

<https://doi.org/10.33472/AFJBS.6.13.2024.4135-4148>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## EXPLORATION OF DIANTHRONES ISOLATED FROM *CASSIA AURICULATA*. L TO ALLEVIATE OXIDATIVE IMPAIRMENT, ANTIBACTERIAL AND ANTIARTHRITIC ACTIVITY.

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### Article Info

Volume 6, Issue 13, July 2024

Received: 04 June 2024

Accepted: 05 July 2024

Published: 31 July 2024

*doi:* [10.33472/AFJBS.6.13.2024.4135-4148](https://doi.org/10.33472/AFJBS.6.13.2024.4135-4148)

### ABSTRACT:

*Cassia auriculata* fractions and its isolated novel compound dianthrone A and B were subjected to *in vitro* antioxidant and antibacterial activity against pathogenic bacteria. The results elucidated that the methanol fraction and the isolated compounds demonstrated dose dependent antioxidant activity when tested with DPPH with an inhibition of 3-10 % at 2.5 mg/ml concentration and displays highest inhibition at 20mg/ml with 26-82%. In FRAP, the highest reducing ability was found at 31-78% at 20 mg/ml concentration. The results of enzymatic antioxidant such as SOD (38.42, 32.64 and 28.42 units); catalase with 46.34, 47.44 and 39.62 $\mu$ mol/min mL; GPX with 89.16, 92.14 and 90.66 was observed for *Cassia auriculata* fractions and dianthrone A & B. Similarly, methanolic fraction and the isolated compound dianthrone A and B revealed maximum zone of inhibition with 23.39 mm against *Streptococcus mutans* whereas, the isolated compounds demonstrated significant activity with an average of 11-22 mm zone against tested species at the concentration of 200 $\mu$ g/mL. Protein denaturation study revealed *Cassia auriculata* fractions, dianthrone A and B treatment inhibits protein denaturation there by reducing the effect of arthritis and inflammation. The current study corroborates our previous research findings exploring the potential of *Cassia auriculata* a promising remedy for various ailments.

**Key words:** Dianthrone; antioxidant; antibacterial activity; agar well diffusion method; inhibition zone.

## 1. INTRODUCTION

Traditionally used plants and herbs contain numerous amounts of phytochemicals and other important secondary metabolites to cure various dread full diseases since used from the ancient times. Investigating the advancement of using plants, the studies demonstrated the feasibility of using medicinal related plant in the traditional system <sup>[1]</sup>. They are classified based on the number and type of constitutive atoms and the structure of their basic skeleton secondary metabolites, derived from plants, form the foundation for a substantial proportion of the commercial medications used today to treat a wide range of diseases <sup>[2]</sup>. *Cassia auriculata* belongs to the largest number of plants, including trees and herbs. They are cultivated in the various part of India and central parts because of their medicinal properties <sup>[3,4]</sup>. It is rich in source of pharmaceuticals, because of the important occurrence of phytochemicals like anthraquinones, flavonoids, lipids, benzocoumarin and chromones as per the previous studies. *Cassia auriculata* commonly known as Tanner's Cassia or Senna *auriculata* is a plant species they are popularly known for their medicinal properties like anti-microbial, antioxidant, antitumor, anti-diabetic and anti-parasitic properties <sup>[5,6]</sup>.

The extract and the fractional compounds from *Cassia auriculata* explore an effective activity on antioxidant, antimicrobial and anti-inflammatory can help to treat these diseases in distinctive way to those of modern medicines <sup>[4]</sup>. Excessive release of free radicals during process of metabolism has the capacity to disrupt the signaling pathway of the body that leads to change in the structure of DNA, protein and other lipid contents, this alteration can result in the development of tumors, aging, swelling of joints, arthritis, and other harmful diseases <sup>[7,8]</sup>. Arthritis causes tissue damage and inflammation that may be acute, chronic, or degenerative based of the region of discomfort and its causative agent in the body which may have chance to develop into a serious disease in the affected part due to uncontrolled amplification of inflammatory response <sup>[9,10]</sup>. The ability of *Cassia auriculata* act as antioxidant, anti-inflammatory, and antimicrobial source needs to be studied further which possess increased resistance to diversity of microbes to keep down usage of contemporary drugs <sup>[11,12]</sup>.

