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Original Article

# Anti-inflammatory and Antioxidant Activities of *Symplocos cochinchinensis* (Lour.) Moore ssp. *Laurina* (Retz.) Nooteb Leaf Extract

Ly Hai Trieu[1], Phan Cong Sanh[2], Nguyen Thanh Huy[2], Tran Ngoc Toan[3], Lam Bich Thao[1], Le Thi Kim Oanh[1], Le Thanh Minh[4], Le Van Minh[1,*]

[1]*Research Center of Ginseng and Medicinal Materials, 41 Dinh Tien Hoang, District 1, Ho Chi Minh City, Vietnam*

[2]*Management Board of Cham Islands MPA, 3 Nguyen Hue, Hoi An City, Quang Nam Province, Vietnam*

[3]*The Greenviet Biodiversity Conservation Centre, K39/21 Thanh Vinh 1, Son Tra, Da Nang City, Vietnam*

[4] *Ho Chi Minh City Institute of Public Health, 59 Hung Phu, District 8, Ho Chi Minh City, Vietnam*

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**Abstract:** *Symplocos cochinchinensis (S. cochinchinensis*) leaves have been used by Vietnamese in herbal medicines to support the treatment of infirmities. The purpose of this study was to determine some phytochemicals, assess *in vitro* anti-inflammatory and antioxidant capacities of *S. cochinchinensis* leaf extract to provide information for further research. The study results show that *S. cochinchinensis* leaves contain polyphenols, flavonoids, and saponins by chemical reactions and thin layer chromatography profiling. The total polyphenol, flavonoid, and saponin contents were 517.71 ± 19.50 mg GAE/g d. w., 1.12 ± 0.01 mg QE/g d. w., and 32.18 ± 0.24 mg/g d. w., respectively. *S. cochinchinensis* leaf extract exhibited anti-inflammatory effect via *in vitro* protein denaturation inhibition, hemolysis inhibition by heat and hypotonicity with $IC_{50}$ values of 63.20, 36.32, and 42.55 µg/mL, respectively. *S. cochinchinensis* leaf extract also displayed antioxidant potential with $IC_{50}$ values of 189.09 µg/mL (DPPH inhibition), 60.67 µg/mL (ABTS inhibition), and 140.53 µg/mL (Reducing power). Taken together, *S. cochinchinensis* leaves have great potential for anti-inflammatory, antioxidant activities and some compounds in pure form should be isolated and studied.

*Keywords: S. cochinchinensis* leaves, anti-inflammatory activity, antioxidant activity.

* Corresponding author.
*E-mail address:* lvminh05@gmail.com

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## 1. Introduction

Inflammation is a complex mechanism of vascular tissues to respond to detrimental stimuli. Inflammation is involved with a number of phenomena such as pain, membrane changes, increase of protein denaturation, and vascular permeability. Simultaneously, it has extensively proven to be associated with oxidative stress [1]. In the pathological classification, inflammation can be categorized into acute and chronic. In acute inflammation, there is an increase in the motion of plasma and white blood cells from the bloodstream into damaged tissues, which is the body's initial response to detrimental stimuli. A series of biochemical reactions then take place and activate the inflammatory response, which are related to the vascular system, immune system, and many cells in damaged tissues. When long-drawn inflammation leads to chronic inflammation, then the cells present in the inflammation area will mature and it is identified by the simultaneous demolition and wound healing process [2]. Non-steroidal anti-inflammatory drugs are ubiquitously applied for inflammation control. However, these drugs cause some side effects, particularly for stomachs such as stomach ulcers and stomach aches. Plants are an abundant source for new compounds with anti-inflammatory properties [3]. Consequently, the discovery and research of natural compounds from plants that have anti-inflammatory activity are increasingly expanding.

Besides, natural-derived products rich in antioxidant compounds have been gained more and more attention because of their beneficial functions on human health, especially their ability to fight off oxidative damage. Medicinal plants have been applied to treat human pathologies in worldwide for a long time since they are a potential source for antioxidant compounds, studies of their antioxidant activities have increased significantly [4].

