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Phytochemical Profiling, Antioxidative and Antibacterial Efficacy of Leaf Aqueous Extract of *Cassia siamea*

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Abstract: In human race, the medicinal plants have shown to cure and prevent diverse illnesses from long period. *Cassia siamea* is well-known in the family of Fabaceae. The various parts of this medicinal plant are applied in the traditional form of healing practices. In the present study, we have prepared the aqueous extract of leaf of *Cassia siamea* and evaluated its antioxidative and antibacterial potency. The basic phytochemical investigation authenticated the presence of flavonoids, phenols, steroids, alkaloids, tannins, proteins, saponins, terpenoids and carbohydrates. The DPPH radical scavenging assay illustrated the dose-mannered inhibition of the DPPH radical by the aqueous extract of leaf of *Cassia siamea* with 76.66% inhibition at 125 µg/ml (IC_{50} dose: 82.06 µg/ml). The hydroxyl radical assay, presented 62.67% of inhibition of hydroxyl radicals by the aqueous extract of leaf of *Cassia siamea* at the utilized higher dose with an IC_{50} value of 84.06 µg/ml. The antibacterial assay performed using agar-well diffusion methodology reflected the bactericidal role of the aqueous extract of leaf of *Cassia siamea* with higher inhibitory zone at 80 µg/ml dose, for *Bacillus cereus* (7.3 ± 0.62 mm) and *Escherichia coli* (9.2 ± 0.70 mm). The bactericidal activity was also dose-based.

Keywords: *Cassia siamea*, Antioxidative, Antibacterial, DPPH, Hydroxyl radical

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Introduction

To cure human diseases, medicinal plants have inevitable role from historical period, in addition to offering basic necessities. They have been in practice in various cultures throughout the globe

due to their unique phytochemical contents which owns potent physiological functioning into the dwelling body (Packirisamy *et al.*, 2023).Usually, tannins, phenolic compounds, alkaloids and flavonoids are the powerful bioactive ingredients aiding in health benefits in versatile ways. Moreover, the phyto-drugs are safer with negligible aftermath, budget-friendly and easily availability. Major population percentage (80%) uses phyto-drugs for health healing (Malik *et al.*, 2023).Numerous scientific experimentations have made the application of indigenous medicinal plants and their constituents as a potent drug source. Indian system of indigenous medicines (Unani, Siddha and Ayurveda) have proven the utility of medicinal plants as a preventive and curative tool for health complications. Diverse sections of medicinal plants are adopted to heal various human aliments (Chaudhari *et al.*, 2010).

In the present days, the infectious diseases spread by the pathogenic bacteria is hugely rising (Tiwari *et al.*, 2017; Shelke *et al.*, 2024). The bacterial strains had adopted new resistive mode for surviving creating the risk of multi-drug resistance bacterial strains. As per the report of World Health Organization, numerous bacterial strains are losing their susceptibility towards the antibiotics and this has created the concern for life-harming infectious diseases. The failure of the commercial marketed anti-infective drugs to eradicate the infectious bacteria have triggered the scientific community to design an efficacious drug to overcome the multi-drug resistance issue of the pathogenic bacterial strains and to solve the issue from its root (Revathi *et al.*, 2023). Moreover, the anti-infective drugs when taken for longer duration can pose negative health impacts such as destroying the natural gut flora, inflammatory reactions, abdominal discomforts, immune-suppression and damage to the vital organs (kidney and liver)(Serene *et al.*, 2018).The phyto-contents of the medicinal plants are attributed to be the efficacious source of lysing the bacteria (Rej *et al.,* 2014).