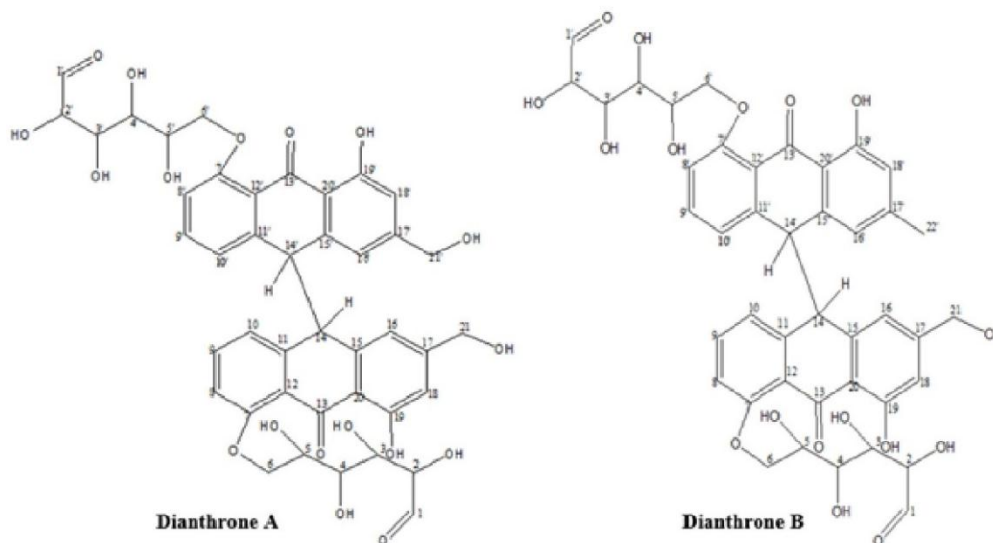
The plant *Cassia auriculata* was the subject of a previous study <sup>[4]</sup> that focused on methanolic fractions and compounds isolated from it. One isolated compound from *Cassia auriculata* has shown promise in treating Hyperglycemia, a condition marked by elevated blood glucose levels. *Cassia auriculata* fractions also exhibit strong antidiabetic activity and can lower the risk associated with treatment with *Ca* fractions. In a different study, the integration of biological activity tests and phytochemical analysis attempts to offer a comprehensive assessment of *C. auriculata* L. buds and flowers' antidiabetic potential, paving the way for further investigation and possible therapeutic uses <sup>[6]</sup>. The research results highlight the unique medicinal potential of new molecules derived from *Ca*. Beyond their antidiabetic benefits, the current research intends to demonstrate useful features of these fractions and molecules. In particular, it looks at their possible antibacterial (ability to combat microbes), antioxidant (capacity to counteract free radicals), and anti-inflammatory (ability to lessen inflammation) qualities.

## 2. MATERIALS AND METHODS

### a. Collection, Preparation and isolation of dianthrone

Flowers of *Cassia auriculata* were gathered in and around the Vellore District in Tamil Nadu, India. The flowers were then washed, dried in the shade, ground into a powder using an electric

blender, then extracted and purified in accordance with the procedure described in [4]. Figure 1 depicts the dianthrone structure



**Figure 1. Structure of Dianthrone A and B**

## b. Chemicals and solvents

All chemicals and solvents were procured from S.D. Fine Chemicals, Mumbai and Fischer Inorganic and Aromatic Limited, Chennai, India

## c. *In vitro* assays

### i. DPPH radicals scavenging assay

The methanolic fraction and isolated chemicals from *Cassia auriculata* were evaluated for their radical scavenge capacity using a slightly modified version of the DPPH (2,2-diphenyl-1-picrylhydrazyl) test [12]. 1.0 mL of a 0.8 mM/L DPPH was added to 1.0 mL of the fraction and separated compound individually (concentrations ranging from 2.5 to 20 mg/mL), well mixed, and then incubated for an additional 30 minutes. At 517 nm, absorbance was measured, and the percentage of inhibition was calculated

### ii. Ferric ions ( $\text{Fe}^{3+}$ ) reducing antioxidant power assay (FRAP)

With a few minor adjustments, Oyaizu et al. from 1986 [13] was utilized to gauge the potential of the separated components and methanolic fraction of *Cassia auriculata*. 1% potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] solution (2.5 mL), Phosphate buffer (2.5 mL, 0.2 M), 1.0 mL of *Cassia auriculata* methanolic fraction, and isolated chemicals (concentrations ranging from 20 to 100  $\mu\text{g}/\text{mL}$ ) were well mixed and incubated at 50°C for 20 minutes. After adding 2.5 mL of 10% trichloroacetic acid to the resulting solution and centrifuging it for 10 minutes at 3000 rpm, 2.5 mL of deionized water and 0.5 mL of 0.1%  $\text{FeCl}_3$  were added. At 700 nm, absorbance was measured, and the percentage of inhibition was calculated

### iii. Hydroxyl radical scavenging assay

With a small modification, the standard technique [14] was used to assess the hydroxyl-scavenging capacity of the separated components and methanolic fraction of *Cassia auriculata*. The stock was freshly prepared by following the procedure using 200  $\mu\text{L}$  of 2.8 mM 2-deoxy-2-ribose, 400  $\mu\text{L}$  of 200 mM  $\text{FeCl}_3$ , 1.04 mM EDTA, 200  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (1.0 mM), 200  $\mu\text{L}$  ascorbic acid (1.0 mM) and various concentrations of the *Ca* extract and incubated for 1 hour at the room temperature. TBA was used to quantify the deoxyribose degradation. After adding the TCA solution, the color changed at 90°C for 25 minutes. After cooling the reaction mixture,

the optical density was determined at 532 nm by comparing it to a blank solution. A positive control was employed, mannitol <sup>[14]</sup>.

