

Evaluation Of The Antimicrobial, Anti-Inflammatory And Antioxidant Activities Of *Acalypha Indica* Leaf Extract Incorporated Into Chitosan Gel: An In Vitro Study

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Abstract

Background: Plant-derived bioactive materials are increasingly investigated for antimicrobial, antioxidant and inflammation-related properties. *Acalypha indica* L. is a medicinal herb of interest, while chitosan is a biodegradable polymer widely used as a carrier for bioactive agents. The present study evaluated *A. indica* leaf extract incorporated into 2% chitosan gel.

Materials and Methods: *A. indica* leaves were collected and ground using a mortar and pestle. The processed material was centrifuged at 8000 rpm for 5 minutes and the supernatant was collected. The extract was mixed with 2% chitosan and stirred for 6 hours; the resulting gel was stored at 4°C. Antimicrobial activity was evaluated against *Acinetobacter baumannii* and *Streptococcus mutans*. Antioxidant and anti-inflammatory activities were evaluated at 20, 40, 60, 80 and 100 µg/mL using ascorbic acid and diclofenac sodium as the respective reference standards.

Results: The formulation produced inhibition zones of 21 mm against both *A. baumannii* and *S. mutans*, compared with 23 mm for the positive controls. Radical scavenging increased from 11.33% to 63.42% across 20-100 µg/mL, compared with 21.02% to 89.64% for ascorbic acid. Anti-inflammatory inhibition increased from 12.38% to 63.88%, compared with 21.82% to 87.32% for diclofenac sodium.

Conclusion: *A. indica* leaf extract incorporated into chitosan gel demonstrated preliminary antimicrobial, antioxidant and anti-inflammatory activities under the tested in-vitro conditions. Further standardized studies are required to establish reproducibility, phytochemical composition, formulation stability and biological safety.

Keywords: *Acalypha indica*; chitosan; antimicrobial activity; antioxidant activity; anti-inflammatory activity; DPPH; in vitro.

Introduction

The increasing interest in naturally derived bioactive compounds has encouraged the investigation of medicinal plants for antimicrobial, antioxidant and inflammation-related effects. Plant extracts contain chemically diverse constituents, and their measured activity is influenced by the species, plant part, extraction technique, concentration and formulation matrix [1-3].

Acalypha indica L. (family Euphorbiaceae) is a medicinal herb used in traditional practice. The present investigation focuses specifically on its leaf preparation. Because biological activity cannot be assumed solely from traditional use, experimental evaluation of a defined formulation is required before further biomedical application can be considered.

Antimicrobial screening is particularly relevant to oral and biomedical research. *Streptococcus mutans* is an important organism in cariogenic biofilms, whereas *Acinetobacter baumannii* is a clinically relevant Gram-negative pathogen. Agar diffusion methods provide a practical preliminary assessment of antibacterial activity, although inhibition-zone size is affected by both microbial susceptibility and diffusion of the tested material through the agar [4].

Oxidative stress contributes to several pathological processes. The DPPH radical scavenging assay is widely used as a preliminary method for evaluating the free-radical-scavenging capacity of natural products and formulations [5]. In-vitro anti-inflammatory screening assays likewise provide an initial comparison with established reference compounds, but their results should not be directly extrapolated to clinical efficacy [6-8].

Chitosan is a biodegradable, biocompatible polysaccharide with gel-forming, film-forming and drug-delivery properties. It has been extensively investigated as a carrier for natural compounds and other bioactive agents [9,10]. Incorporation into a chitosan matrix may influence handling, retention, diffusion and release of plant-derived constituents.

Therefore, the present study was undertaken to evaluate the in-vitro antimicrobial, antioxidant and anti-inflammatory activities of *A. indica* leaf extract incorporated into a 2% chitosan gel.

Materials and Methods

Study design

The present study was conducted as an in-vitro experimental study to evaluate the antimicrobial, antioxidant and anti-inflammatory activities of *Acalypha indica* leaf extract incorporated into a chitosan gel.

