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Research Article

Evaluation of antioxidant and Anti-inflammatory activities of Cassia siamea Lam. and Asystasia gangetica (L.) T. Anders

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Abstract
Antioxidant systems play a crucial role in protecting membranes from the deleterious free radicles. Natural, plant derived antioxidants are now preferred to the synthetic ones because of safety concerns. Leaf extracts of *Cassia siamea Lam*. and *Asystasia gangetica (L.) T. Anders* were tested for antioxidant and anti-inflammatory components and compared with those of the standard free radicle scavenging compounds like BHT, toluene and Ascorbic acid. Methanolic leaf extracts exhibited strong membrane stabilizing activity. They were also found to be effective in inhibiting heat induced hemolysis, protein denaturation, COX and LOX activities. *Cassia siamea* exhibited high levels antioxidant components and a more significant radicle scavenging and membrane stabilizing activity when compared to *Asystasia gangetica.*

Key words: *Asystasia gangetica, Cassia siamea*, Reactive oxygen species, Total antioxidant activity.

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Introduction

In cells, free radicals produced as reactive oxygen species during various metabolic processes, take electrons from nearby molecules to replenish the loss (Davies, 1994). It can cascade and will lead to the damage of macro molecules (DNA, RNA, lipids, glycoproteins, proteins and enzymes) that hastens ageing (Arouma, 2003). All aerobic organisms are equipped with potential antioxidant defense systems to combat with damage caused by the free radicals (Chae *et al*., 2004). Many evidences indicate that the consumption of foodstuffs, herbals enriched with antioxidant phytonutrients are found to be beneficial in management of diseases due to oxidative damage. Antioxidants protect biological systems by preventing excessive oxidations and plants are known to produce natural antioxidants (Cuendet *et al*., 2000).

Inflammation is the body's natural defense mechanism against harmful stimuli, including pathogens, damaged cells, and irritants. While essential for healing, prolonged or excessive inflammation is linked to various diseases, such as arthritis, asthma, cardiovascular disorders, and neurodegenerative conditions (Medzhitov, 2008). Consequently, there is a high demand for safe, effective anti-inflammatory agents. Although synthetic drugs like NSAIDs and corticosteroids are widely used, they can cause significant side effects with long-term use, including gastrointestinal issues and liver toxicity (Vane and Botting, 1998). This has fueled interest in plant-based alternatives, which often contain bioactive compounds with reduced adverse effects.

Cassia siamea and *Asystasia gangetica* are two plants traditionally used in folk medicine for treating inflammation. *Cassia siamea Lam* (family: Fabaceae) also known as *Senna siamea*, is recognized for its analgesic and anti-inflammatory effects (Khare, 2007). Research has shown that its leaves contain flavonoids, alkaloids, and tannins, which are believed to contribute to its medicinal properties (Singh *et al*.,2010). Similarly, *Asystasia gangetica* of the Acanthaceae family is traditionally applied in treating inflammation, wounds, and infections. Its leaves are rich in bioactive compounds like saponins and phenolics, known for their anti-inflammatory activity (Chanda and Parekh, 2008). Methanolic extraction is widely used to isolate these bioactive compounds, as it effectively concentrates a broad spectrum of phytochemicals for pharmacological testing (Sharma *et al*., 2018). The study focuses on evaluating the antioxidant and anti-inflammatory activities of leaf extracts from *Cassia siamea* and *Asystasia gangetica* through *in vitro* assays, comparing their effectiveness and identifying bioactive components. By investigating plant extracts, the present research aims to advance the development of plant-based therapies for managing inflammation, providing safer alternatives to conventional drugs with minimal side effects.

Materials and Methods

Plant material: The leaves of the two plants *Cassia siamea* and *Asystasia gangetica* were collected from Andhra university campus, Visakhapatnam.

Preparation of methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica*

The leaves of *Cassia siamea* and *Asystasia gangetica* were shade-dried and then ground into a powder. Separately, 1000 milliliters of methanol were used to steep 250 grams of dried and powdered leaf samples from both plants. After 15 days, the solution was gathered by filtering it through cheesecloth and Whatman filter paper. The filtrates were dried in a rotary evaporator below 50°C to produce a concentrated semisolid mass of the extracts. Methanol leaf extracts are further used for anti-inflammatory experiments.

Aqueous extracts were used for the analysis of enzymatic and non-enzymatic antioxidants, as well as for the assessment of antioxidant potential.

Chemicals

All chemicals used were of analytical grade and were obtained from commercial sources.

Enzymatic antioxidants

The antioxidant enzymes like Superoxide dismutase, Catalase, peroxidase and ascorbic acid oxidase were determined according to the standard methods (Beauchamp and Fedovich, 1976; Malik and Singh,1980) and the enzyme activities were expressed as units per milligrams of protein

Non enzymatic antioxidants

Total phenols and Flavonoids

The total phenols and Flavonoids in the extracts were determined according to the standard methods (Singleton and Rossi, 1965; Bao *et al*., 2005) and expressed as milligrams of gallic acid equivalents (GAE) and quercetin equivalents (QE) per gram tissue respectively.

Estimation of Ascorbic acid: Ascorbic acid content was determined by the method of Sadasivam and Balasubramanian (1987). The ascorbic acid content was expressed as milligrams per gram tissue.