**iv. Catalase assay**

The test mixture included 0.04 mL of separated chemicals and methanolic fractions from *Cassia auriculata*, 0.4 mL of 15 mM/L H<sub>2</sub>O<sub>2</sub>, and 2.6 mL of 50 mM/L potassium phosphate buffer (pH 7.0). The decrease in absorbance at 240 nm coincided with the breakdown of H<sub>2</sub>O<sub>2</sub>. U/(mg protein) was used to express the enzyme activity. 1 mM/L of H<sub>2</sub>O<sub>2</sub> reduction per minute per milligram of protein is the definition of one U <sup>[15]</sup>

**v. Superoxide dismutase**

1 mL of NBT solution (150 µM) made in phosphate buffer with a pH of 7.4, 1 mL of NADH solution (468 µM) prepared in the same phosphate buffer with a pH of 7.4, and methanolic extracts of *Cassia auriculata* at different concentrations (25-100 µg/mL) were included in the reaction mixture. Lastly, 100 µL of PMS solution (made at 60 µM in phosphate buffer, pH 7.4) was added to the mixture to speed up the process. After five minutes of incubation at 25°C, the absorbance of the reaction mixture was measured at 560 nm using methanol as the control. Every test was run in three duplicates. By contrasting the outcomes of the control test sample, percentage inhibition was computed <sup>[16]</sup>.

**vi. Glutathione peroxidase assay**

Effect of *Cassia auriculata* methanolic fractions and isolated compounds on enzymatic antioxidant Glutathione peroxidase was assessed using the method of Rotruck et al., <sup>[17]</sup> with slight modification.

**d. In vitro anti-arthritic activity- Protein Inhibition assay**

With the use of 1N HCl, the reaction mixture made up of separated compounds (2.5–20 mg/mL) and methanolic fractions of *Cassia auriculata* as well as 1% bovine albumin fraction aqueous solution was brought to pH 6.3. After 20 minutes of incubation at 37 °C, the samples were subjected to a 51°C for 20 min, after which they were analyzed spectrophotometrically at 660 nm. It was estimated what percentage of protein denaturation was inhibited <sup>[18]</sup>.

**e. Anti-inflammatory assay**

With a few minor adjustments, protein inhibition assay was performed using Mizushima and Kobayashi's methodology. 0.45 mL of bovine serum albumin (5% aqueous solution) and 0.05 mL of *Cassia auriculata* fractions and separated chemicals (2.5–20 mg/mL) made up the reaction mixture (0.5 mL). After a little quantity of 1 N HCl was added to the pH to bring it down to 6.3, the samples were heated for 30 minutes at 57°C after being incubated for 20 minutes at 37°C. After the samples had cooled, each tube was filled with 2.5 mL of phosphate buffer saline (pH 6.3). At 416 nm, turbidity was measured using spectrophotometry. Instead of using extracts in the product control test, 0.05 mL of distilled water was utilized, and bovine serum albumin was absent <sup>[19]</sup>. The percentage inhibition of protein denaturation was calculated.

**f. Test microorganisms and antibacterial activity**

*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyrogens*, *Escherichia coli*, *Salmonella paratyphi*, *Bacillus sphaericus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris* were among the microorganisms obtained from MTCC and ATCC for the current investigation. An analysis was conducted on the antibacterial activity of the fractions, dianthrone A and B <sup>[20]</sup>.

**g. Statistical analysis**

Results were represented in Mean ± S.D. and statistically evaluated with SPSS\16.0 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test.

### 3. Results

#### a. Effect of *Cassia auriculata* fractions, dianthrone A and B on non-enzymatic and enzymatic antioxidant

*Ca* fractions and dianthrone A and B compounds were used to assess DPPH free radicals at various concentrations in a dose-dependent manner (Figure 2). At 2.5 mg/ml concentration, the scavenging activity was found to be 3–10%, and it gradually increases as concentration rises. With an inhibition of 26–82%, the maximum radical scavenging activity was attained at 20 mg/ml. Reductases found in extracts of *Ca* secondary metabolites are responsible for the variance in reducing activity, since they neutralize free radicals by bringing them into balance. Similar to this, the findings of the proven decreasing ability offer guidance for boosting it, since it increases from 6–13% to 31–78% at 2.5–20 mg/ml concentrations. Despite having a little lower reducing capacity than the typical ascorbic acid value, the results indicate the possibility of antioxidant characteristics. Conversely, Figure 3 shows that the isolated molecules of dianthrone A and B have greater potential, with 77% and 75% inhibition, respectively.