Naturally, within the biological system, free radicals are continuously formed through various processes such as phagocytosis, respiratory chain reactions and prostaglandin formation involving the role of enzymes. Additionally, exogenic factors such as pollutants, smoking, ozone and X-rays also serves as stimulant for free radical genesis (Lobo *et al*., 2010). Too much free radicals within the system upsurges the oxidative type of stress which induces the deteriorations of the macromolecular structures such as proteins, nucleic acid (DNA) and lipids in the living system (Farag *et al.*, 2020). This can stimulate the occurrence of cancer, chronic inflammatory conditions, liver diseases, renal diseases, cardiovascular diseases, diabetes mellitus, auto-immune conditions (rheumatoid arthritis), cataract and lung diseases (Chaudhary *et al.,* 2023). The compounds with the efficiency of delaying the oxidation and halting the generation or propagation of free radicals are termed as antioxidants. They aid in cleaving the auto-oxidative form of cascade reaction. Naturally, the medicinally valued plants are filled with antioxidant traits owned to the high phenolic contents which efficaciously delivers their hydrogen atom to the free radicals and hinders its baneful impact (Stankovic *et al.*, 2016; Fierascu *et al.,* 2018). The plant-derived antioxidative compounds display quenching of singlet/triplet oxygen, decomposition of peroxide and halting the functioning of oxidative enzymes (Choi *et al.*, 2002). Due to the toxic impacts of synthetically availed antioxidants, the plant sourced antioxidants are in trends (Lourenco *et al*., 2019).

Cassia genus (family Fabaceae) has nearly 260 species distributed in the Asian countries. *Cassia siamea* is native to Africa and America. In the traditional healing practices, it is used for treating wounds, anthrax, warts and poisonous snake bites. Also, the various sections of the plants are applied to treat rhinitis, menstrual pain, malaria, urinary tract infections, toothache, abdominal pain, diabetes mellitus and jaundice. The leaf has shown to possess antimicrobial, antiproliferative, antilipemic and antioxidant properties (Gebrehiwot *et al.*, 2024). Various parts of this plant have disclosed the existence of phyto-

contents like phenolic acids, steroids, flavonoids, saponins, tannins and terpenoids (Koffi *et al.*, 2019).

In the present study we have applied the aqueous extract of leaf of *Cassia siamea* and evaluated the antioxidative as well as antibacterial efficacy, as very few reports are available on the pharmacological efficacy of this plant.

Materials and Methods

The leaves from the plant *Cassia siamea* were collected in a plastic container. In the laboratory, the healthy and the uninfected leaves were separated manually, washed and rinsed in the distilled water and shade-dried for three weeks. Then blended powder of leaves was obtained by milling the crushed leaves in the mixer. Exactly, 1 L of the distilled water and 50 g of the leaf powder was carefully loaded in the Soxhlet equipment. The soxhlation was executed for 24 h, and the light yellowish-brown coloured liquid gathered was collected and filtered through Whatman No.1 filter paper. The filtrate was placed in a rotary evaporator to exclude the liquid content. The sticky yellowish-brown residue acquired was scraped and retained in a sterile glass vial followed by refrigeration (4 ℃) for further experiment (Trivedi *et al.*, 2024).

Phytochemical qualitative profiling (*Manohar and Raja, 2021*; *Sriyanti et al., 2022*; *Janani et al., 2023*; *Manjeera and Sundararajan, 2024*)

20 mg of the aqueous extract of leaf of *Cassia siamea* was mixed in distilled water and then the diluted extract was applied for spotting the phytochemical ingredients by adopting following standard testing methods:

(A) *Alkaloid screening by Mayer's test*

To spot the alkaloids, 2 ml of the aqueous extract of leaf of *Cassia siamea* was reacted with Mayer's reagent (1 ml). A whitish/creamy coloured precipitate appeared signified alkaloid's presence.

(B) *Flavonoids screening by Shinoda test*

For this test 1 ml of the aqueous extract of leaf of *Cassia siamea* was slightly warmed with 5 drops of ethanol and added magnesium ribbon (0.5 g) along with 2 drops of concentrated hydrochloric acid and the pink colour emergence indicated the flavonoids.

(C) *Saponin screening by foam test*

The aqueous extract of leaf of *Cassia siamea* (1 ml) was diluted with 3 ml of distilled water in a sterile test tube and the tube was constantly shaken. Persistent bubble or foam formation dictated saponin content.

(D) *Tannin screening by gelatin test*

The aqueous extract of leaf of *Cassia siamea* (2 ml), 10% sodium chloride (1 ml) and 1% gelatin (1 ml) were reacted together. A visible whitish precipitation notified tannin existence.

(E) *Phenol screening by Ellagic acid test*

To 1 ml of the aqueous extract of leaf of *Cassia siamea*, equal volume of 5% glacial acetic acid was mingled along with 0.5 ml of sodium nitrite (5%). Phenol's presence was justified by muddy-brown colouration.

