincingly onto the known phytochemistry of *A.indicum* and broader flavonoid/phenolic antimycobacterial pharmacology, providing a coherent explanation for the rank order of MICs observed and a rational basis for prioritizing semi-polar fractions for further bioassay-guided isolation research.

CONCLUSION

This study found that *Abutilon indicum* (L.) 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. Overall, *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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