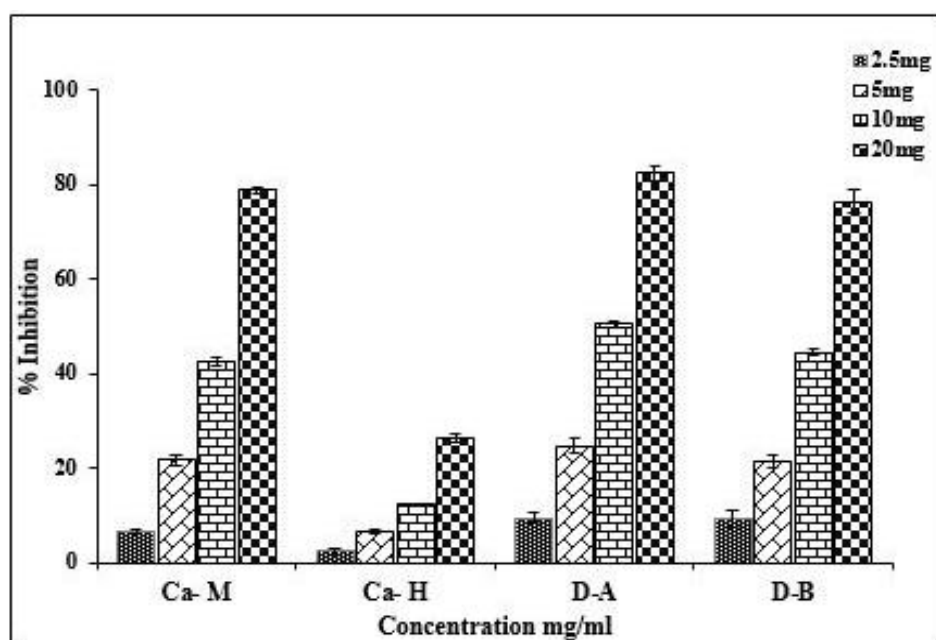


Figure 2. DPPH radical scavenging potential of *Ca* fractions and Dianthrone

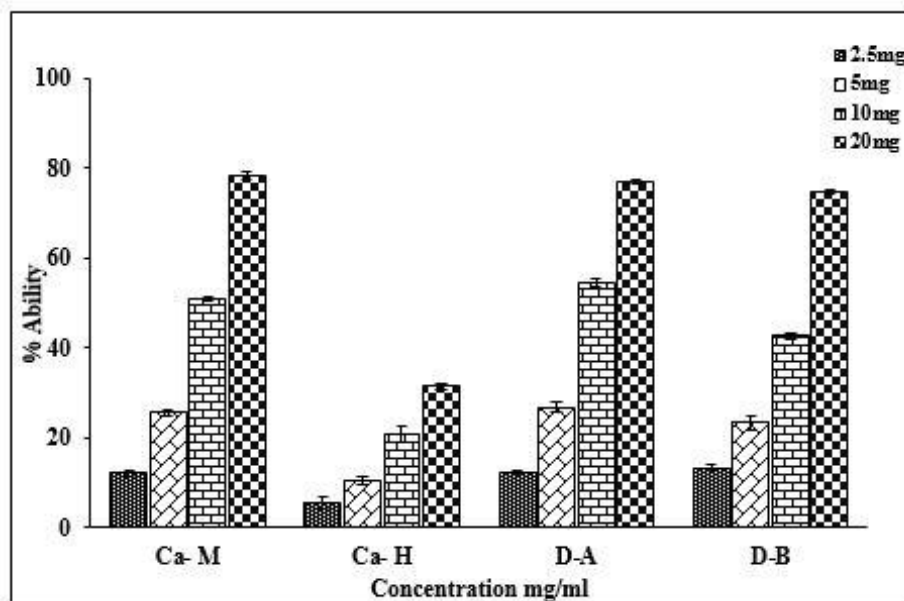


Figure 3. Ferric reducing-antioxidant power of *Ca* fractions and Dianthrone

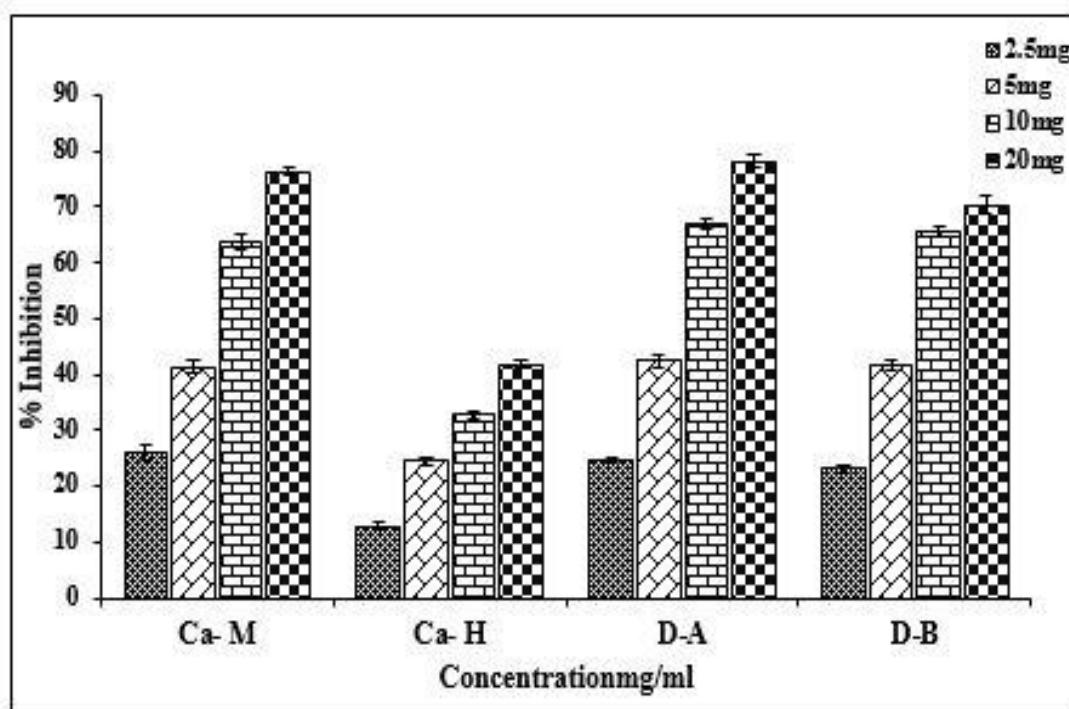


Figure 4. Hydroxyl radical scavenging potential of *Ca* fractions and Dianthrone

The capacity to reduce hydroxyl radicals by 42–78% is demonstrated by *Ca* fractions and their constituents at concentrations of 20 mg/ml, while the least amount of inhibition was found at 2.5 mg/ml, exhibiting just 13-26% inhibition. It is clear from the findings shown in figure 4 that the extracts of calcium and its constituents have a strong ability to reduce hydroxyl ions. The components of *Ca* extracts are also subjected to enzymatic antioxidants such as SOD, catalase, and GPX, whose results are shown in Table 1. The SOD readings for the Ca-M fraction showed 38.42 units of SOD activity, whereas the separated compounds showed 32.64 and 28.42 units of activity. In a similar vein, the methanol fraction's specific catalase activity was determined to be 46.34  $\mu\text{mol}/\text{min mL}$ , whereas the separated compounds A and B