*S. cochinchinensis* (Lour.) Moore belongs to the family, Symplocaceae and it is largely discovered in East Asia. The leaf of the plant has been proven to have antioxidant, antidiabetic, antilipidemic, anti-inflammatory, antitumor, and antimicrobial amongst other medicinal properties [5-7]. In addition, preliminary phytochemical analysis has revealed the presence of polyphenols, tannins, iridoids, flavonoids, and saponins in the *S. cochinchinensis* leaves [8]. However, despite the widespread folklore uses of *S. cochinchinensis* leaves in the management of various diseases, there have been few studies to evaluate and provide information on the biological properties of *S. cochinchinensis* leaves. Thus, this study will proceed to determine the anti-inflammatory and antioxidant capacities of *S. cochinchinensis* leaves by some *in vitro* assays. Furthermore, this study determines the phytochemical characteristics of *S. cochinchinensis* leaves by qualitative chemical reactions, thin layer chromatography, and total flavonoid and saponin contents.

## 2. Materials and Methods

### *2.1. Chemicals and Reagents*

Ethanol (98% *v/v*) was purchased from OPC Pharmaceutical Company. TLC silica gel 60 $F_{254}$ (Merck), egg albumin (GRM6421, ≥ 78%, HIMEDIA), diclofenac sodium (TLC ≥ 98%), methanol (HPLC ≥ 99.9%), ascorbic acid (vitamin C, HPLC ≥ 99%), Folin-Ciocalteu's phenol reagent, aluminum chloride (99.999% trace metals basis), quercetin (HPLC ≥ 98%), gallic acid (HPLC ≥ 98%), trichloroacetic acid (HPLC ≥ 99%), 1,1-diphenyl-2-picrylhydrazyl reagent (Quality level 200), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (HPLC ≥ 98%) were supplied by Sigma-Aldrich® Co. Ltd (USA).

### *2.2. Medicinal Plant Preparation*

The plant *S. cochinchinensis* was collected on August 2019 from Cham Island, Quang Nam Province, Vietnam. The Greenviet Biodiversity Conservation Centre and Southern Institute of Ecology, Vietnam Academy of Science and Technology identified and verified the samples; a voucher specimen was also deposited

for *Symplocos cochinchinensis* **(**Lour.) Moore ssp. *Laurina* (Retz.) Nooteb.

In order to remove dirt on the surface of leaves, they are washed with tap water, then distilled water. They were then air dried to the standard of losing weight due to drying in accordance with the Vietnam Pharmacopoeia 5$^{th}$ Edition. The dried materials were ground into powder and kept in sealed bag (Sample code: TTS-SC-0819) at Research Center of Ginseng and Medicinal Materials Ho Chi Minh City.

### *2.3. Extraction*

Material powder was extracted with 45% ethanol (material-solvent ratio of 1/15 (g/mL)) for 24 hours at room temperature using a device called percolator apparatus. During the extraction process, the extract was obtained at a rate of 1-3 mL/min and concentrated by a rotary evaporator under reduced pressure at 60 °C to gain total extract. The percentage yield of total extract of *S. cochinchinensis* leaves reached 23.04%. The total extract was conserved in vials and stored at 4 °C in a refrigerator and dissolved in a suitable solvent to yield a stock solution.

### *2.4. Determination of Physicochemical Parameters*

Physicochemical parameters were performed according to regulations in Vietnamese Pharmacopoeia 5$^{th}$ Edition about the loss on drying, the total ash content, and the hydrochloric acid-insoluble ash [9].

### *2.5. Qualitative Determination of Flavonoids*

The ethanol extract of *S. cochinchinensis* leaves were tested for the presence of flavonoids by the following chemical reactions: Alkaline reagent test, ferric chloride test, lead acetate solution test, and Shinoda's test [9].

### *2.6. Qualitative Determination of Saponins*

The ethanol extract of *S. cochinchinensis* leaves were tested for the presence of saponins by the following chemical reactions: Foam test, Fontan-Kaudel's test, and Liebermann-Burchard's test [9].

### *2.7. Thin Layer Chromatography (TLC) Analysis for Flavonoids and Saponins*

The stock solutions of the dried powdered material were prepared for flavonoids and saponins. The solvent systems with differing polarities were created and saturated for 30 min before deploying chromatography. This analysis was accomplished using TLC aluminium-backed plates, silica gel 60 $F_{254}$ (stationary phase) and solvent systems (mobile phase). The detection methods and solvent systems for flavonoids and saponins were shown in Table 1. The colored bands appeared were determined and the retention factor ($R_f$) was recorded.