Estimation of Total Protein: The total protein was estimated by the method of Lowry *et al.*(1953). The protein concentration was expressed as milligrams per gram tissue.

Antioxidant ability assays

The total antioxidant capacity was expressed as ascorbic acid equivalent (AAE) and Gallic acid equivalent (GAE) in milligrams of ascorbic acid and Gallic acid respectively per gm sample (Prieto *et al*., 1999). Reducing ability was tested with increasing concentrations of plant extracts using BHT as a positive control (Ferreira *et al*., 2007) and expressed as Vitamin E equivalents (Vit E Eq) in mg per gm sample. The ability of the plant extracts to inhibit different free radicles was expressed as the inhibition percentage (I) which was calculated as follows (Blois, 1958).

$$I = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

Membrane stabilization Assay

The human red blood cell (HRBC) membrane stabilization method has been used to study the anti-inflammatory activity (Gandhidasan *et al*., 1991).

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = Optical density of Test sample/Optical density of Control x 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 - Optical density of Test sample/Optical density of Control x 100

Heat induced hemolysis

Percent membrane stabilization was determined by the method of Shinde *et al*., (1999).

% Inhibition=100-(A1 – A2)/ (A0) x 100

where A1 is the absorbance of the sample;

A2 is the absorbance of the product control; A0 is the absorbance of the positive control.

Inhibition of protein denaturation

The percentage inhibition of protein denaturation was carried out by standard method (Vallabh *et al*.,2009). Percentage inhibition = 100-(O.D. of test – O.D. of product control)/ O.D. of Control x 100

Assay of Cyclooxygenase (COX) inhibition

The assay mixture consisted of glutathione, hemoglobin, enzyme, and 0.1M Tris-HCl buffer. The test was initiated with the addition of arachidonic acid and was terminated by adding 0.2 ml of 1-0% TCA in 1N HCl, mixing, and adding 0.2 ml of Thiobarbituric acid (TBA) after 20 minutes of incubation at 37°C. The contents were then centrifuged for three minutes at 1000 rpm after being cooked for twenty minutes in a boiling water bath and chilled. The supernatant's COX activity was evaluated at 632 nm (Viji and Helen, 2008). To inhibit cyclooxygenase, leaf extracts (100 and 500 µg/ml) were added to the assay mixture, and the test was carried out as instructed. The results were expressed as % Cyclooxygenase inhibition.

Assay of 5- Lipoxygenase inhibition

The reaction was carried out in a quartz cuvette at 25°C with a 1 cm light path. The test combination consists of 2.75 ml of Tris buffer with a pH of 7.4, 50 µl of the enzyme, and 0.2ml of sodium linoleate and the optical density was measured at 234 nm (Viji and Helen, 2008). To inhibit lipoxygenase, leaf extract (100 and 500 µg/ml) was added to the assay mixture, and the test was carried out as a result. The inhibitory effect of the investigated leaf extracts was expressed as a percentage of enzyme activity inhibited.

Results

High levels of enzymatic antioxidants were observed in *Cassia siamea*, with the exception of ascorbic acid oxidase, which was found in higher amounts in *Asystasia gangetica*. (Table 1). The amount of phenols and flavonoids was higher in *Cassia siamea* (108.33±1.44 GAE and 73.34±2.88 QE respectively) as compared to *A. gangetica* (26.34±0.577 GAE and 2.67± 0.144 QE) (Fig. 1A). Ascorbic acid content was low in *A. gangetica* (55.084±1.128 mg/gm tissue) as compared to *C. siamea* (126.67±3.53 mg/gm tissue) (Fig. 1B) and the protein content in *C. siamea* and *A. gangetica* was found to be 14.67 and 4.25 mg/gm of fresh sample, respectively.

Antioxidant ability was determined by the total antioxidant activity, reducing power and radical scavenging capacity. The total antioxidant activity was found to be higher in *C. siamea* with 115±2.5 AAE/gm and 162.5±2.5 GAE/gm (Fig. 2) compared to *A. gangetica* (15.25±0.5 AAE/gm and 20.4±0.655 GAE/gm). *C. siamea* exhibited higher reducing power (59.34 ± 1.154 Vit E Eq/g tissue) compared to *A. gangetica* (11.67 ± 0.577 Vit E Eq/g tissue), with concentration-dependent activity (Fig. 3A, 3B), though BHT remained the most potent reducing agent.

Radical scavenging activity in all the assays increased with increasing the concentration of plant extract except for DPPH radical in *C. siamea* which showed almost similar inhibition at both the concentrations (Table 2). *Cassia siamea* showed a higher scavenging potential of 64.28±3.208 % as compared to *A. gangetica* with 29.41±0.763 % and the strongest inhibition was obtained with BHT and Ascorbic acid.

Percentage of inhibition of hydroxyl radical is highest in positive controls BHT and ascorbic acid with 76.12±1.041 % and 72.30±0.522 % respectively as compared to the plant species of *C. siamea* (55.60±4.4 37%), and *A. gangetica* (12.50±0.782 %). Lipid peroxidation inhibition in plant extracts *C. siamea* and *A. gangetica* was 58.30±4.093 % and 28.57±0.675 % respectively, when compared to positive controls BHT and ascorbic acid with 80.20±0.614 % and 69.20±3.071 % of inhibition respectively.