indicated 47.44 and 39.62  $\mu\text{mol}/\text{min mL}$  activity, respectively. The activity of methanolic extracts of glutathione peroxidase (GPX) was 89.16 for dianthrone A and B, and 92.14 and 90.66 for the latter two. Hexane fraction shows extremely little activity for all evaluated antioxidants across all the data.

**Table 1. Effect of dianthrones A and B isolated from *Cassia auriculata* on enzymatic antioxidant.**

Sample	SOD Units/mg protein	CAT $\mu\text{mol}/\text{min mL}$	GPX $\mu\text{g}$ of glutathione oxidized/min/mg protein
Ca- Methanol fraction	38.42 $\pm$ 1.22	46.34 $\pm$ 0.42	89.16 $\pm$ 0.34
Ca- Hexane fraction	8.32 $\pm$ 0.48	18.64 $\pm$ 0.46	32.14 $\pm$ 0.16
Pure Compounds - dianthrone			
A	32.64 $\pm$ 0.18	47.44 $\pm$ 1.26	92.14 $\pm$ 1.12
B	28.42 $\pm$ 0.34	39.62 $\pm$ 0.64	90.66 $\pm$ 0.84

SOD: Super Oxide Dismutase, CAT: catalase activity. GPX: Glutathione Peroxidase.