(F) *Steroids and terpenoid screening by Salkowski's test*

2 ml of aqueous extract of leaf of *Cassia siamea* was treated with three drops of sulphuric acid in addition to 1 ml of chloroform. A reddish to dark brownish coloured precipitate denoted steroids and terpenoid's existence.

(G) *Carbohydrate screening by Benedict's test*

2 ml of Benedict's reagent was boiled with 1 ml of aqueous extract of leaf of *Cassia siamea* for 5 min in a boiling water bath. The bluish colour alteration to brick-reddish attributed for the carbohydrates.

(H) *Protein screening by Xanthoproteic test*

3 drops of concentrated nitric acid were mingled with 2 ml of aqueous extract of leaf of *Cassia siamea*. A light yellowish to deep yellowish colour development showed protein in the tested leaf extract.

Quantitative analysis of phyto-content of aqueous

extract of leaf of Cassia siamea

(a) *TPC* (*Total phenolic content*) *quantification*

F-C (Folin-Ciocalteu) reagent was applied to quantify the TPC of aqueous extract of leaf of *Cassia siamea*. Precisely, aqueous extract of leaf of *Cassia siamea* (0.3 ml) was blended with 2.25 ml of Folin-Ciocalteu phenol reagent and maintained for 5 min to react. Then, 2.25 ml of sodium carbonate solution was added to the content and retained at room temperature for 90 min for the reaction. Finally, the absorbance of the reacted mixture was taken spectrophotometrically at 725 nm. Gallic acid (0 – 200 µg/ml) was used to plot the standard curve and from the graph, the TPC was measured and presented as mg of GAE (gallic acid equivalent)/g of leaf weight (Raghuvanshi *et al.*, 2022).

(b) *Alkaloid quantification*

The content of alkaloid in the aqueous extract of leaf of *Cassia siamea* was measured using the protocol given by Ullah Khan *et al.* (2023) with slight modification. 5 g of the aqueous extract of leaf of *Cassia siamea* was mingled with acetic acid (10%, 200 ml made in ethanol). The content was left to react for 4 h at ambient temperature. Post incubation, the content was filtered and the filtrate was reduced to one-quarter of the entire filtrate volume by placing on a hot water bath at 60 ℃. Then added 10 drops of ammonium hydroxide to the concentrated filtrate until the precipitation developed. In a sterile conical flask, the precipitate appeared was transferred and washed gently, applying diluted solution of 20 ml of ammonium hydroxide (0.1 M) and filtered. The final precipitate was collected, dried and weighed. The quantity of alkaloids was calculated by the following formula:

Alkaloid content = (Residue weight/ Weight of the leaf extract taken) x 100

(c) *Total flavonoid content* (*TFC*) *quantification*

Using aluminium chloride assay, the TFC in the aqueous extract of leaf of *Cassia siamea* was determined. For the quantification of TFC, exactly, 0.5 ml of aqueous extract of leaf of *Cassia siamea* prepared as 0.1 mg/ml was further diluted to 2 ml using distilled water and carefully mixed with 1.2% w/v of aluminium chloride solution (0.5 ml) and 120 mM of potassium acetate (0.5 ml). The whole mixture was retained for 30 min at ambient condition and the absorbance was noted at 425 nm. As standard quercetin was applied and the TFC was presented as mg QE/g (mg of quercetin equivalent/g of leaf material)(Takaidza *et al.,* 2018).

(d) *Tannin quantification*

To quantify the tannin in the aqueous extract of leaf of *Cassia siamea*, Folin-Ciocalteu protocol was applied as executed in the previous work with mild changes. The aqueous extract of leaf of *Cassia siamea* (100 µl prepared as 10 mg/ml) was poured into a sterile 20 ml standard flask along with distilled water (7.5 ml). Then added 0.5 ml of Folin-Ciocalteu reagent in the reacting mixture followed by addition of 10 ml of sodium carbonate (35%). The content was made up to 20 ml applying distilled water. The entire mixture was shaken well and retained in dark condition for half an hour. The absorbance was taken spectrophotometrically at 725 nm. Tannic acid was used as standard. The entire tannin content was quantified and given as mg of tannic acid equivalent/g of leaf extract (mg of TAE/g (Malada *et al.*, 2022).