Table 1. The solvent systems and detection methods in TLC analysis for flavonoids and saponins

| Metabolites | Mobile systems | Detection methods |
|---|---|---|
| Flavonoids | *n*-butanol: Acetic acid: Water (7: 1: 2, *v/v*) | UV light at 254 nm.<br>Spraying 5% $FeCl_3$ reagent and observing under visible light. |
| | Toluene: Ethyl acetate: Methanol: Formic acid (6: 6: 2: 1, *v/v*) | |
| | Ethyl acetate: Methanol: Formic acid: Acetic acid: Water (80: 10: 1: 1: 8, *v/v*) | |
| Saponins | Toluene: Ethyl acetate (7: 3, *v/v*) | Spraying 10% $H_2SO_4$ reagent, then drying at 105 °C for 10 min and observing under visible light. |
| | Toluene: Methanol: Formic acid (8: 1: 0.3, *v/v*) | |
| | Toluene: Ethyl acetate: Formic acid (3: 2: 1, *v/v*) | |

### 2.8. Determination of Total Polyphenol Content

The total polyphenol content was estimated by a previously described method using Folin-Ciocalteu's reagent with some modifications [10]. Briefly, 0.2 mL test extract was combined with 0.5 mL of Folin-Ciocalteu's reagent in 6 mL of distilled water. Subsequently, 1.5 mL of 20% *w/v* $Na_2CO_3$ solution was poured into this mixture right after 5 min, the volume reached 10 mL by distilled water. The reaction was kept for 2 hr at room temperature in the dark. The absorbance was recorded at 758 nm and all experiments were made in set of triplication. Total polyphenol content was calculated using the linear regression equation of gallic acid ($Y = 0.0019x - 0.2135$, $R^2 = 0.991$) and expressed as mg gallic acid equivalent per 1 gram of dry weight (mg GAE/ g d. w.).

### 2.9. Estimation of Total Flavonoid Content

The flavonoid content was investigated on the basis of spectral colorimetric method with aluminum chloride reagent [11]. To briefly illustrate, 1 mL of $AlCl_3$ (2% *w/v*) was incorporated with 1 mL diluted extract or standard quercetin solutions separately, and with methanol, the mixture was reached up to 10 mL in quantity. Subsequently, the solution was vortexed and kept at 25 $^oC$ for 15 min. The optical density of all tests was calibrated at 416 nm. The measurements were carried out in triplicate. Total flavonoids content was estimated using the linear regression equation of the quercetin ($Y = 0.0219x - 0.0554$, $R^2 = 0.998$) and presented as mg quercetin equivalent/g of dry weight (mg QE/g d. w.).

### 2.10. Determination of Total Saponin Content

The extract for saponins estimation was prepared from 20 g of material powder in methanol. The concentrated extract was then mixed with diluted water put into a 100 mL. Diethyl ether was then added and strongly shaken in the separating funnel. The aqueous layer was collected while the diethyl ether layer was removed. This process was repeated until the diethyl ether layer was depleted. Saturated-*n*-butanol was added to the aqueous layer and continued to shake vigorously. The *n*-butanol layer was recovered and the extraction process was repeated until the saponins was exhausted (cheked by thin layer chromatography). After that, the combined *n*-butanol extracts were washed with diluted water three times. The *n*-butanol extract was evaporated and dried to a constant weight. The measurement was repeated three times and values are presented as mg/g of dry weight.

### 2.11. In Vitro Anti-inflammatory Assays

#### 2.11.1. Inhibition of Protein Denaturation

Anti-inflammatory capacity was evaluated via inhibition of protein denaturation based on the method described previously [12] with slightly adjustments. To briefly illustrate, 2 mL extract at different concentrations or diclofenac sodium (positive control) was incorporated with 2.9 mL of PBS (pH = 6.4). After that, 100 µL of egg albumin was added and the mixture was incubated for another 15 min at (37±1) °C. The reaction mixture was continuously kept at 70 °C for 10 min for protein denaturation to be formed via using a water bath. The mixture after cooling, its absorbance is acquired at 660 nm. The tests were carried out in triplicate.