Data are expressed as Mean  $\pm$  SD (n=3)

**b. Antibacterial potential of *Cassia auriculata* fractions, dianthrone A and B.**

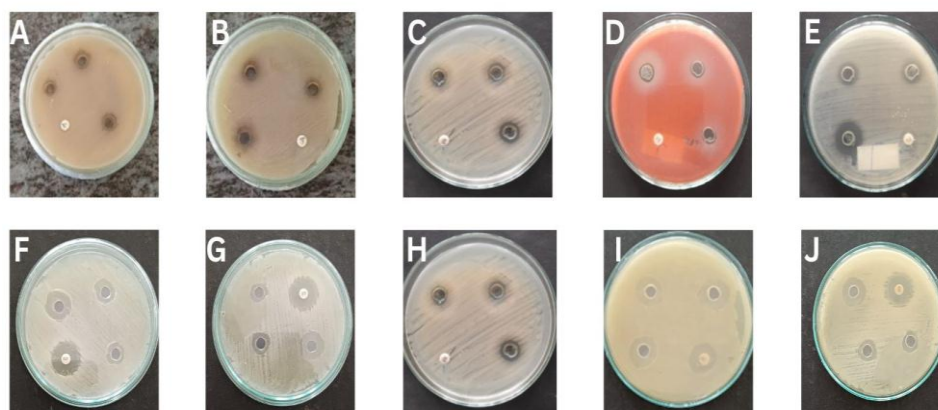
In tests using many bacterial strains, calcium fractions and their compounds demonstrated a maximum inhibition of 23.39 mm for *S. mutans* at 200  $\mu\text{g}/\text{ml}$  of methanolic extract, and 19.28 and 20.24 mm for Dianthrone A and B compounds at 200  $\mu\text{g}/\text{ml}$  concentration, respectively. At 100  $\mu\text{g}/\text{ml}$  concentration, hexane fraction does not exhibit any inhibition; however, at 200  $\mu\text{g}/\text{ml}$ , it did exhibit considerable inhibition. The compound A dianthrone showed the maximum zone of inhibition at *S. pyrogens*, measuring 22.3 mm, whereas compound B showed the highest zone of inhibition at 20.63 mm. The maximum inhibitory effect for compound B was seen at 21.91 mm for *S. marcescens*. *P. vulgaris* had a minimum zone of inhibition of 11.98 mm for the methanolic fraction. Analogously, dianthrone A, the isolated chemical, showed strong action against *P. vulgaris*, with a minimum inhibition zone of 11.34 mm and a range of 12.07 – 22.35 for other harmful bacterial strains. As for dianthrone B, it indicated a minimum inhibition of 7.54 mm against *P. vulgaris* and a zone of 14.69 – 21.91 mm against other bacterial species. Table 2 displays the results of the examination of antibacterial activity against certain harmful bacterial species.

**Table: 2. Antibacterial potential of dianthrone A and B isolated from *Cassia auriculata* against pathogenic microorganisms**

Organism	Zone of Inhibition (in mm)								C (10µg/ml)
	Methanol fractions		Hexane fraction		Dianthrone				
	100µg/ml	200µg/ml	100µg/ml	200µg/ml	A		B		
				100µg/ml	200µg/ml	100µg/ml	200µg/ml		
<i>S. aureus</i>	5.62±0.14	18.23±0.65	-	2.96±0.14	6.24±0.18	17.89±0.13	6.84±0.48	19.06±0.21	15.46±0.12
<i>S. mutans</i>	7.24±0.21	23.39±0.33	-	5.3±0.19	6.98±0.24	19.28±0.31	7.24±0.64	20.84±0.31	14.27±0.14
<i>S. pyrogens</i>	7.04±0.13	21.44±0.49	-	6.76±0.02	7.66±0.42	22.35±0.2	6.82±0.24	20.63±0.09	20.42±0.29
<i>S. marcescens</i>	6.88±0.24	20.01±0.34	-	5.65±0.26	6.84±0.48	20.03±0.6	7.14±0.21	21.91±0.3	21.83±0.1
<i>B. sphaericus</i>	7.14±0.22	20.58±0.24	-	3.06±0.07	-	12.07±0.17	7.42±0.16	16.03±0.08	15.36±0.09
<i>E. coli</i>	5.48±0.14	18.37±0.18	-	2.43±0.30	-	13.41±0.25	6.88±0.22	16.19±0.06	16.14±0.08
<i>S. paratyphi</i>	6.24±0.16	19.52±0.37	-	-	5.84±0.16	18.85±0.09	7.24±0.18	16.11±0.06	18.06±0.05
<i>P. aeruginosa</i>	-	16.89±0.09	-	-	6.21±0.14	18.51±0.1	7.82±0.16	17.32±0.01	9.98±0.12
<i>K. pneumoniae</i>	8.42±0.16	21.19±0.21	-	-	6.44±0.26	17.87±0.4	5.42±0.44	14.69±0.1	11.32±0.03
<i>P. vulgaris</i>	-	11.98±0.30	-	3.24±0.28	-	11.34±0.28	-	7.54±0.09	9.56±0.17

All values are mean of three replicates. ‘-’ No activity, C (Chloramphenicol).