As depicted in the Table 2, both the plant extracts could chelate the metal complex and this property might help in preventing the lipid peroxidation. The percentage of metal chelating capacity in *A. gangetica* and *C. siamea* was 37.80±1.470 % and 25±2.984 % respectively as compared to 93.18±0.874 % inhibition for positive control EDTA.

The inhibition of hydrogen peroxide radical was highest in BHT with 61.23% as compared to 43% and 10.9% for the plant extracts of *C. siamea* and *A. gangetica* respectively (Fig. 4).

Table 1 Levels of Enzymatic antioxidants

Plant	SOD		Catalase		Peroxidase		Ascorbic acid oxidase	
	Units/ mg protein	Units/ gm tissue	Units/ mg protein	Units/ gm tissue	Units/ mg protein	Units/ gm tissue	Units/ mg protein	Units/ gm tissue
C. siamea	15.965 ±0.544	234.206 ±7.987	39.238 ±1.293	575.626 ±18.980	1.397 ± 0.201	20.498 ±2.958	0.092 ±0.012	1.356 ±0.176
A.gangetica	1.747 ±0.190	7.441 ±0.787	37.254 ±3.39	166.665 ±14.433	0.358 ±0.016	1.523 ±0.069	1.003 ±0.353	4.264 ±1.501

(All the values are an average of three determinations and expressed as mean ± S.D.)

Table 2 Radical scavenging capacity of *Cassia siamea* and *Asystasia gangetica*

Plant	Conc. (mg/ml)	% of inhibition			
		DPPH radical	Hydroxyl radical	Lipid peroxidation	Ferrozine-Fe^{2+}complex
C.siamea	2.5	63.33±4.801	44.45±6.325	41.66±4.326	-
	10	64.28±3.208	55.60±4.437	58.30±4.093	25±2.984
A.gangetica	2.5	21.58±1.322	6.25±1.023	14.20±1.432	-
	10	29.41±0.763	12.50±0.782	28.57±0.675	37.80±1.470
BHT*	1	78.30±1.388	76.12±1.041	80.20±0.614	NT
Ascorbic acid*	1	77.08±1.438	72.30±0.522	69.20±3.071	NT
EDTA*	1	NT	NT	NT	93.18±0.874

Radical scavenging capacity is expressed as percentage of inhibition and each value is an average of three determinations and expressed as mean ± S.D.

NT- Not tested, - Result not obtained; * used as a positive control

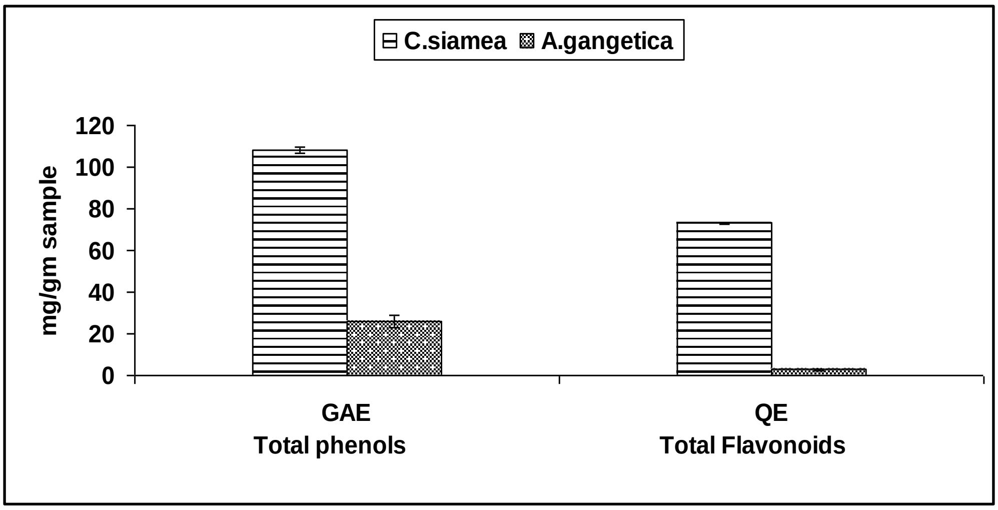

Fig 1A Total Phenols and Flavanoid content of *Cassia siamea* and *Asystasia gangetica* leaf extracts

Total phenols and Total flavonoids are expressed as mg of Gallic acid equivalents (GAE) and mg of Quercetin equivalents (QE) respectivel. All the values are average of three determinations and expressed as mean ± S.D.