#### 2.11.2. Membrane Lysis Assay

i) Preparation of red blood cells (RBCs) suspension

The blood was taken from the tails of healthy *Swiss albino* mice (20-25 g) and transfered to the centrifuge tubes containing anticoagulant. After centrifuging (3000 rpm for 10 min), RBCs were washed with normal saline for three times (equal volume). The blood was suspended in normal saline to reach a concentration of 10% (*v/v*) [13];

ii) Heat induced haemolysis

The resulting reaction mixture consists of 1 mL different concentrations of test extract or diclofenac sodium (positive control) and 1 mL of 10% RBCs suspension were put into centrifuge tube. For the control sample, the extract was

replaced with saline [13]. The centrifuge tubes were kept at 56 ºC for 30 min and then were cooled by tap water. Subsequently, these tubes were centrifuged at 2500 rpm for 5 min and the optical density of the supernatants was recorded at 560 nm. All measures were done in triplicate;

iii) Hypotonicity-induced haemolysis

The reaction mixture consists of 0.5 mL test extract at variety of concentrations or diclofenac sodium (positive control), 1 mL of phosphate buffer, and 2 mL of hyposaline were mixed with 0.5 mL of RBCs suspension [2]. After incubating (at 37 ºC for 30 min), the reaction mixtures were centrifuged at 3000 rpm and the supernatant liquid was then decanted. The optical density was acquired at 560 nm to determine haemoglobin content. All tests were performed in triplicate. The proportion of protein denaturation/haemolysis inhibition was estimated using the expression:

$$\%\ inhibition = \frac{A_c - A_t}{A_c} \times 100$$

In which, $A_t$ and $A_c$ are absorbance of test sample and control sample, respectively. Simultaneously, the $IC_{50}$ value of test samples were also determined based on the equation illustrating the correlation between the test substance concentration and the percentage of inhibitory activity using Graphpad software.

### *2.12. In Vitro Antioxidant Activity Assays*

#### *2.12.1. 1,1-diphenyl-2-picrylhydrazyl Radical Quenching Assay*

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was applied to evaluate antioxidant capacity of *S. cochinchinensis* leaf extract based on a previously described method [12]. To briefly illustrate, reaction mixture (4 mL) consisting of 500 µL of different concentrations of test extract or ascorbic acid (positive control) and 500 µL of DPPH reagent (0.6 mM) in methanol was incubated at 25 ºC for 30 min in the dark. After that, the absorbance was acquired at 515 nm and all tests were done in triplicate.

#### *2.12.2. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation Decolorization Assay*

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antioxidant test was carried out according to the following description: In order to prepare the stock ABTS solution, 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate solution of equal volume and this mixture was then incubated in the dark for 16 hours at room temperature. After that, 1 mL of stock ABTS solution was diluted in 50 mL of methanol to reach an absorbance of 0.70 ± 0.05 units at 734 nm using a spectrophotometer. This solution was applied for testing. 20 µL of the test sample at various concentrations or ascorbic acid (positive control) was mixed with 980 µL of ABTS solution, the absorbance was measured at 734 nm after 6 min at room temperature. All tests were made in triplicate and an average of each sample was calculated [14]. The results were displayed as an $IC_{50}$ value for each sample from proportion of the radical quenching activity, which was calculated by the formula:

$$\%\ \text{scavenging effect} = \frac{A_c - A_t}{A_c} \times 100$$

In which, $A_c$ and $A_t$ are the absorbance of the control sample (without test extract) and the test sample (with test extract), respectively.

Antioxidant activity was also assessed through $IC_{50}$ (Half inhibitory concentration) which is the concentration of antioxidant that need to inhibit 50% of free radicals. The $IC_{50}$ value is determined based on the equation illustrating the correlation between the test substance concentration and the percentage of antioxidant activity using Graphpad software.

#### *2.12.3. Reducing Power (RP) Assay*

The reducing ability of the extract was assessed as described by Oyaizu [15]. Briefly, 200 µL of sample at the concentrations examined was added with 500 µL of PBS phosphate buffer solution (pH = 6.6) and 500 µL of $K_3[Fe(CN)_6]$ (carried out in the dark), incubated at 50 ºC, 30

minutes. After that, each tube was cooled and then added with 0.5 mL of 10% trichloacetic acid solution and centrifuge 3000 cycles for 10 min. Then, 500 µL of the above solution (the supernatant) was taken into a new eppendorf, 100 µL of $FeCl_3$ solution and 500 µL of distilled water were added. Measuring absorbance at a wavelength of 700 nm, the optical density value OD reflects the reducing ability of the sample. The antioxidant ability of the test sample was judged via optical density and $EC_{50}$ values. The lower the optical density value, the weaker the reduction activity of the sample. $EC_{50}$ value is concentration of effective antioxidant for optical density reaches 0.5, which was calculated through the equation illustrating the correlation between the concentration of the sample and the its optical density using Graphpad software.