A- *S. aureus*, B- *S. mutans*, C- *S. pyrogens*, D- *S. marcescens*, E- *B. sphaericus*, F- *E. coli*, G- *S. paratyphi*, H- *P. aeruginosa*, I- *K. pneumonia*, J- *P. vulgaris*

**Figure 5. Antibacterial potential of Ca fractions and Dianthrone**

**c. *In vitro* anti-inflammatory and antiarthritic effect of *Cassia auriculata* fractions and dianthrone.**

Studies on albumin denaturation were conducted to determine the anti-inflammatory potential of calcium extracts and their constituents in a dose-dependent manner. When the quantity of the extract was gradually raised, the percentage of inhibition likewise rose, reaching 92.6% at 25 µg/ml of methanol extract. The initial observation of inhibition was 18.6% at 25 µg/ml. When compared to methanolic extracts, compounds A and B exhibit lower activity, with the maximum inhibitions being 76.8% and 74.2%, respectively. Table 3 provided a complete insight of *Ca* extracts' and its components' anti-inflammatory capabilities.

The anti-arthritis efficacy of individual compounds and fractions of *Cassia auriculata* on the *in vitro* suppression of protein denaturation is shown in Table 4. At a concentration of 200 µg/mL, the investigated fractions and dianthrone showed strong inhibition of denaturation. The methanol fraction showed the most substantial inhibition of protein denaturation, with 92.63% activity, followed by 76.8% and 74.2% inhibition on dianthrone A and B. With an inhibition of 24.8%, the minimum inhibition was noted at 25µg/ml.

**Table 3. *In vitro* anti-inflammatory activity of dianthrone A and B isolated from *Cassia auriculata* on albumin denaturation**

Sample	Concentration (µg/ml) (% of inhibition)			
	25	50	100	200
<i>Ca</i> - Methanol extract	18.6	38.8	64.6	92.6
<i>Ca</i> - Hexane fraction	7.4	14.6	26.8	39.8
Standard drug -Diclofenac sodium	89.6	99.6	99.6	100
<b>Pure Compounds - dianthrone</b>				
<b>A</b>	20.6	41.2	61.4	76.8
<b>B</b>	19.8	31.4	52.6	74.2

**Table 4. In vitro anti-arthritic potential of dianthrone A and B isolated from *Cassia auriculata* on inhibition of protein denaturation**

Sample	Concentration ( $\mu\text{g/ml}$ ) (% of inhibition)			
	25	50	100	200
Ca- Methanol extract	24.8	46.2	74.8	94.8
Ca- Hexane fraction	10.1	22.6	34.6	48.5
Standard drug -Diclofenac sodium	90.6	98.4	100	100
<b>Pure Compounds - dianthrone</b>				
<b>A</b>	20.4	41.6	60.4	72.6
<b>B</b>	22.6	43.4	62.8	80.4

#### 4. Discussion

The necessity of finding new medication sources to treat and battle diseases has increased due to multidrug resistance, which has allowed conventional medicine to reclaim its position in the treatment of diseases [20]. Plants possessing phytoconstituents in their secondary metabolites that exhibit anti-inflammatory, anti-cancer, anti-proliferative, and antioxidant activities have been discovered and isolated [21, 22]. According to this line of research, *Cassia auriculata* uses its bioactive ingredients to treat a variety of illnesses. Figure 1 shows the results of a prior investigation on the separation and purification of two active constituents, dianthrone A and B, which showed strong diabetic action. *Cassia auriculata* is a traditional medication that has been used for many years against many studies. The goal of the current study, which is an extension of our earlier research, is to provide scientific support for the traditional use of isolated dianthrone and *Cassia auriculata* for their antibacterial, anti-inflammatory, antioxidant, and antiarthritic properties.

Strong antioxidant qualities are well recognized to be associated with the distinct structural characteristics seen in secondary metabolites from conventional plants [23]. A free radical with a deep violet colour is called DPPH. By neutralizing the DPPH, the mechanism of DPPH causes a colour change from violet to yellow. The Ca fractions' antioxidant qualities are indicated by the colour shift. [24]. The FRAP test works by converting  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by the creation of a blue colour when a reducing agent is present. The compounds may function as reductones because of their high reducing power, which indicates their strong antioxidant qualities and indicates the ferric ion reducing power of the fractions [13]. In contrast to different research, which employed *Asparagus horridus* methanolic extract, the maximum percentage of inhibition was seen at 25 mg/ml, with 50% of inhibition for both the FRAP and DPPH assays. Our present investigation shows that the percentage of inhibition was 82% and 78% at a dose of 20 mg/ml. The findings showed that the Ca fraction had excellent antioxidant capabilities. [25]. The findings are consistent with the earlier research, which found that secondary metabolites such phenols, saponins, aromatic compounds, and flavonoids are mostly responsible for the antioxidant activity [23]. Antioxidants, by their capacity to scavenge radicals or through an antioxidant defense system, protect humans from pathological diseases caused mostly by free radicals. Because hydroxyl ions are extremely reactive species, they can harm other biomolecules as well as proteins and DNA. It was crucial to neutralize these ions in order to stop the issues caused by oxidative stress. The white and pink *Nelumbo nucifera* Gaertn flowers were the subject of the previous investigation. The concentration ranged from 10–400 $\mu\text{g}$ , and at 400 $\mu\text{g/ml}$ , the greatest scavenging activity was 60.53% and 46.72%, respectively. On the