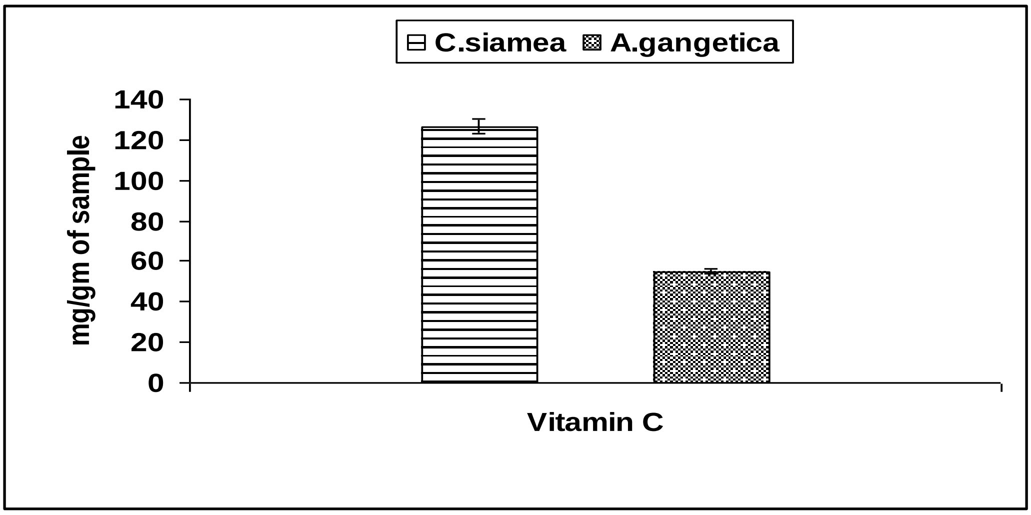

Fig. 1B Vitamin C content in *Cassia siamea* and *Asystasia gangetica* leaf extracts

Vitamin C is expressed as mg/gm of sample. All the values are an average of three determinations and expressed as mean ± S.D

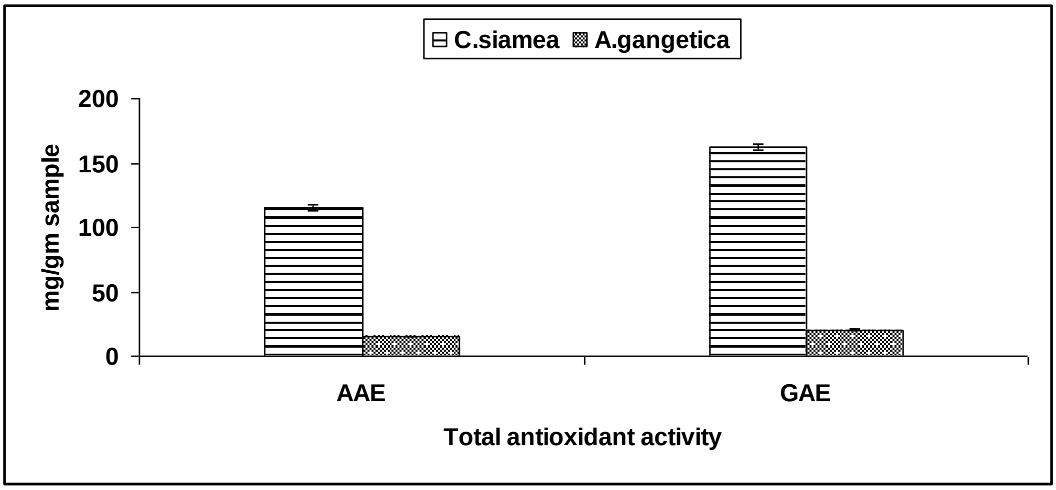

Fig. 2 Total antioxidant activity of *Cassia siamea* and *Asystasia gangetica* leaf extracts
Total antioxidant activity is expressed both as mg of ascorbic acid equivalents (AAE) and as mg of Gallic acid equivalents (GAE)

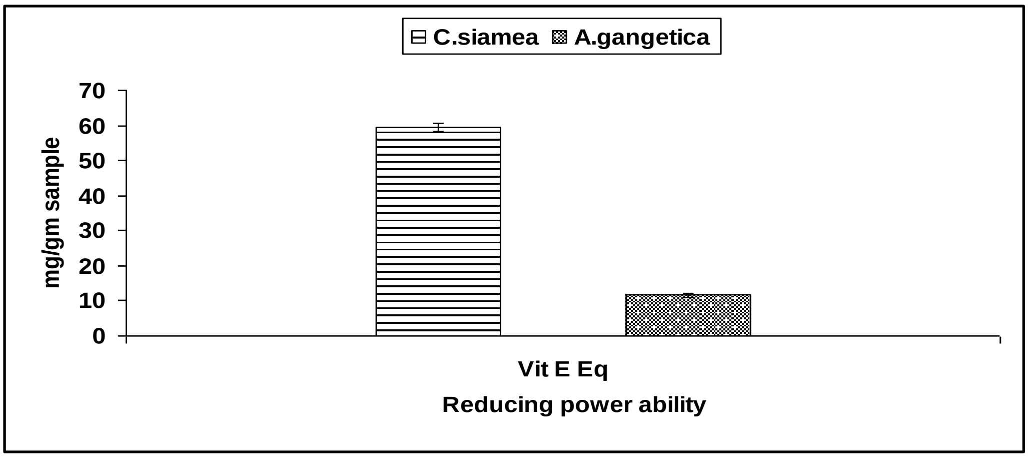

Fig. 3A Reducing power ability of *Cassia siamea* and *Asystasia gangetica* leaf extracts
Reducing power ability is expressed as mg of Vitamin E equivalents (Vit E Eq).
The values are the average of three determinations and are expressed as mean ± S.D.

