

Phytochemical Profiling and In Vitro Anti-Inflammatory Potential of *Stachytarpheta indica* (L.) Vahl Leaf Extracts

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ABSTRACT

Medicinal plants represent a valuable source of bioactive compounds with therapeutic relevance, particularly in the management of inflammatory disorders. This study investigated the phytochemical profile and in vitro anti-inflammatory activity of aqueous and methanolic leaf extracts of *Stachytarpheta indica* (L.) Vahl. Leaves were collected from Bhopal, authenticated botanically, and extracted using infusion (aqueous) and Soxhlet (methanolic) methods. Qualitative phytochemical screening revealed notable solvent-specific variation, with aqueous extracts containing tannins, saponins, glycosides, phenolics, flavonoids, alkaloids, and terpenoids, whereas methanolic extracts lacked tannins, saponins, and glycosides. Total phenolic content (TPC) was higher in the aqueous extract (0.0753 mg GAE/mL) compared to the methanolic extract (0.0718 mg GAE/mL), while total flavonoid content (TFC) was greater in the methanolic extract (0.4068 µg QE/mg) than the aqueous fraction (0.287 µg QE/mg). Anti-inflammatory potential assessed by the heat-induced protein denaturation assay demonstrated significant inhibition by the aqueous extract (42.16%) relative to the methanolic extract (6.32%), with aspirin showing 79.1% inhibition. The enhanced activity of the aqueous extract correlates with elevated phenolic-tannin presence and their known protein-stabilizing effects. Overall, the findings highlight *S. indica* as a promising source of natural anti-inflammatory agents and support the relevance of aqueous preparations in traditional medicine.

Keywords: *Stachytarpheta indica*, Phytochemical profiling, Phenolic content, Anti-inflammatory activity, Protein denaturation assay

1. INTRODUCTION

Medicinal plants remain an essential source of therapeutic agents and continue to play a central role in primary healthcare, particularly in developing countries where traditional remedies are widely utilized (World Health Organization, 2013). Their pharmacological properties are largely attributed to the presence of diverse secondary metabolites such as phenolics, flavonoids, tannins, terpenoids, and alkaloids, which contribute to antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities (Harborne, 1998; Mandal et al., 2017; Panche et al., 2016). Among these, phenolic compounds and flavonoids are of particular interest due to their capacity to modulate inflammatory pathways by scavenging reactive oxygen species, suppressing pro-inflammatory mediators, and stabilizing cellular membranes (Rice-Evans et al., 1997; Cushnie & Lamb, 2005).

Inflammation is a complex biological response to injury, infection, or chemical insult and is mediated by cytokines, prostaglandins, and reactive species. Although non-steroidal anti-

inflammatory drugs (NSAIDs) remain the primary treatment for inflammatory disorders, their long-term use is associated with adverse effects including gastrointestinal irritation and nephrotoxicity (Vane & Botting, 1998). Consequently, plant-derived anti-inflammatory agents with favorable safety profiles have gained significant scientific attention.

Stachytarpheta indica (L.) Vahl (family Verbenaceae) is a perennial herb widely distributed across tropical and subtropical regions. Ethnobotanical surveys report its traditional use in fever, gastrointestinal disturbances, respiratory infections, skin ailments, and inflammatory conditions (Barrett, 1994; Nath et al., 2013). Pharmacological studies have confirmed antibacterial, wound healing, hepatoprotective, and anti-inflammatory activities in species of the genus *Stachytarpheta* (Liew & Yong, 2016; Agampodi et al., 2022). Despite these findings, there is limited systematic research on the phytochemical composition of *S. indica* leaves and their relationship to anti-inflammatory potential.

Therefore, the present study aims to evaluate the phytochemical profile of aqueous and methanolic leaf extracts of *S. indica* and investigate their *in vitro* anti-inflammatory activity using a heat-induced protein denaturation assay.

2. MATERIALS AND METHODS

Plant Survey, Identification, and Collection

The medicinal plant *Stachytarpheta indica* (L.) Vahl (Verbenaceae) was selected for this study on the basis of its reported ethnomedicinal relevance and pharmacological potential (Nath, et al., 2013). Field surveys were conducted in rural regions surrounding Bhopal, Madhya Pradesh, India, where the species was observed in its natural habitat. Morphological features including leaf arrangement, inflorescence, stem texture, and floral traits were used for preliminary identification following standardized botanical descriptions. The species was authenticated by a botanist from the Department of Botany, and a voucher specimen was prepared and deposited. Herbarium sheets were prepared according to the procedures described by Johansen (1940), ensuring proper documentation for future reference.