Preparation of *Acalypha indica* leaf extract and chitosan gel

Acalypha indica plant material was collected and the leaves were separated. The leaves were ground using a mortar and pestle. The processed material was centrifuged at 8000 rpm for 5 minutes and the supernatant was collected. A 2% chitosan preparation was prepared separately. The plant extract was mixed with 2% chitosan and stirred for 6 hours. The resulting chitosan-*A. indica* gel was stored at 4°C until further evaluation.

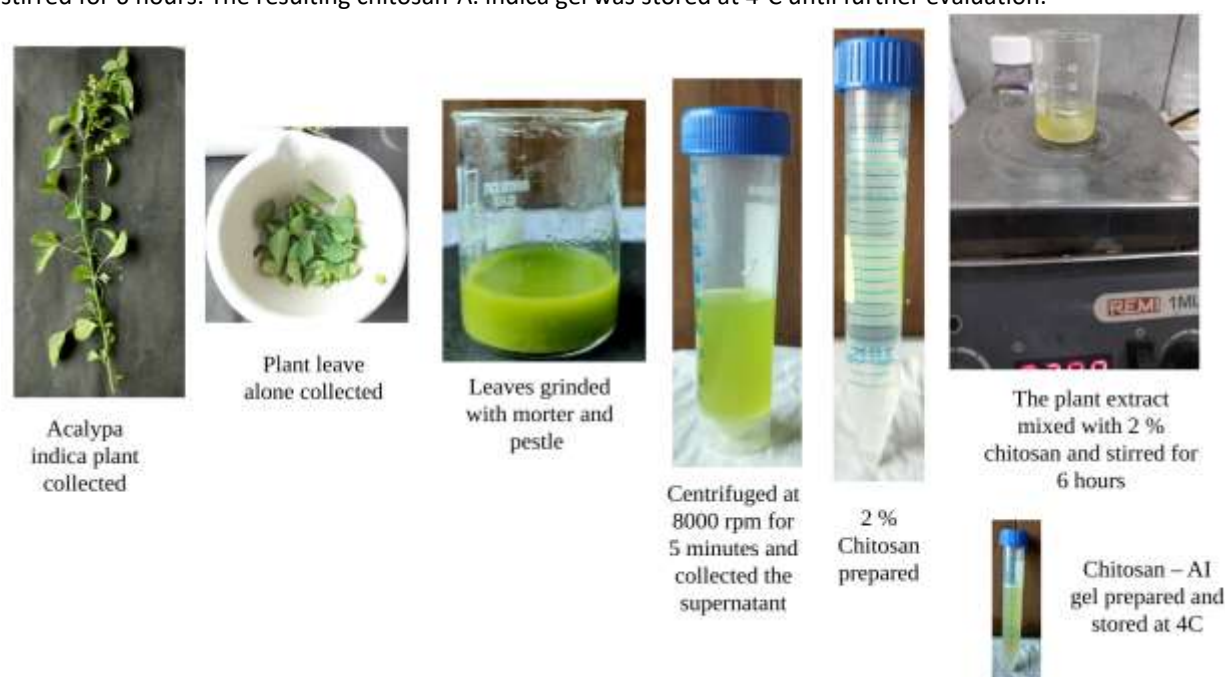


Figure 1. Experimental workflow for preparation of *Acalypha indica* leaf extract and incorporation into 2% chitosan gel (methodology image from the experimental PPT).

Antimicrobial activity

The antimicrobial activity of the prepared chitosan–*Acalypha indica* formulation was evaluated using the agar well diffusion assay. Mueller–Hinton agar was used as the culture medium. Strains of *Acinetobacter baumannii* and *Streptococcus mutans* were obtained from the Department of Microbiology, Saveetha Medical College and Hospital. Following inoculation, wells were prepared in the agar and the test formulation was introduced. Antimicrobial activity was assessed by measuring the zones of inhibition in millimetres and comparing them with the positive control [4].