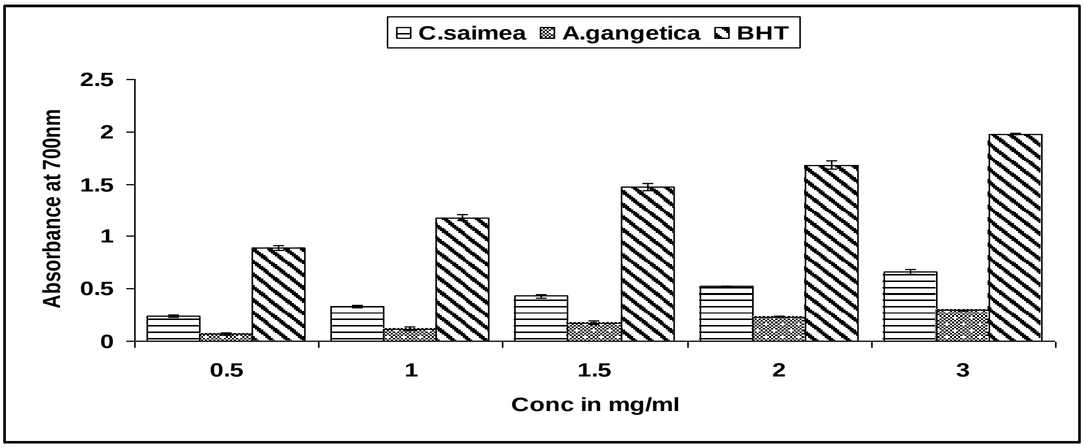

Fig. 3B Reducing ability of *Cassia siamea* and *Asystasia gangetica* leaf extracts
Reducing ability (Fe^{3+}-Fe^{2+} transformation) of *C.siamea*, *A.gangetica*, and BHT at different concentrations presented as OD values at 700nm. The values are the average of three determinations and are expressed as mean ± S.D.

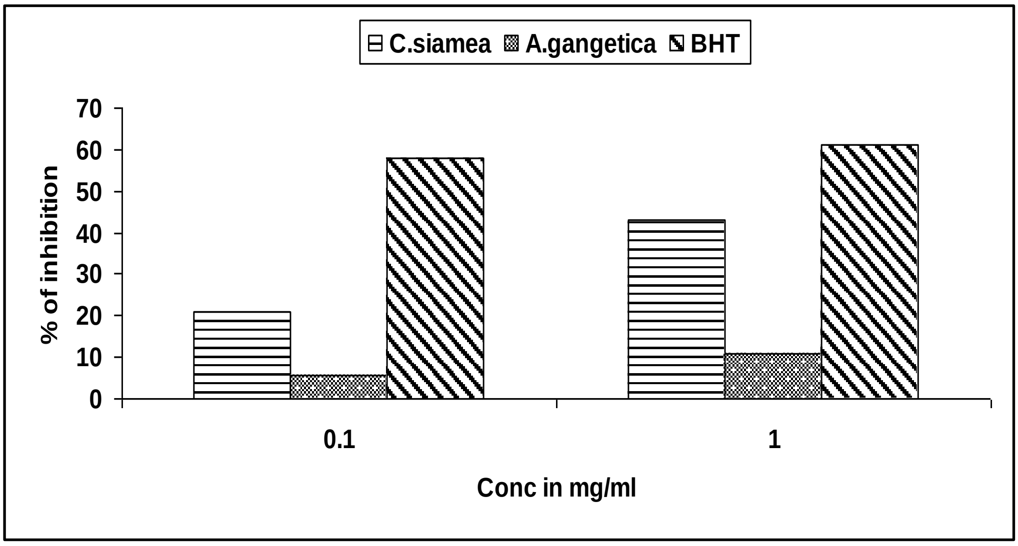

Fig. 4 H_2O_2 scavenging activity of *C.siamea, A.gangetica* leaf extracts

H_2O_2 radical scavenging activity of *C.siamea*, *A.gangetica* and BHT at two different concentrations. Each value is an average of three determinations

***In vitro* anti-inflammatory activity Inhibition of hypotonicity induced hemolysis**

The methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica* were effective in inhibiting the hypotonicity induced hemolysis at different concentrations (100-500 µg/ml). Methanolic leaf extracts of both the plants (100-500µg/ml) inhibited the hypotonicity induced hemolysis of RBCs to varying degree (Fig. 5). Of the two plant extracts, methanolic extract of *Cassia siamea* showed 75% inhibition at a concentration of 500µg/ml. Diclofenac sodium, standard drug showed the maximum inhibition, 94 % at 100µg/ml.

Inhibition of Heat induced hemolysis

Stabilization of the RBCs membrane was studied further to establish the mechanism of anti- inflammatory action of methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica* . Both the extracts were effective in inhibiting the heat induced hemolysis at different concentrations (100-500 µg/ml). Both the extracts (100-500µg/ml) inhibited the heat induced hemolysis of RBCs to varying degrees (Fig. 6). The methanolic leaf extract of *Cassia siamea* demonstrated significant inhibition (77%) at a concentration of 500 µg/ml, though its inhibitory effect was less pronounced compared to aspirin, a standard drug, which exhibited 94% inhibition at a lower concentration of 100 µg/ml.

Inhibition of protein denaturation

Both the methanolic leaf extracts were effective in inhibiting heat induced albumin denaturation at different concentrations (Fig. 7). Maximum inhibition of protein denaturation (80%) was observed with methanolic leaf extract of *Cassia siamea* at 500µg/ml. Diclofenac sodium, a standard anti-inflammatory drug showed the maximum inhibition, 95% at 100µg/ml concentration.