Preparation of Plant Material

Fresh and disease-free leaves were collected, thoroughly washed under running tap water, and shade-dried at ambient temperature for approximately one week. Shade drying was preferred to prevent degradation of thermo-sensitive constituents, as recommended for pharmacognostic evaluations (Kothari et al., 2012; Azwanida, 2015). The dried leaves were ground into fine powder using an electric grinder and stored in air-tight containers protected from moisture and light until use.

Extraction Procedures

Two extraction techniques were adopted to obtain aqueous and methanolic fractions. For aqueous extraction, 20 g of powdered leaf material were infused in 200 mL of freshly boiled distilled water for 30 minutes and subsequently filtered through muslin cloth followed by Whatman No. 1 filter paper. For methanolic extraction, 20 g of defatted leaf powder were subjected to Soxhlet extraction using 80% methanol (200 mL) for 24 hours at 60°C, as described by Handa et al. (2008). Methanol was selected due to its ability to recover diverse

phenolic compounds and flavonoids efficiently (Do et al., 2014). Filtrates were concentrated using a water bath followed by vacuum drying. Extraction yield was calculated using:

$$\text{Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of crude powder}) \times 100$$

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of both extracts was performed following standard phytochemical methods described by Harborne (1998) and Sofowora (2013). Specific chemical tests were carried out for major secondary metabolites including alkaloids (Dragendorff's test), tannins (ferric chloride test), flavonoids (lead acetate test), saponins (froth test), terpenoids (Salkowski test), and glycosides (Benedict's reagent test). The presence or absence of phytoconstituents was noted based on characteristic colour changes or precipitate formation (Tenguria, et al., 2013).

Determination of Total Phenolic Content (TPC)

Total phenolic content was quantified using the Folin–Ciocalteu method according to Singleton and Rossi (1965), with minor modifications. Briefly, diluted extract samples were mixed with Folin–Ciocalteu reagent and sodium carbonate solution, incubated in the dark for 60 minutes, and absorbance was measured at 650 nm using a UV–Vis spectrophotometer. A calibration curve was constructed using gallic acid standards, and results were expressed as mg gallic acid equivalents (GAE) per gram of extract (Tenguria, et al., 2012; Tenguria, et al., 2013).

In Vitro Anti-Inflammatory Assay (Protein Denaturation Assay)

Anti-inflammatory activity was evaluated using the albumin denaturation assay described by Chandra, et al. (2012) and Bailey-Shaw, et al. (2017). Extracts at a concentration of 1 mg/mL were mixed with bovine serum albumin (1%) and incubated at 37°C for 20 minutes, followed by heating at 70°C for 5 minutes. After cooling, absorbance was measured at 660 nm. Percentage inhibition of protein denaturation was calculated as:

$$\text{Inhibition (\%)} = [(\text{Ab}_{\text{Scontrol}} - \text{Ab}_{\text{Sample}}) / \text{Ab}_{\text{Scontrol}}] \times 100$$

3. RESULT AND DISCUSSION

Extract Yield and Organoleptic Characteristics

Extraction yields and organoleptic properties provide initial insight into solvent-specific extractability and phytochemical solubility. As shown in **Table 1**, the aqueous extract of *Stachytarpheta indica* leaves produced a substantially higher yield (44.5%) compared to the methanolic extract (20.2%). Higher extraction efficiency in water suggests the predominance of hydrophilic constituents such as phenolics, tannins, saponins, and carbohydrates, a trend consistent with literature on polar solvent extraction of medicinal plants (Kumar et al., 2021; Sasidharan et al., 2011).

The greenish-brown tint and sticky texture of the methanolic extract suggests extraction of chlorophyll derivatives, sterols, and semi-polar phenolics, consistent with earlier reports on methanol-based plant extractions (Azwanida, 2015). The darker color in the aqueous extract may indicate polymerized tannins and oxidized phenolics, as observed in infusion-based methods. These results support traditional herbal preparation methods (decoctions/infusions), where water acts as the primary solvent.