(e) *Saponin quantification*

To quantify the saponin in the aqueous extract of leaf of *Cassia siamea*, vanillin-sulphuric acid colorimetric protocol was adopted. In brief, the aqueous extract of leaf of *Cassia siamea* (50 µl) was gently mixed with distilled water (250 µl). To this, added vanillin reagent which was prepared by diluting vanillin (800 mg) in 99.5% of ethanol (10 ml). To this added, 2.5 ml of 72% sulfuric acid. The whole mixture was mixed thoroughly and retained for 10 min at 60 ℃ on a water bath. The mixture was cooled in the ice-cold water and the absorbance was noted at 544 nm. Diosgenin (Dg) was applied as a standard and the entire saponin content was measured and presented as mg of

Dg/g of leaf extract (Snehalatha and Rasmi, 2021).

Antioxidant assays

DPPH assay

The antioxidative potency of the aqueous extract of leaf of *Cassia siamea* was measured applying the standard DPPH protocol as per method of Maharani and Anna Sheba (2019) with slight amendments. 1 ml of varying doses (25, 50, 75, 100, 125 µg/ml) of aqueous extract of leaf of *Cassia siamea* was mingled with 1 ml of freshly made solution of 0.004% w/v of methanolic solution of DPPH and incubated for 20 min. Post-incubation the absorbance of the reacted mixture was measured at 517 nm by spectrophotometer. Here, ascorbic acid was used as reference positive control. The inhibition achieved for the DPPH radical activity in percentage was calculated by adopting the following formula:

% of DPPH racial quenching = $[(ABS_{(C)} - ABS_{(TS)})/ABS_{(C)}] \times 100$

Where, $ABS_{(C)}$ signify the control absorbance; $ABS_{(TS)}$ signify aqueous extract of leaf of *Cassia siamea* absorbance

Hydroxyl radical scavenging assay

The antiradical efficacy of the aqueous extract of leaf of *Cassia siamea* was evaluated by method of Pavithra and Vadivukkarasi (2015) with slight amendments. 1 ml of varying doses of aqueous extract of leaf of *Cassia siamea* (25, 50, 75, 100, 125 µg/ml), one ml of 0.85% dimethyl sulphoxide made in phosphate buffer (0.1 mol/l, pH 7.4), EDTA (0.5 ml, 0.018%) and 1 ml of iron-EDTA solution (made by adding 0.26% of EDTA and 0.3% ferrous ammonium sulphate) were blended together along with ascorbic acid (0.22%, 0.5 ml). The test tubes were retained at 80 ℃ in the hot water bath for 15 min. Termination of the reaction was performed by adding 17.5% of ice-cold 1 ml of tricarboxylic acid (TCA). Then to the tube mixture added three ml of Nash reagent [prepared by adding glacial acetic acid (3 ml), ammonium acetate (75%) and acetyl acetone (2 ml); the entire content was diluted to 1 L utilizing distilled water]. The tube content was incubated for 15 min under ambient condition for the formation of yellowish colour. Subsequently, the intensity of the appeared colour was checked at 412 nm in a spectrophotometer. For positive reference, ascorbic acid was utilized. The hydroxyl radical quenching was measured adopting following formula:

Hydroxyl radical decline in % = $[(ABS_{(C)} - ABS_{(TS)})/ABS_{(C)}] \times 100$

Where, $ABS_{(C)}$ signify the control absorbance; $ABS_{(TS)}$ signify aqueous extract of leaf of *Cassia siamea* absorbance

Antibacterial assay

Adopting well-known agar-well diffusion methodology with slight changes, the bactericidal potency of the aqueous extract of leaf of *Cassia siamea* was assessed (Manore *et al.*, 2012). By pouring 20 ml of Mueller-Hinton (M-H) agar medium in the petri-plates under sterile environment, the MH plates were made and retained to solidify for 10 min in the LAF (laminar air flow chamber). Exactly, 100 µl of the sterile and well grown bacterial culture (*Bacillus cereus* and *Escherichia coli*) was gently and uniformly layered on the surface of agar utilizing a L-shaped sterile glass rod. Then, the 6 mm wells were designed in the MH agar plates, with the help of a sterile steel well-borer. Varying doses of aqueous extract of leaf of *Cassia siamea* (20, 40, 60 and 80 µg/ml) was loaded into each well of individual bacterial strains and retained uninterrupted in a bacterial incubator (37 ℃) for one day. The inhibitory zones, appeared surrounding each well was noticed and measured carefully in millimetres (mm). Here, Ciprofloxacin (10 µg/ml) was implemented for reference need as a positive control.