### *2.13. Statistical Analysis of Data*

The data were statistically analyzed by t-test and expressed in terms of mean ± SEM (Standard error of the mean) using Graphpad Prism software (version 8.0.2, Inc., La Jolla, CA, USA).

## 3. Results

### *3.1. Physicochemical Parameters*

Some physicochemical parameters including loss on drying, total ash and hydrochloric acid-insoluble ash values of raw materials of *S. cochinchinensis* leaves were determined (Table 2). Generally, these parameters reached the standard according to regulations in Vietnamese Pharmacopoeia 5$^{th}$ Edition.

Table 2. Some physicochemical parameters of raw materials of *S. cochinchinensis* leaves

| Parameters | Raw materials (mean ± SEM, $n$ = 3) | Reference standard of Vietnamese Pharmacopoeia 5$^{th}$ Edition |
|---|---|---|
| The loss on drying (%) | 9.73 ± 0.41 | ≤ 13% |
| The total ash content (%) | 7.85 ± 0.23 | 4–12% |
| The hydrochloric acid-insoluble ash (%) | 0.36 ± 0.03 | ≤ 2.4% |

Table 3. The qualitative results of flavonoids and saponins in *S. cochinchinensis* leaves

| Phytochemicals | Test/reagents | Finding/result |
|---|---|---|
| Flavonoids | Alkaline reagent test | + |
| | Ferric chloride test | + |
| | Lead acetate solution test | + |
| | Shinoda's test | + |
| Saponins | Foam test | + |
| | Fontan-Kaudel's test | + |
| | Liebermann-Burchard's test | + |
| *(+): positive* | | |

### *3.2. Qualitative Determination of Flavonoids and Saponins by Chemical Reactions and Thin Layer Chromatography (TLC) Analysis*

The result by chemical reactions showed that *S. cochinchinensis* leaf extract had a positive reaction with specific reagents of flavonoids and saponins (Table 3). This indicated that the presence of flavonoids and saponins in *S. cochinchinensis* leaves. Therefore, *S. cochinchinensis* leaf extract was further analyzed by TLC.

The successfully developed TLC plate was observed at 254 nm and after 5% $FeCl_3$ reagent was sprayed on TLC plate generated different band colors and positions from each other for flavonoids. Similarly, the successfully developed TLC plate was also sprayed with 10%

sulfuric acid reagent, then dried at 105 °C for 10 min generated different band colors under visible light and positions from each other for saponins. The TLC profiling results indicated the presence of various phytochemicals within the *S. cochinchinensis* leaf extract. The $R_f$ values of flavonoids and saponins in solvent systems were shown in Table 4.

Table 4. The $R_f$ values on TLC profiling of flavonoids and saponins

| Solvent system | Number of spots and their $R_f$ value | |
|---|---|---|
| | Flavonoids | Saponins |
| 1 | 0.57, 0.66 and 0.72 | 0.53, 0.61 and 0.83 |
| 2 | 0.16, 0.29 and 0.82 | 0.35, 0.47 and 0.76 |
| 3 | 0.35, 0.45 and 0.66 | 0.20, 0.43 and 0.76 |

### *3.3. Total Polyphenol, Flavonoid, and Saponin Contents*

Results showed that the total polyphenol, flavonoid, and saponin contents of *S. cochinchinensis* leaves were 517.71 ± 19.50 mg GAE/g d. w. (dry weight), 1.12 ± 0.01 mg QE/g d. w., and 32.18 ± 0.24 mg/g d. w., respectively.

### *3.4. Anti-inflammatory Activity of S. cochinchinensis Leaf Extract*

The extract of *S. cochinchinensis* leaves was able to prevent protein denaturation and hemolysis in a dose-dependent manner. The inhibitory effect of *S. cochinchinensis* leaves at different concentrations (12.5-200 µg/mL) is displayed in Figure 1A. The proportion of inhibition of protein denaturation, hemolysis by heat, and hemolysis by hypotonicity of *S. cochinchinensis* leaf extract was within the range from 18.58 ± 0.59% to 73.49 ± 1.86%, 22.00 ± 0.26% to 85.23 ± 0.72%, and 23.05 ± 0.61% to 82.71 ± 1.53%, respectively. The inhibitory rate of the positive control was shown in Figure 1B. The $IC_{50}$ values of the extract of *S. cochinchinensis* leaves in the assays was lower than that of diclofenac sodium (Table 5).