Table 1. Extraction yield and organoleptic properties of *S. indica* leaf extracts

Extract Type	% Yield	Colour	Texture	Odour
Aqueous Extract	44.5%	Dark brown	Crystalline	Organic, burnt
Methanolic Extract	20.2%	Greenish-brown	Sticky, coarse	Organic, pleasant

Phytochemical Screening

Qualitative phytochemical screening revealed notable differences between the two solvent systems (Table 2). While the aqueous extract tested positive for all screened phytochemical classes, the methanolic extract lacked tannins, saponins, and glycosides. This pattern indicates solvent-dependent extractability, wherein water preferentially solubilizes highly polar bioactive compounds such as tannins, glycosides, and saponins, whereas methanol favors the extraction of semi-polar constituents including flavonoids, alkaloids, and terpenoids. The observed absence of tannins, saponins, and glycosides in the methanolic fraction is consistent with their high polarity and limited solubility in semi-polar organic solvents (Harborne, 1998; Makkar et al., 2007).

From a pharmacological perspective, the detected phytochemicals are noteworthy due to their established biological roles: tannins contribute to protein binding, enzyme inhibition, and anti-inflammatory effects (Scalbert, 1991); flavonoids exhibit membrane-stabilizing and radical-scavenging properties (Panche et al., 2016); saponins modulate membrane permeability and inflammatory responses (Ojo, 2022); and terpenoids act through diverse anti-inflammatory and antioxidant pathways (Huang et al., 2022). Taken together, the polarity-driven enrichment of tannins and other phenolic constituents in the aqueous extract suggests a stronger protein-stabilizing and anti-inflammatory potential, which is particularly relevant to heat-induced protein denaturation models used in this study.

Table 2. Qualitative phytochemical profile of *S. indica* leaf extracts

Phytochemical Group	Aqueous Extract	Methanolic Extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	–
Glycosides	+	–
Terpenoids	+	+
Saponins	+	–

(“+” present; “–” absent)

Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu assay and expressed as mg Gallic Acid Equivalent (GAE) per mL. The final values were calculated by comparing sample absorbance readings with those of the gallic acid calibration curve (see Table 3 and Figure 1).

According to the TPC outcomes presented in Table 4, the aqueous extract exhibited a slightly higher phenolic content than the methanolic fraction. This observation is consistent with previous reports indicating that water efficiently extracts hydrophilic phenolic acids such as chlorogenic, caffeic, and gallic acids (Singleton & Rossi, 1965; Do et al., 2014). Phenolic compounds are well-recognized modulators of inflammatory processes through multiple biochemical mechanisms, including the inhibition of COX and LOX enzymes, suppression of

prostaglandin synthesis, stabilization of cellular and lysosomal membranes, and scavenging of reactive free radicals (Rice-Evans et al., 1997; Mandal et al., 2017). Consequently, the comparatively elevated TPC in the aqueous extract provides a plausible biochemical basis for its superior anti-inflammatory performance observed in subsequent assays.

Table 3: Gallic acid as standard concentration vs absorbance at 650 nm to plot standard curve for estimation of phenolics in samples Using Folin-Coeucaltue’s Method.

S.N.	GA Concentration in mg/ml	Absorbance at 650 nm
1.	2	1.891
2.	1	0.976
3.	0.5	0.457
4.	0.25	0.228
5.	0.125	0.128

Instrument Used: Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305.

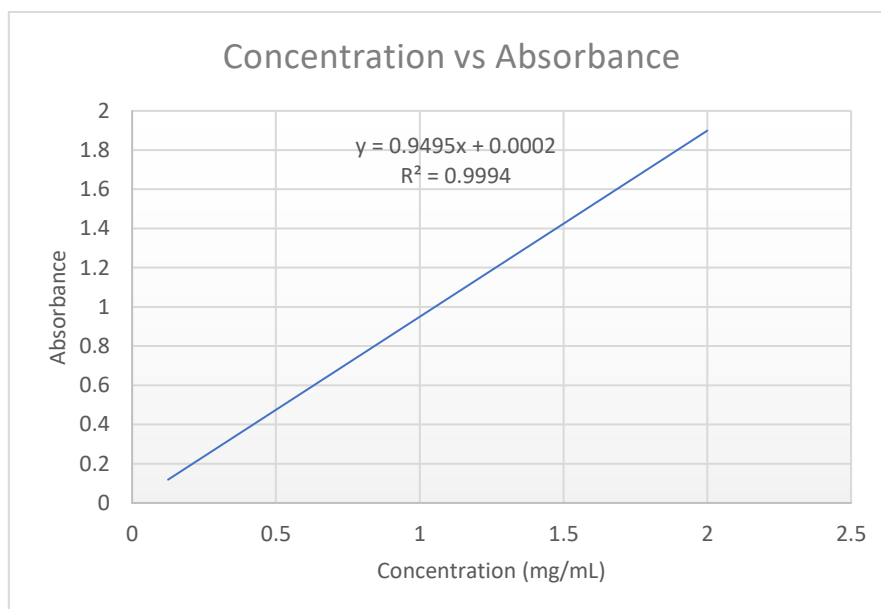


Figure 1: Standard Plot for known concentration of Gallic acid Standard at 650 nm. The Graph is obtained from Excel 2013 linear regression function