Antioxidant activity

Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay at concentrations of 20, 40, 60, 80 and 100 µg/mL. Ascorbic acid was used as the reference standard. The antioxidant response was expressed as percentage radical scavenging activity and compared with ascorbic acid at the corresponding concentrations [5].

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the albumin denaturation assay at concentrations of 20, 40, 60, 80 and 100 µg/mL. Diclofenac sodium was used as the reference standard. Anti-inflammatory activity was expressed as percentage inhibition of protein denaturation and compared with diclofenac sodium at the corresponding concentrations [6-8].

Statistical analysis

The supplied experimental results were evaluated descriptively. As the source dataset did not provide replicate numbers, standard deviation, standard error or other measures of variability, inferential statistical analysis was not performed and statistical significance was not inferred.

Results

Antimicrobial activity

The chitosan-A. indica formulation demonstrated antibacterial activity against both tested organisms. A zone of inhibition of 21 mm was recorded against *A. baumannii* and 21 mm against *S. mutans*. The corresponding positive-control value was 23 mm for both organisms (Table 1; Figure 2).

Table 1. Antimicrobial activity of the chitosan-Acalypha indica formulation

Organism	Sample (mm)	Positive control (mm)
<i>Acinetobacter baumannii</i>	21	23
<i>Streptococcus mutans</i>	21	23



s.no	Organisms	Sample	PC
1	<i>Acinetobacter baumannii</i>	21	23
2	<i>Streptococcus mutans</i>	21	23

Figure 2. Agar-plate images and recorded inhibition-zone values from the antimicrobial experiment.

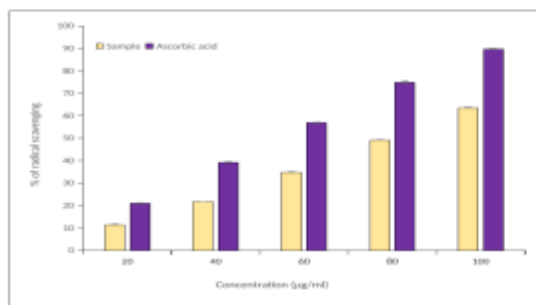
Antioxidant activity

The formulation showed a concentration-related increase in radical-scavenging activity. The recorded values were 11.33%, 21.64%, 34.75%, 49.02% and 63.42% at 20, 40, 60, 80 and 100 µg/mL, respectively. Ascorbic acid showed corresponding values of 21.02%, 39.20%, 56.97%, 74.97% and 89.64%. The formulation remained below the reference standard at each tested concentration (Table 2; Figure 3).

Table 2. Antioxidant activity

Concentration (µg/mL)	Sample (%)	Ascorbic acid (%)
20	11.33	21.02

40	21.64	39.2
60	34.75	56.97
80	49.02	74.97
100	63.42	89.64



S.no	Concentration	Sample	Ascorbic acid
1	20	11.33	21.62
2	40	21.64	39.2
3	60	34.75	56.97
4	80	49.02	74.97
5	100	63.42	89.64

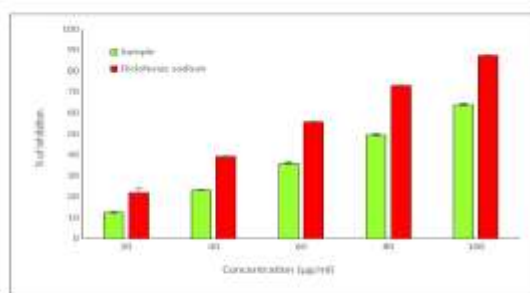
Figure 3. Antioxidant activity of the formulation compared with ascorbic acid at increasing concentrations.

Anti-inflammatory activity

The anti-inflammatory response also increased with concentration. Percentage inhibition increased from 12.38% at 20 µg/mL to 63.88% at 100 µg/mL. Diclofenac sodium increased from 21.82% to 87.32% across the same concentration range. The formulation showed lower values than diclofenac sodium at all tested concentrations (Table 3; Figure 4).