Cyclooxygenase (COX) inhibitory assay

The effect of methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica* on production of prostaglandins were determined by the inhibition of Cyclooxygenase activity. From (Fig. 8), it is clear that methanolic leaf extracts have good cyclooxygenase inhibitory activity in comparision to standard Ibuprofen. Of the two leaf extracts, methanolic leaf extract of *Cassia siamea* showed maximum inhibition of 76% at 500 µg/ml concentration. The standard Ibuprofen showed 94% inhibition at a concentration of 100µg/ml.

Assay of 5-Lipoxygenase (LOX) Inhibition

Methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica* were checked at 100, 250 and 500µg/ml for anti-lipoxygenase activity. Methanolic leaf extract of *Cassia siamea* showed maximum inhibition of 72% at 500 µg/ml than the *Asystasia gangetica* leaf extract (Fig. 9). The standard Ibuprofen showed 95% inhibition at a concentration of 100µg/ml. The results obtained from the present study support anti-inflammatory potential of methanolic leaf extracts of both the plants

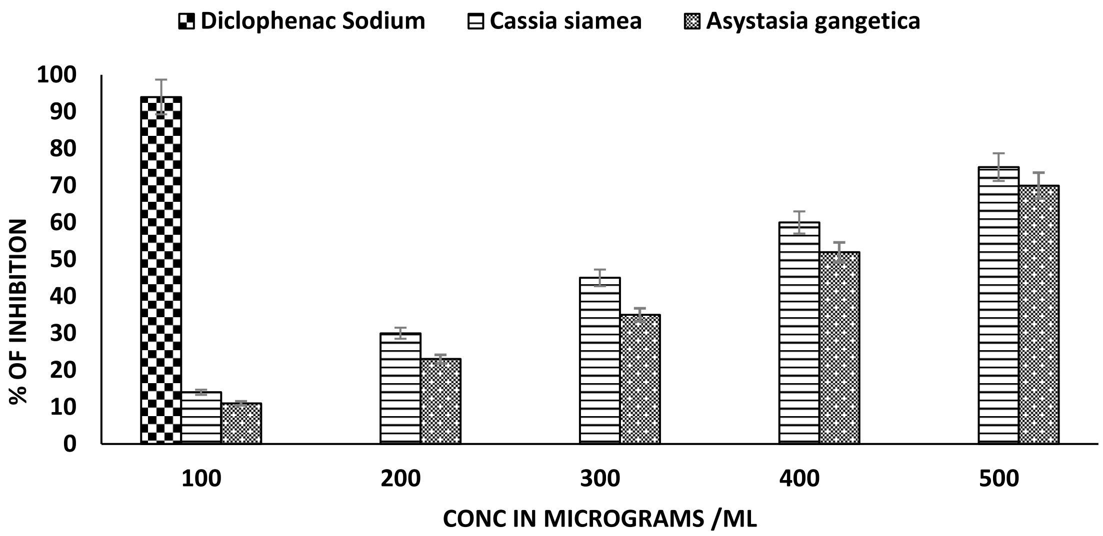

Fig.5 Effect of methanolic leaf extracts of *C.siamea*, *A.gangetica* on RBC membrane stabilization

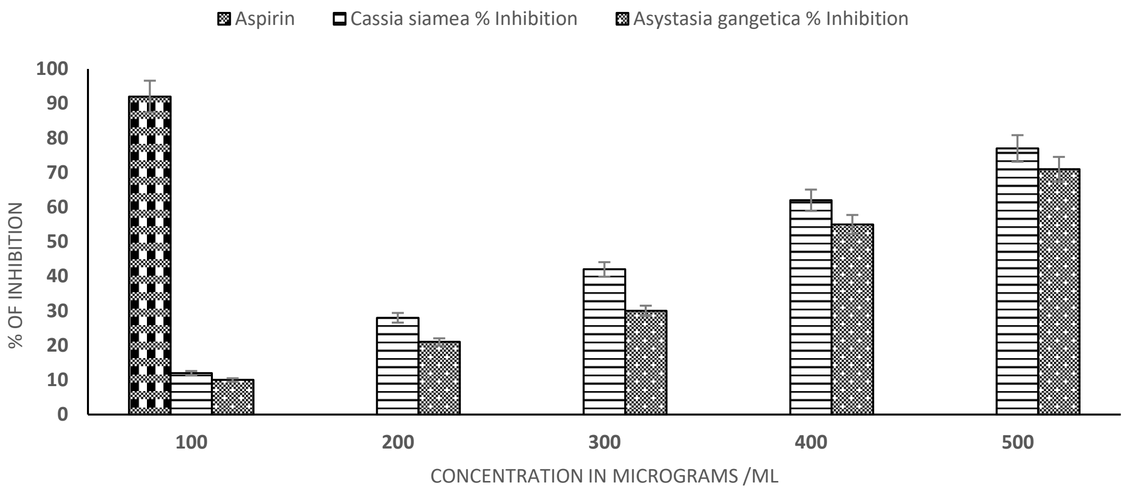

Fig.6 Effect of methanolic leaf extracts of *C.siamea*, *A.gangetica* on Heat induced hemolysis