Table 4. TPC of *S. indica* extracts

Extract Type	Absorbance (650 nm)	TPC (mg GAE/mL)
Aqueous Extract	0.715	0.0753
Methanolic Extract	0.682	0.0718

Total Flavonoid Content (TFC)

TFC was measured using the aluminium chloride complexation assay and expressed as µg QE/mg. Final concentrations were calculated by referencing sample absorbance values to the quercetin calibration curve (see Table 5 and Figure 2). As shown in Table 6, the methanolic extract exhibited a higher flavonoid concentration compared to the aqueous extract, which is consistent with earlier findings demonstrating the efficiency of methanol in extracting

flavonoid-rich fractions from plant materials (Chang et al., 2002; Kumar et al., 2021). Flavonoids are recognized for their multifaceted anti-inflammatory mechanisms, including suppression of NF-κB activation, downregulation of COX-2 and inducible nitric oxide synthase (iNOS), stabilization of cellular membranes, and metal-chelating antioxidant activity (Cushnie & Lamb, 2005; Panche et al., 2016). These observations indicate that both extracts possess bioactive constituents relevant to inflammation, although the distribution of specific phytochemical groups varies according to solvent polarity.

Table 5: Quercetin as standard concentration vs absorbance at 420 nm to plot standard curve for estimation in samples Using AlCl₃ precipitation Method

S.N.	Concentration (µg/ml)	Absorbance (λ)
1.	625	0.087
2.	1250	0.151
3.	2500	0.296
4.	5000	0.671
5.	10000	1.280

Instrument Used: Single beam visible range digital microprocessed spectrophotometer from Electronic India model EI-2305.

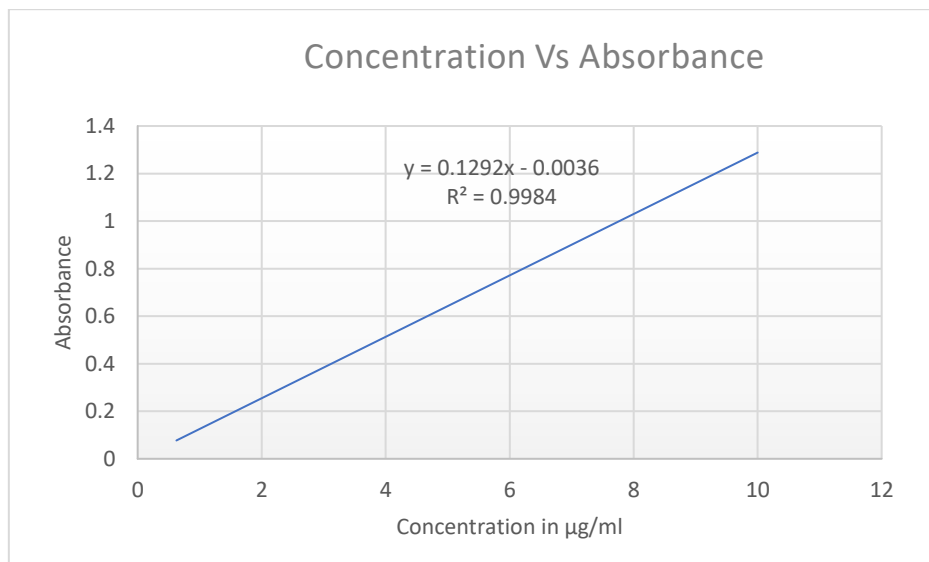


Figure 2: Standard Plot for known concentration of Quercetine Standard. The Graph is obtained from Excel 2013 linear regression function

Table 6. TFC of S. indica extracts

Extract Type	Absorbance (420 nm)	TFC (µg QE/mg)
Aqueous Extract	0.367	0.287
Methanolic Extract	0.522	0.4068