Table 3. Anti-inflammatory activity

Concentration (µg/mL)	Sample (%)	Diclofenac sodium (%)
20	12.38	21.82
40	23.11	39.04
60	35.9	55.71
80	49.52	73.04
100	63.88	87.32



S.no	Concentration	Sample	Diclofenac sodium
1	20	12.38	21.82
2	40	23.11	39.04
3	60	35.9	55.71
4	80	49.52	73.04
5	100	63.88	87.32

Figure 4. Anti-inflammatory activity of the formulation compared with diclofenac sodium at increasing concentrations.

Discussion

The present in-vitro study evaluated a chitosan-based formulation containing *Acalypha indica* leaf extract for three biologically relevant properties: antimicrobial activity, free-radical-scavenging activity and inhibition of albumin denaturation. The formulation demonstrated measurable antibacterial activity against both *Acinetobacter baumannii* and *Streptococcus mutans* and showed a progressive increase in antioxidant and anti-inflammatory responses as the concentration increased from 20 to 100 µg/mL. Taken together, these findings indicate that the combined chitosan-A. indica formulation possesses multiple in-vitro biological activities; however, the results should be interpreted as preliminary screening evidence rather than proof of clinical efficacy.

The antimicrobial component of the study was evaluated by the agar well diffusion method using Mueller-Hinton agar. The test formulation produced a zone of inhibition of 21 mm against both *A. baumannii* and *S. mutans*, compared with 23 mm for the respective positive controls. The small numerical difference between the test formulation and positive control indicates appreciable growth inhibition under the conditions of the present experiment. Agar diffusion techniques are useful for initial antimicrobial screening, but the diameter of the inhibition zone is influenced not only by intrinsic antimicrobial activity but also by factors such as inoculum density, agar composition, molecular size and diffusion characteristics of the test material [4]. This consideration is particularly relevant for a gel formulation because viscosity and polymer-extract interactions can modify diffusion through the agar matrix.

The activity against *S. mutans* is relevant from an oral-health perspective because this organism is an important constituent of cariogenic dental biofilms. The observed response suggests that the formulation may merit further investigation in oral or topical applications. Activity against *A. baumannii* additionally indicates that the antibacterial effect was not restricted to the oral test organism. Nevertheless, the present agar diffusion findings alone cannot define the bacteriostatic or bactericidal concentration of the formulation. Subsequent studies should therefore determine minimum inhibitory concentration and minimum bactericidal concentration and should include extract-only and chitosan-only groups to distinguish the contribution of each component.

The antioxidant activity was assessed using the DPPH radical scavenging assay, a widely used method for preliminary assessment of the hydrogen- or electron-donating capacity of natural products [5]. The formulation demonstrated a concentration-dependent response, increasing from 11.33% radical scavenging at 20 µg/mL to 63.42% at 100 µg/mL. In comparison, ascorbic acid increased from 21.02% to 89.64% across the same concentration range. The progressive rise in activity with increasing concentration supports a dose-responsive antioxidant effect of the formulation under the experimental conditions.

Although the antioxidant values of the test formulation were consistently lower than those of ascorbic acid, direct equivalence with a pure reference antioxidant should not be expected. Ascorbic acid is a defined low-molecular-weight antioxidant, whereas the experimental sample consists of a complex plant extract incorporated into a chitosan matrix. The observed response may reflect the collective action of phytochemical constituents present in the leaf extract as well as their accessibility within the polymeric carrier. Similar concentration-dependent antioxidant responses have been described in in-vitro evaluations of plant-derived formulations [6,7]. However, the present study did not perform phytochemical profiling; therefore, attribution of the observed effect to a particular phenolic, flavonoid or other constituent would be speculative.