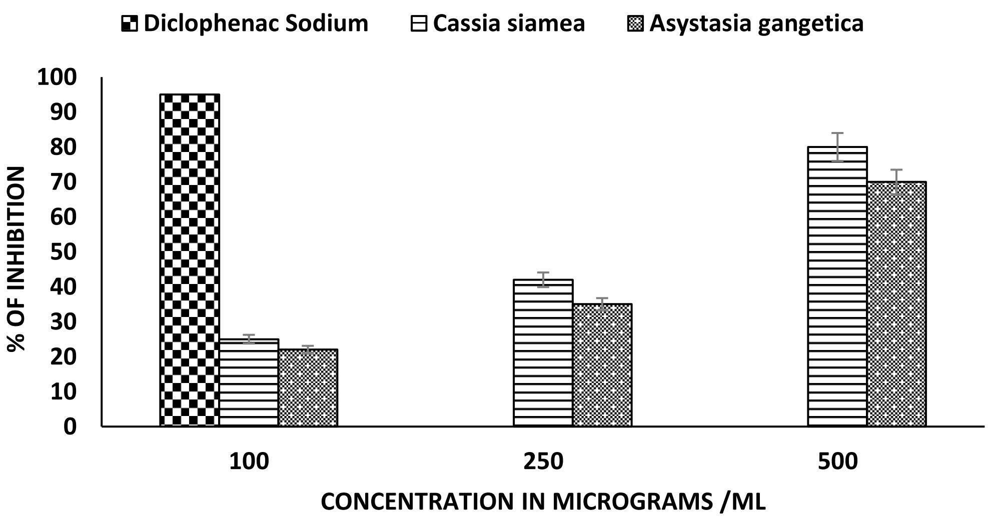

Fig.7 Effect of methanolic leaf extracts of *C.siamea*, *A.gangetica* on Protein denaturation

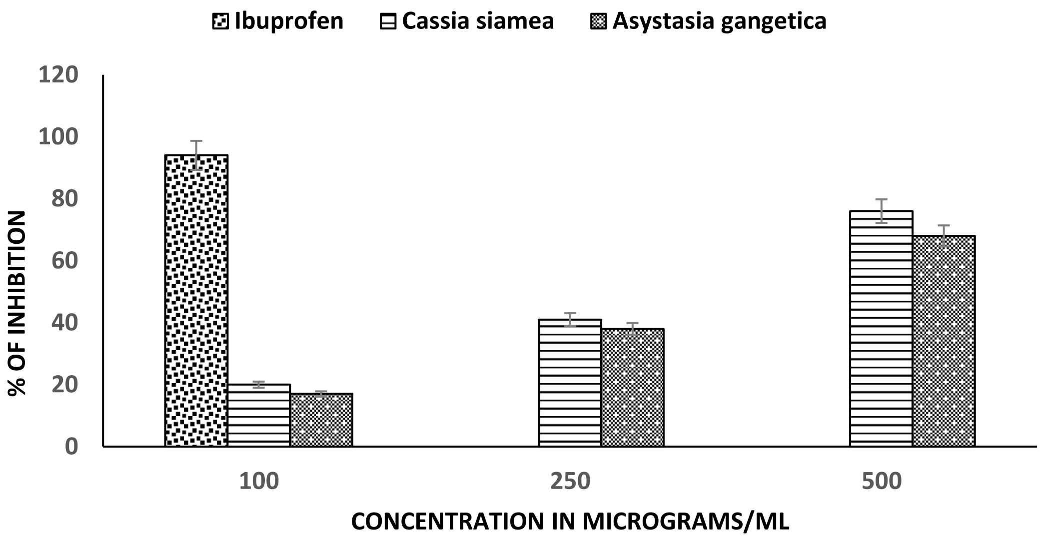

Fig.8 Effect of methanolic leaf extracts of *C.siamea*, *A.gangetica* on Cyclooxygenase (COX) inhibition

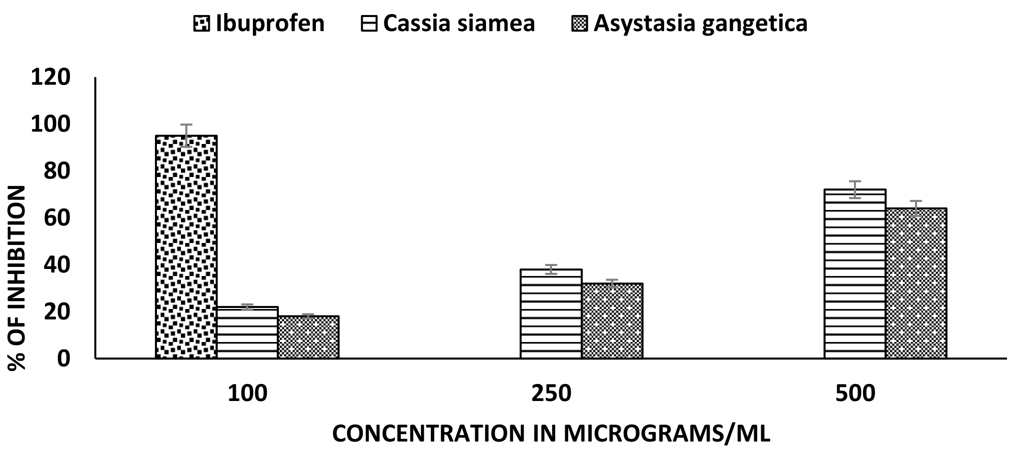

Fig.9 Effect of methanolic leaf extracts of *C.siamea*, *A.gangetica* on Lipoxygenase (LOX) inhibition

Discussion

The antioxidant and anti-inflammatory activities observed in the aqueous and methanolic leafextracts of *Cassia siamea* and *Asystasia gangetica* indicate their potential as natural therapeuticagents. These activities are closely linked to their enzymatic and phytochemical profiles. Significant levels of SOD, Catalase, peroxidase and Ascorbic acid oxidase in the plant extracts indicate their strong antioxidative potential, aligning with similar findings reported in grapes (Rani *et al*., 2004) .