In Vitro Anti-Inflammatory Activity

Protein denaturation is a clinically relevant model of inflammation because denatured proteins trigger auto-antibody production and inflammatory cascades (Mizushima & Kobayashi, 1968). The aqueous and methanolic extracts were compared against Aspirin as standard. As indicated in Table 7 and Figure 3, the aqueous extract demonstrated substantially higher inhibition of albumin denaturation (42.16%), whereas the methanolic extract produced only minimal inhibition. This difference is consistent with solvent-dependent phytochemical distribution, wherein the aqueous extract contains tannins, saponins, glycosides, and phenolic compounds, while the methanolic extract is enriched with flavonoids, alkaloids, and terpenoids. Among these constituents, tannins are particularly relevant to this assay due to their well-documented protein-precipitating and membrane-stabilizing properties, which directly contribute to the protection of proteins against heat-induced denaturation (Scalbert, 1991; Shinde et al., 1999). Similar trends have been reported in earlier studies, where polar, water-based extracts exhibited superior anti-inflammatory or membrane-stabilizing activity compared to organic solvent extracts, a relationship attributed to higher concentrations of phenolic and tannin-rich fractions (Chandra et al., 2012; Mohamed, et al., 2020). Taken together, these observations support the hypothesis that the aqueous extract of *S. indica* possesses greater in vitro anti-inflammatory potential, likely mediated through phenolic-tannin driven protein stabilization.

Table 7. Inhibition of albumin denaturation by *S. indica* extracts

Sample	Concentration	Absorbance (660 nm)	% Inhibition
Control	—	0.823	0%
Aspirin	10 mg/mL	0.172	79.1%
Aqueous Extract	10 mg/mL	0.476	42.16%
Methanolic Extract	10 mg/mL	0.771	6.32%

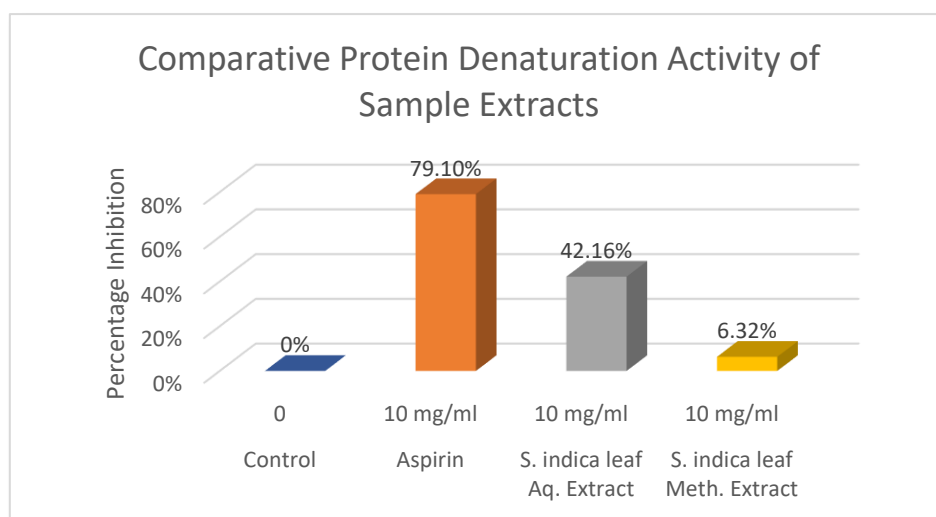


Figure 3: Graphical representation of in vitro anti-inflammatory activity based on protein denaturation inhibition for aqueous and methanolic extracts compared to aspirin.

4. CONCLUSION

The present study provides scientific evidence supporting the anti-inflammatory potential of *Stachytarpheta indica* leaf extracts and demonstrates solvent-dependent variation in phytochemical composition. The aqueous extract exhibited superior extraction yield, higher phenolic content, and stronger inhibition of heat-induced protein denaturation when compared to the methanolic fraction. These effects can be attributed to the presence of hydrophilic secondary metabolites such as tannins, saponins, and phenolic acids, which possess documented protein-stabilizing and anti-inflammatory properties. In contrast, the methanolic extract, although richer in flavonoids, showed limited anti-inflammatory activity under the assay conditions. Collectively, the results validate traditional water-based preparations of *S. indica* and suggest that phenolic-rich fractions warrant further biochemical and pharmacological investigation. Future research should include mechanistic studies across additional inflammation models, cytotoxicity evaluation, and bioassay-guided fractionation to identify specific active compounds.

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