other hand, our investigation showed that a large proportion of inhibition, namely 78%, was detected at 20 mg/ml [26].

Superoxide radicals (SOx) are extremely toxic and can be a powerful precursor to harmful ROS, which is why researchers are focusing so much on natural resource scavengers these days. SOD, the most significant antioxidant defense enzyme, has been shown to destroy SOx, but it also produces hydrogen peroxide as an intermediate, which lessens the toxic effect [24]. Antioxidants, both enzymatic and non-enzymatic, reduce the harm caused by free radicals and eliminate their toxic effects. One of the enzymes that supports the defensive system is catalase. Studies have demonstrated that glutathione peroxidase functions as a strong radical scavenger, a heavy metal binding precursor, and a membrane stabilizer [27].

Drug resistant against major pathogenic microorganism due to usage of major classes of antibiotic has led an emergency to the scientific community to identify molecules to these resistant microorganisms [10]. Subjecting the fractions and the isolated compounds dianthrone A and B to antibacterial activity against selected pathogenic bacterial species indicated an on-par results with the reference drug and the results are represented in Table 2. From the present study of methanolic fractions of *Ca* demonstrate highest percentage of zone of inhibition was found in the maximum concentration of 80 µg/ml for all the 10 bacteria. The antibacterial activity was compared with the previous study, they used 4 different species, the findings revealed that the zone of inhibition was increased when the concentration of the extract increases [28]. Auto antigens are produced in certain arthritic condition and inflammation is mainly due to *in vivo* denaturation of proteins [29], thereby inhibiting protein denaturation may lead to decrease in the production of auto antigen. The methanolic fractions of *Ca* shows notable anti-inflammatory properties, the extract exhibits 18.6% of inhibition at the lowest concentration and the highest percentage of inhibition was observed at 200 µg/ml with the percentage inhibition of 92.6%. The present investigation is consistent with the earlier work, which found that the methanolic extract exhibits considerable anti-inflammatory effect in a dose-dependent manner, ranging from  $23.38 \pm 0.28\%$  to  $84.2 \pm 1.15\%$ . These results demonstrate the extract's possible anti-inflammatory action by showing that when the extract's concentration rises, so does its activity [28]. Using the bovine serum technique, protein denaturation inhibition was used as a proxy for *in vitro* anti-arthritic effectiveness. Comparing our study to another, which examined the antiarthritic activity of ethanolic extracts from *Cassia fistula* Linn leaves, the latter found that the highest percentage of inhibition was only observed at 1000 µg/ml. In contrast, our study shows the highest percentage of antiarthritic activity at 200 µg/ml with a percentage inhibition of 92.63%. The findings indicate that calcium and its compounds have enhanced anti-arthritic potential in a dose-dependent manner [30]. The two most often prescribed medications for arthritis and inflammation are DMARDs (disease-modifying anti-rheumatic medications) and NSAIDs (non-steroidal anti-inflammatory medicines); nevertheless, there are still gaps in the scientific community's knowledge on safer medications, and natural sources are encouraged [31, 32].

## 5. Conclusion

The studies explore that dianthrone isolated from *Cassia auriculata* L. yielded promising results across various fields, including mitigation of oxidative impairment, antibacterial efficacy, and antiarthritic potential. The ability of *Cassia auriculata* L. to alleviate oxidative impairment suggests a potential role in opposing oxidative stress-related diseases and promoting overall health. The antibiotic activity of the compound highlights their capacity to inhibit bacterial growth suggesting that it can be used for the development of novel antibacterial agent. Their ability to lessen inflammation and alleviate arthritic symptoms suggests a promising role in the treatment and management of arthritis disease, improving the quality of life for affected individuals. These findings provide a strong foundation for further research aimed at clarifying their mechanisms of action and exploring their potential therapeutic role in various field.

## 6. Conflicts of Interest

No conflict of interest

## 7. Acknowledgement

Authors are thankful to the Management and Principal of K. S. Rangasamy College of Technology, Tiruchengode and DST- FIST, DBT STAR-scheme, DBT PG programme, New Delhi, for providing the infrastructure and facilities to carry out this research work successfully.

## 6. REFERENCE

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