Results and Discussion

The phytochemical testing performed indicated the presence of the active phyto-metabolites (Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Carbohydrates, Steroids and terpenoids in the aqueous extract of leaf of *Cassia siamea*

Table1: Phyto-contents found in the aqueous extract of leaf of *Cassia siamea*

Leaf secondary metabolites	Report
Alkaloids	Copiously evidenced
Flavonoids	Copiously evidenced
Saponin	Moderately evidenced
Tannin	Moderately evidenced
Phenol	Moderately evidenced
Steroids and terpenoids	Moderately evidenced
Carbohydrates	Slightly evidenced

Table 2: The quantification of the phyto-ingredients of the aqueous extract of leaf of *Cassia siamea*

Phyto-contents	mg/g
TPC	67.24
Alkaloid	20.99
Flavonoid	48.60
Tannin	15.02
Saponin	10.77

(Table 1). The quantification of some of the phyto-ingredients in the aqueous extract of leaf of *Cassia siamea* is depicted in Table 2.

Quantification of leaf extract

The quantification of some of the phyto-ingredients in the aqueous extract of leaf of *Cassia siamea* is depicted in Table 2.

DPPH radical scavenging assay

The ability of the aqueous extract of leaf of *Cassia siamea* to quell the impact of DPPH radical was checked. At the used minimum concentration to maximum concentration, the DPPH percentage inhibition was 18.27%, 26.78%, 38.23%, 61.63% and 76.66%. As the concentration of the aqueous extract of leaf of *Cassia siamea* raised the inhibitory percentage of DPPH radical was also escalated. The IC_{50} for the aqueous extract of leaf of *Cassia siamea* was 82.06 µg/ml and for the ascorbic acid it was 74.90 µg/ml. Overall, the aqueous extract of leaf of *Cassia siamea* illustrated dose-based DPPH radical inhibition role (Table 3).

DPPH is recognized as a stabilized nitrogenous free radical owning purple colouration. Its interactivity with the antioxidative compounds, causes reduction promoting the acceptable of hydrogen atom/electrons from the antioxidative compounds and turning the purplish coloured DPPH to yellowish coloured compound (2, 2-diphenyl-1-picrylhydrazine)(Elzaher *et al.*, 2024). The DPPH purple colour decolourization to yellowish has direct proportionality to the antioxidant's efficacy (Saeed *et al.*, 2012). The plant's phenolic compounds have aromatic or benzene ring in their structure along with hydroxyl groups (one or two OH groups), with functional groups such as glycosides, ester and methyl esters. The OH groups are amenable for the antioxidative functioning of the plant extracts (Nimse and Pal, 2015).

Hydroxyl radical scavenging assay

The inhibitory role of aqueous extract of leaf of *Cassia siamea* on the hydroxyl radical was assessed. At the used lower dose of 25 µg/ml, the aqueous extract of leaf of *Cassia siamea* caused 16.13% of inhibition of hydroxyl radical. Hydroxyl radical inhibition of 62.67% was visualized when

Table 3: DPPH radical scavenging percentage presented by the aqueous extract of leaf of *Cassia siamea*

Concentration (µg/ml)	% inhibition of DPPH radical by aqueous extract of leaf of *Cassia siamea*	% Inhibition of DPPH by ascorbic acid (Positive control)
25	18.27 ± 0.56	29.69 ± 0.95
50	26.78 ± 0.77	44.96 ± 0.68
75	38.23 ± 0.71	61.19 ± 0.65
100	61.63 ± 0.62	71.54 ± 0.76
125	76.66 ± 0.89	94.63 ± 0.94

Table 4: Hydroxyl radical scavenging percentage presented by the aqueous extract of leaf of *Cassia siamea*

Concentration (µg/ml)	% inhibition of hydroxyl radical by aqueous extract of leaf of *Cassia siamea*	% Inhibition of hydroxyl radical by ascorbic acid (Positive control)
25	16.13 ± 0.64	27.88 ± 0.81
50	24.15 ± 0.96	34.46 ± 0.63
75	31.80 ± 0.88	49.99 ± 0.72
100	45.74 ± 0.73	70.48 ± 0.89
125	62.67 ± 0.99	80.09 ± 0.85

125 µg/ml of the aqueous extract of leaf of *Cassia siamea* was used. The IC_{50} dose for the aqueous extract of leaf of *Cassia siamea* was 84.06 µg/ml and for ascorbic acid it was 77.61 µg/ml (Table 4).