### *3.5. Antioxdiant Activity of S. cochinchinensis Leaf Extract*

Antioxidant ability of *S. cochinchinensis* leaf extract was examined via an array of assays compared to ascorbic acid (positive control) (Figure 2). Results divulged that the extract showed the DPPH radical quenching property in a concetration-dependent manner, which was over 50% at 250 µg/mL (61.22 ± 1,06%) with an $IC_{50}$ value of 189.09 µg/mL, which was comparable with ascorbic acid (4.20 µg/mL) (Figure $2A_1$ and Table 5).

In Figure $2A_2$, *S. cochinchinensis* leaf extract was discovered to have potential in quenching the ABTS radical. The percentage inhibition of this radical was concentration-dependent. At 64 µg/mL, the inhibition of the extract was 50.84 ± 6.03%. The $IC_{50}$ of positive control (ascorbic acid) was 9.59 µg/mL while that of the extract was 60.67 µg/mL (Table 5).

The antioxidant proficiency of the *S. cochinchinensis* leaf extract as examined by the reducing power assay was also depicted in Figure $2A_3$. In this assay, the absorbance of *S. cochinchinensis* leaf extract was found to be 0.501 ± 0.009 at 150 µg/mL with an $EC_{50}$ value of 140.53 µg/mL. The reference; ascorbic acid had an $EC_{50}$ value of 4.19 µg/mL (Table 5).

In general, the *S. cochinchinensis* leaf extract showed potent antioxidant activities with $IC_{50}$ values (compared by assay) in the order of ABTS < reducing power < DPPH. Therefore, the *S. cochinchinensis* leaf extract exhibited a more typical antioxidant activity in the direction of ABTS cation decolorization property.

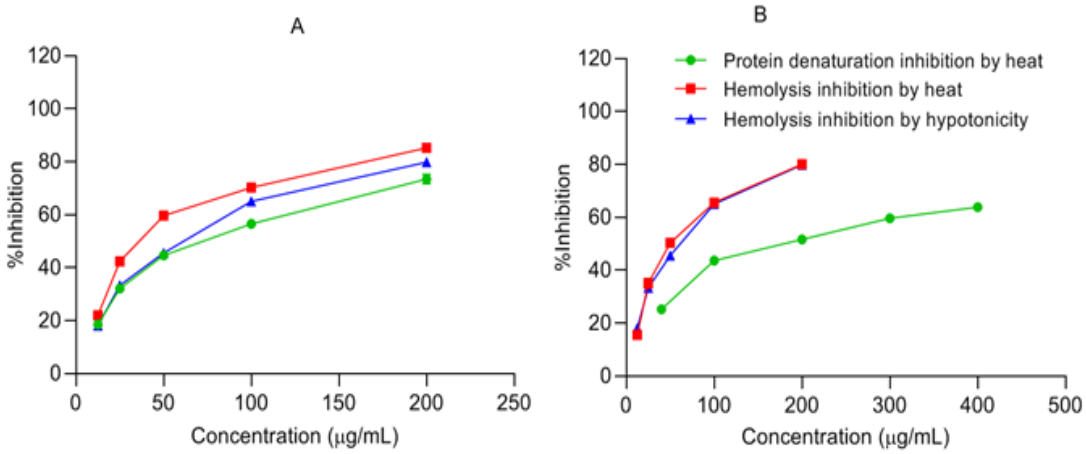


Figure 1. Effects of *S. cochinchinensis* leaf extract on inhibition of protein denaturation and hemolysis. Percentage inhibition at different concentrations of *S. cochinchinensis* leaf extract (A) and positive control (Diclofenac sodium) (B). Mean ± SEM ($n$ =3).