The neutralization of free radicals by *Cassia siamea* and *Asystasia gangetica* may be attributed to their substantial levels of bioactive compounds, including phenols, flavonoids, alkaloids, tannins, saponins and Ascorbic acid which are known for their roles in combating oxidative stress and modulating inflammatory responses (Singh *et al*., 2010; lhami Gülçin, 2005)

The therapeutic use of plants in treating inflammatory conditions is well-documented in traditional medicine across cultures. Plants provide a rich source of bioactive compounds, which have been shown to modulate the inflammatory response with fewer adverse effects than synthetic drugs (Tiwari *et al*., 2011).

Research has shown that extracts of *Cassia siamea* leaves contain bioactive compounds such as flavonoids, tannins, and alkaloids, which exhibit strong anti-inflammatory effects (Singh *et al*., 2010). In one study, methanolic extracts of *Cassia siamea* leaves showed a dose-dependent reduction in inflammation in animal models, attributed to the presence of flavonoids and alkaloids that inhibit pro-inflammatory enzymes like cyclooxygenase and lipoxygenase (Akinmoladun *et al*., 2016).

The findings in the present study suggest that *Cassia siamea* may offer a natural alternative for managing inflammation without the side effects associated with conventional drugs.

Studies indicated that *Asystasia gangetica* leaves contain saponins, flavonoids, and phenolic compounds, all of which have been associated with anti-inflammatory effects (Akinmoladun *et al*., 2016).

Chanda and Parekh (2008) demonstrated that the methanolic leaf extract of *Asystasia gangetica* effectively reduces inflammation in animal models by suppressing cytokine production and inhibiting pro-inflammatory enzymes. These properties highlight the plant's potential for development as a plant-based anti-inflammatory agent.

Both *Cassia siamea* and *Asystasia gangetica* have been shown to act on key inflammatory pathways, primarily by inhibiting the release of pro-inflammatory mediators. The bioactive compounds in Cassia siamea, particularly flavonoids and tannins, are known to inhibit the activity of cyclooxygenase (COX) enzymes, which play a crucial role in prostaglandin synthesis, a mediator of inflammation (Tiwari *et al*., 2011). Similarly, *Asystasia gangetica's* phenolic compounds exhibit anti-inflammatory effects by inhibiting pro-inflammatory cytokines such as TNF-α and IL-6, which are pivotal in the development of chronic inflammation (Akinmoladun *et al*., 2016).

Several studies have compared the efficacy of plant-based anti-inflammatory agents to that of synthetic drugs, revealing that natural extracts often produce fewer adverse effects while providing effective symptom relief. Nworu et al. (2007) reported that plant extracts containingflavonoids and tannins could reduce inflammation comparably to NSAIDs, without causing gastrointestinal irritation[28]. Given these benefits, there is significant interest in developing therapies based on extracts of *Cassia siamea* and *Asystasia gangetica*, as these plants are bothwidely available and traditionally used in treating inflammation.

The results in the present indicate that both *Cassia siamea* and *Asystasia gangetica* have significant antioxidant and anti-inflammatory properties, with potential applications as naturalalternatives to synthetic drugs. *Cassia siamea* may offer stronger COX inhibition due to its higher concentration of flavonoids (Nworu *et al*., 2007). Together, these findings suggest a complementary effect, making these plants promising candidates for combined use in managing inflammation and oxidative stress, particularly in chronic inflammatory diseases where both oxidative damage and inflammatory mediators play a critical role.

This research also emphasizes the importance of further investigation into the specific bioactive compounds within these plants, which could lead to the isolation of potent agents for use in pharmaceuticals. The relatively low toxicity of plant-based extracts compared to synthetic drugs highlights the potential of *Cassia siamea* and *Asystasia gangetica* as sources for safer anti-inflammatory therapies (Sharma *et al*., 2018).

Conclusion

The present study highlights the significant antioxidant and anti-inflammatory activities of aqueous and methanolic leaf extracts of *Cassia siamea* and *Asystasia gangetica* respectively, supporting their traditional use in managing inflammation-related conditions. Given the side effects of long-term synthetic drug use, these extracts offer promising natural alternatives. Further research to isolate specific bioactive compounds could lead to the development of safer,more effective plant-based therapies. In conclusion, *Cassia siamea* and *Asystasia gangetica* show strong potential as natural antioxidant and anti-inflammatory agents, supporting their role in alternative medicine for chronic inflammatory diseases.

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Declaration of Conflicts of Interests

he author declares that there is no conflict of interest.

Declarations

The author declare that all works are original and this manuscript has not been published in any other journal.

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