Within the living bodies, the hydroxyl radicals are immensely reactive and extremely potent in causing damage to the bio-organic compounds in the cells. On combination with DNA nucleotides, these radials cleave the strands bringing the risk of carcinogenesis, mutation and cellular toxicity (Khan *et al.*, 2012). Plant flavonoids are dynamic hydroxyl radical quencher, along with other bioactive secondary metabolic compounds (Lalhminghlui and Jagetia, 2018).

Antibacterial assay

The antibacterial performance of the aqueous extract of leaf of *Cassia siamea* was checked on the *Bacillus cereus* and *Escherichia coli*. The aqueous extract of leaf of *Cassia siamea* illustrated dose-based antibacterial activity towards the screened bacterial strains. With the escalated concentration of aqueous extract of leaf of *Cassia siamea*, the antibacterial activity was also hiked with the enhanced inhibitory zone. At 20 µg/ml of aqueous extract of leaf of *Cassia siamea*, the inhibitory zone against the *Bacillus cereus* was 4.9 ± 0.21 mm and for *Escherichia coli* it was 5.1 ± 0.00 mm. Moreover, at the tested higher dose (80 µg/ml), the inhibitory zone was 9.8 ± 0.12 mm towards *Bacillus cereus* and 11.0 ± 0.38 mm towards

Table 5: Antibacterial efficacy of aqueous extract of leaf of *Cassia siamea*

Concentration (µg/ml)	Inhibition zone width in mm	
	Bacillus cereus	***Escherichia coli***
20	4.9 ± 0.21	5.1 ± 0.00
40	5.1 ± 0.99	6.3 ± 0.23
60	6.0 ± 0.40	7.5 ± 0.86
80	7.3 ± 0.62	9.2 ± 0.70
Positive control as Ciprofloxacin	9.8 ± 0.12	11.0 ± 0.38

Escherichia coli (Table 5).

The phyto-phenolics can lyse the bacterial cells by adopting diverse mechanism (Vietez *et al*., 2017). Studies have justified the impact of tannins, alkaloids, glycosides, steroids, flavonoids, tannins and saponins as potent antibacterial compounds, in addition to their antioxidative function (Wasihun *et al*., 2023). Flavonoids halt the functioning of DNA gyrase enzyme in the bacterial cells thus interrupting the replicative cycle of nucleic acid (Yan *et al.*, 2024). Loss of cellular membrane integrity, cell wall formation inhibition and respiratory chain enzyme functioning are the well-recognized mode taken by the flavonoids to lyse the bacterial cells (Yuan *et al*., 2021). Iron-chelation, cellular membrane disruption, cell wall formation inhibition, fatty acid genesis inhibition, halting the quorum sensing and gene expression attenuation are the pivotal mechanism of tannin on bacterial cell to lyse them efficaciously (Farha *et al*., 2020). Inhibition of vital protein formation, efflux pump, bacterial toxin formation, carbohydrate metabolism and cleavage of the DNA molecules are the alkaloid's mechanism for killing the bacterial cells. Additionally, certain crucial enzymes such as glutamine synthetase and DNA topoisomerase are also inhibited by the alkaloids (Yan *et al*., 2021).

Conclusion

The phytochemical screening, antioxidant assay and antibacterial assay was done to check the pharmacological potency of the aqueous extract of leaf of *Cassia siamea*. In this study, the findings showed that the phytochemicals found in the aqueous extract of leaf of *Cassia siamea* were responsible for the antioxidative and antibacterial activity projected by the extract. The antioxidant and antibacterial activity of the aqueous extract of leaf of *Cassia siamea* were dose-based. More, pharmacological experimentation has to be done to identify the potency of the aqueous extract of leaf of *Cassia siamea* to make its role in various treatments and preventive diseases.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Chemistry, Sacred Heart College (Autonomous), Tirupattur, 635601, Tamil Nadu, India.

Author Contributions

Each author has contributed equally in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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