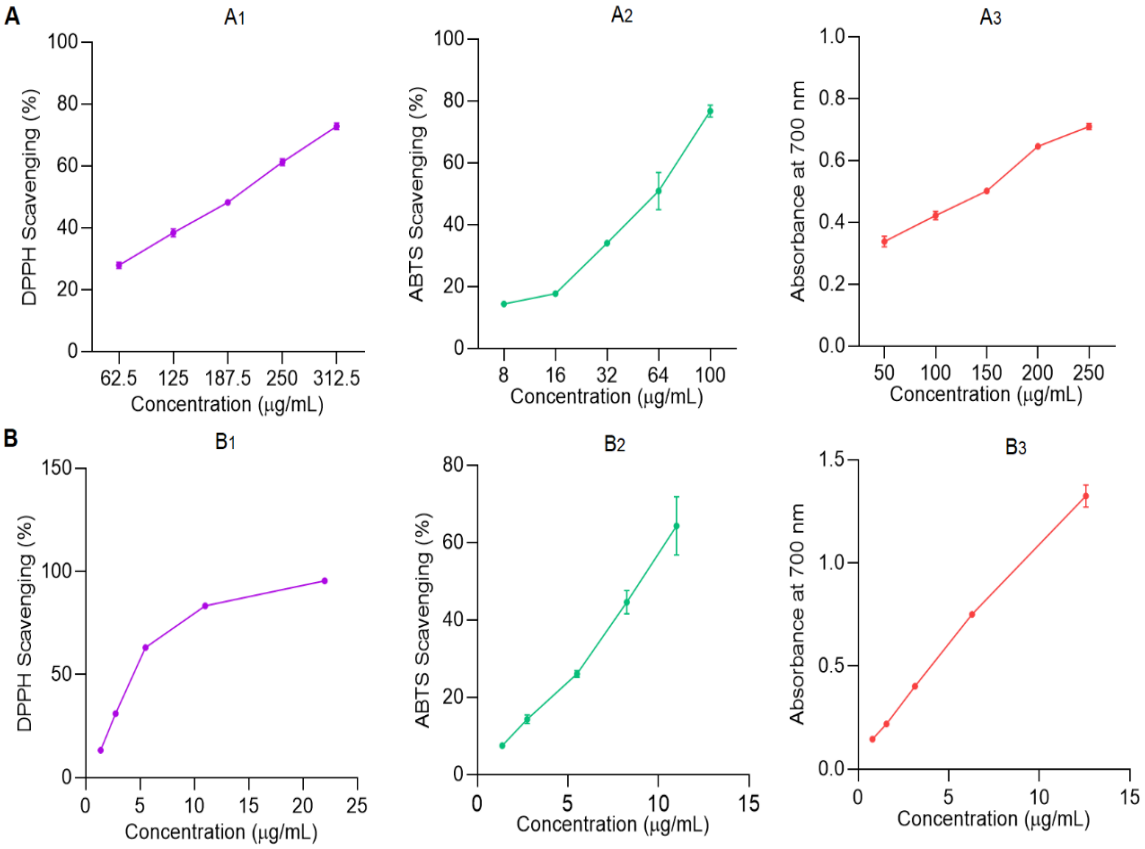


Figure 2. Antioxidant activity of *S. cochinchinensis* leaf extract (A) and ascorbic acid (B) for DPPH radical scavenging activity ($A_1$ - $B_1$), ABTS radical scavenging activity ($A_2$ - $B_2$), Reducing power (RP) activity ($A_3$ - $B_3$). $p$<0.001, a significant difference one by one in each test. Mean ± SEM ($n$ = 3).

Table 5. Determination of anti-inflammatory and antioxidant activities of *S. cochinchinensis* leaf extract

| Activity | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | Extract | Positive control |
| Protein denaturation inhibition | 63.20 ± 0.52 | 175.50 ± 3.45 |
| Hemolysis inhibition by heat | 36.32 ± 0.30 | 49.79 ± 1.18 |
| Hemolysis inhibition by hypotonicity | 42.55 ± 0.20 | 54.99 ± 0.53 |
| DPPH inhibiton | 189.09 ± 1.75 | 4.20 ± 0.04 |
| ABTS inhibiton | 60.67 ± 0.63 | 9.59 ± 0.90 |
| Reducing power ($EC_{50}$) | 140.53 ± 3.28 | 4.19 ± 0.11 |

## 4. Discussion

During the inflammatory process, protein denaturation can occur which is characterized by changes in electrostatic hydrogen, hydrophobic and disulfide bonds. Protein denaturation leads to the autoantigens production in a number of pathologies. Therefore, if protein denaturation is inhibited, inflammation can be prevented [16]. Besides, the infiltration of cells, especially leukocytes, also has an important effect of the inflammatory response. Lysosomal enzymes are released from leukocytes which damage tissue and promote inflammatory events. Among cell types, erythrocyte membrane is similar to lysosomal membrane, so the inhibition of erythrocyte hemolysis can be applied to assess the anti-inflammatory ability. Stabilizing these cell membranes can slow or limit the release of cytoplasmic content that contributes to minimizing tissue damage [17, 18].