The anti-inflammatory potential of the formulation was evaluated by the albumin denaturation assay, with diclofenac sodium used as the reference standard. Protein denaturation is associated with inflammatory processes, and inhibition of experimentally induced protein denaturation is commonly used as a screening approach for anti-inflammatory activity of plant preparations. The test formulation showed a progressive increase in percentage inhibition from 12.38% at 20 µg/mL to 63.88% at 100 µg/mL. Diclofenac sodium showed corresponding values of 21.82% and 87.32%. Thus, the formulation displayed a clear concentration-related inhibitory response, although its activity remained below that of the standard drug at each tested concentration.

The concordant concentration-response patterns observed in the DPPH and albumin denaturation assays are noteworthy because they indicate that the formulation exhibited both radical-scavenging and protein-protective effects as its concentration increased. Oxidative stress and inflammatory processes are biologically interconnected; nevertheless, the present experiments evaluate distinct in-vitro endpoints and do not establish a common

mechanism. The findings should therefore be reported as parallel antioxidant and anti-inflammatory activities rather than as evidence of a specific molecular pathway.

Chitosan was selected as the polymeric carrier for the present formulation. Chitosan is a biodegradable and biocompatible polysaccharide with established film-forming and gel-forming characteristics and has been extensively investigated for biomedical and drug-delivery applications [9,10]. Incorporation of *A. indica* extract into a 2% chitosan gel provides a formulation that can retain and disperse plant-derived constituents while offering handling characteristics suitable for further development. At the same time, chitosan itself can influence the biological and physicochemical behaviour of a formulation. For this reason, future experiments incorporating a chitosan-only control are essential before the observed effects can be attributed specifically to *A. indica*.

The combined findings of the present study are encouraging from a formulation-development perspective. A single preparation demonstrated antibacterial activity against both tested microorganisms and concentration-dependent antioxidant and anti-inflammatory effects. This multi-assay approach is useful during early-stage screening because it identifies whether a formulation warrants more detailed biological characterization. However, the present results do not establish clinical effectiveness, therapeutic dose, tissue compatibility or superiority over existing agents.

Several limitations should be considered. First, the available dataset does not include the number of replicate measurements, standard deviation, standard error, confidence intervals or p-values. Consequently, the differences between the test formulation and reference standards cannot be interpreted as statistically significant. Second, complete extraction parameters, including the exact quantity of leaf material, extraction volume and extraction yield, were not available in the experimental record used for this manuscript. Third, phytochemical characterization was not performed, preventing identification or quantification of the constituents responsible for the observed effects. Fourth, extract-only and chitosan-only controls were not included in the supplied dataset, limiting interpretation of possible additive or carrier-related effects.

Future investigations should therefore standardize the plant material and extraction procedure, document extraction yield, perform qualitative and quantitative phytochemical analysis, and include appropriate formulation controls. Antimicrobial testing should be expanded to MIC and MBC determination and, where relevant, biofilm-based assays. Antioxidant findings may be corroborated using complementary assays rather than relying on DPPH alone. The anti-inflammatory response should also be evaluated using additional biochemical or cell-based models. Replicate experiments with appropriate statistical analysis are necessary to determine the precision and reproducibility of the observed effects. For eventual biomedical or oral application, formulation stability, release characteristics, cytocompatibility and subsequent in-vivo evaluation would also be required.

Within these limitations, the present study provides preliminary evidence that *Acalypha indica* leaf extract incorporated into 2% chitosan gel retains measurable biological activity after formulation. The observed antibacterial effect together with concentration-dependent antioxidant and anti-inflammatory responses supports further systematic investigation of this plant-polymer combination as a candidate for future local or topical biomedical formulations.

Conclusion

The present in-vitro study demonstrated that *Acalypha indica* leaf extract incorporated into 2% chitosan gel exhibited antibacterial activity against *Acinetobacter baumannii* and *Streptococcus mutans* and showed concentration-related antioxidant and anti-inflammatory responses. Although the responses were lower than the respective reference standards, the formulation demonstrated measurable biological activity under the tested conditions. Standardized and replicated studies are required to confirm these findings and to characterize the active constituents, stability and safety of the formulation.

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