Taken together, in this study, an array of assays including protein denaturation, heat-induced hemolysis, and hypotonicity-induced haemolysis was applied to survey the anti-inflammatory property of *S. cochinchinensis* leaf extract. Interestingly, *S. cochinchinensis* leaf extract is in mixed form and has not been purified, it exhibited the protein denaturation and hemolysis inhibitory activities, which was better than the standard drug that has been purified and commercialized. Previous studies had also shown *in vitro* and *in vivo* anti-inflammatory activities of *S. cochinchinensis* leaves [19]. However, the *in vitro* anti-inflammatory capacity of extracts (*n*-hexane, chloroform, ethyl acetate, methanol extracts) in that study was lower than 45% ethanol extract of *S. cochinchinensis* leaves in the present study.

Free radicals have been shown to play a significant part in many diseases. One of the mechanisms by which antioxidants support to the body's immune system to fight free radicals is its ability to quench them [20]. In this study, free radicals scavenging abilities including $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ were investigated. For the antioxidant potential screening of total extracts, the DPPH and ABTS assays are highly valued, which is its electronic donation ability to purge DPPH color and the possibility of decolorizing the blue-green color of $ABTS^{\bullet+}$ solution when adding antioxidants to this solution. The degree of color reduction is proportional to the antioxidants' concentration and capacity. In the presence of a large number of compounds that have free radical quenching property, the absorbance of the reaction sample is remarkably reduced [20]. Our results showed that *S. cochinchinensis* leaf extract has the ability to bleach DPPH and ABTS free radicals.

In the reducing power ability assay, the capacity to change colors will depend on the reducing competence of the test sample. Previous studies have shown that reducing motions are acted on through the action of antioxidants due to the contribution of a hydrogen to inhibit the free radical reaction chain. At this wavelength, the higher the absorbance, the stronger the reduction capacity.

The result illustrated that reducing activity power of *S. cochinchinensis* leaf extract accreted with concentration.

Secondary metabolites or in combination together may have a positive role in biological activities of *S. cochinchinensis* leaves. In this study, *S. cochinchinensis* leaf extract was demonstrated to contain polyphenols, flavonoids, saponins with antioxidant and anti-inflammatory properties. These achieved results were consistent with the research of Sunil et al., [5, 6] which also supported the vital role of polyphenols derived from *S. cochinchinensis* the leaf and bark extracts in India with antioxidant activity through reductive ability, scavenger of DPPH, nitric oxide or inhibition of lipid peroxidation.

Importantly medicinal and pharmacological properties have been demonstrated in many researches which would be attributed to polyphenol compounds in the presence of many plant extracts, for instances, antioxidant, anti-inflammatory, analgesic, antibacterial, hepatoprotective, and antidiabetic activities [21]. Polyphenol compounds play the most crucial role for their antioxidant properties because of their ability to give hydrogen from its hydroxyl group. Flavonoids represent about two-thirds of the polyphenols in the diet, are considered to be the most powerful antioxidants. Flavonoids are phytochemical found in many medicinal herbs and they have a certain role in the cure of human pathologies and the protection of human health such as antioxidant, anti-inflammation, anticancer, antidiabetes, coronary heart disease prevention, hepatoprotection, against neurodegenerative diseases like Alzheimer's and Parkinson's [22]. Besides, saponins have anticancer, anti-inflammatory, analgesic, antinociceptive, immunomodulatory, and anti-stress properties [23].

## 5. Conclusion

*S. cochinchinensis* leaf extract showed considerable antioxidant effects by the ability to scavenge free radicals and anti-inflammatory potential by inhibition of protein denaturation and hemolysis. Further studies should be conducted such as isolating antioxidant and anti-inflammatory compounds, determining their biological effects.

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