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Antioxidant, anti-arthritic and anti-inflammatory activity of methanolic extracts of *Cardiospermum halicacabum* seeds

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Abstract: *Cardiospermum halicacabum* L., belonging to the family Sapindaceae, is a traditional medicinal plant commonly known as balloon vine. Bioactive compounds such as flavonoids, saponins, triterpenes and phenolic acids, which contribute to a wide range of pharmacological activities,have been reported from seeds. This study investigates the antioxidant, anti-arthritic and anti-inflammatory properties of *C. halicacabum* seed extract. Antioxidant activity was evaluated using DPPH and hydroxyl radical scavenging assay, demonstrating strong radical scavenging potential. The anti-arthritic effects assessed through protein denaturation and anti-inflammatory activity by LOX and COX assays revealed significant inhibition of inflammatory mediators. Dose-dependent responses were noted, with higher extract concentrations showing enhanced activity across all tested parameters. Comparative analysis with standard drugs indicated that the extract had comparable efficacy. The results suggest that *C. halicacabum* seed extract can modulate oxidative and inflammatory pathways involved in arthritis pathogenesis. The findings validate the ethnomedicinal use of *C. halicacabum* seeds in the treatment of inflammatory and arthritic conditions and suggest their potential as a natural therapeutic source for oxidative and inflammatory disorders.

Keywords: *Cardiospermum halicacabum*, seed extract, antioxidant, anti-arthritic, anti-inflammatory, Sapindaceae.

1. INTRODUCTION

Seeds are a potent reservoir of biologically active compounds, playing a pivotal role not only in plant reproduction but also in traditional and modern medicinal systems. They are endowed with a variety of secondary metabolites such as flavonoids, phenolic acids, alkaloids, tannins, terpenoids, saponins and essential oils that contribute to their broad spectrum of therapeutic activities (Khan et al., 2021; Elangovan et al., 2022). These compounds have been linked to pharmacological effects including antioxidant, anti-inflammatory, antimicrobial, antidiabetic and anticancer properties (Barbosa et al., 2020).

In traditional medicine systems such as Ayurveda, seeds of various plants have been used to treat ailments like inflammation, skin disorders, gastrointestinal issues and microbial infections (Uddin et al., 2022). Recent scientific investigations support many of these ethnomedicinal uses, demonstrating that seed extracts can modulate biochemical pathways and neutralize oxidative stress—a major contributor to chronic diseases such as diabetes, cardiovascular diseases and cancer (Atiya Praveen et al., 2021; Dowlath et al., 2020).

The Sapindaceae family, comprising over 140 genera and approximately 1900 species, includes several plants traditionally valued for their medicinal properties. Among the various plant parts used, the seeds of Sapindaceae members have gained increasing scientific attention due to their rich phytochemical composition and therapeutic potential (Elangovan et al., 2022). These seeds are known to be abundant in flavonoids, triterpenoids, saponins, phenolic compounds and fatty acids, which contribute to a range of bioactivities, notably antioxidant, anti-inflammatory and anti-

arthritic effects (Dowlath et al., 2020; Gaziano et al., 2019).Antioxidant activity of Sapindaceae seeds is primarily attributed to the presence of phenolic compounds that scavenge free radicals and reduce oxidative stress, a key factor in the development of chronic diseases such as arthritis and cardiovascular disorders (Rokkam et al., 2024).Moreover, anti-inflammatory and anti-arthritic properties of Sapindaceae seeds have been demonstrated in both in vitro and in vivo models. The bioactive constituents, including triterpenes and saponins, modulate inflammatory mediators such as COX-2, TNF-α and IL-6, thus reducing joint inflammation and cartilage degradation (Elangovan et al., 2022; Gaziano et al., 2019).

The seeds of *Cardiospermum halicacabum* are rich in bioactive compounds, including flavonoids, fatty acids, sterols, triterpenes and alkanes. Bioactive compound flavanone (Rupeshkumaret al., 2012), squalene, a triterpene and campesterol (Saravanan et al., 2021)identified in the seed extracts of *Cardiospermum halicacabum* were demonstrated to exhibit antioxidant and anti-inflammatory properties. Oral administration of ethanolic extract of leaves of *C.halicacabum* (125 and 250 mg/kg), inhibited FCA-induced arthritis in rats in a dose-dependent manner(Kumar et al., 2008). Ethanolic fraction of leaf extract (EFC) of *C.halicacabum* reduced the production of pro-inflammatory mediators in RAW 264.7 macrophages and tumour necrosis factor-alpha (TNF-α) in human peripheral blood mononuclear cells stimulated by lipopolysaccharide (LPS), establishing its efficacy in the treatment of rheumatoid arthritis(Venkatesh Babu and Krishnakumari, 2006).Acombination of ethanolic extracts from *Moringa oleifera* and *C.halicacabum* demonstrated a strong anti-inflammatory and anti-arthritic effect in vitro (Balamurugan and Muruganandam, 2021). From the above studies, it is evident that the seeds are less explored for their therapeutic properties. Hence, the present study aims to understand the antioxidant, anti-arthritic and anti-inflammatory activity of*Cardiospermum halicacabum* seed extract.

2. Materials and methods:

2.1. Collection of Plant

The fresh seeds of *Cardiospermum halicacabum* were collected from the moist areas of Nagercoil, Kanyakumari District, Tamil Nadu. The species identification was done by comparing its morphological features and microscopic examination of the anatomy as per the standard methodologies by the Department of Botany, Holy Cross College, Nagercoil.

2. 2. Sample Extraction – Soxhlet Method:

The seeds were crushed into fine powder and rolled in cotton cloth.Extraction was performed using a Soxhlet apparatus (Krishnananda et al., 2017) with solvent methanol (5gm/100 ml). The powdered sample, wrapped in cotton cloth and placed in the Soxhlet thimble, was prevented from clogging the siphon using cotton plugs. Extraction was carried out for 15 cycles andafter extraction, the solvents were evaporated and the crude extracts were stored for further analysis.

2.3. Antioxidant Activity

2.3.1. DPPH radical scavenging activity

A 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay(Williams et al.,1995)was performed to assess the *invitro* free-radical scavenging activity of the extract fractions with slight modifications.The stock solution was prepared by dissolving 24 mg DPPH with 100 ml of ice-cold ethanol (99.5%) instead of methanol as it would enhance the solubility and stability. The working solution was prepared by diluting DPPH solution with ethanol and 3 ml aliquot of this solution was mixed with 1 ml of sample at various concentrations (20, 30and 40 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 minutes at room temperature and the absorbance was measuredat 517 nm. The control was prepared without any sample and scavenging activity based on the percentage of DPPH radical scavenging was calculated using the following formula:

Percentage of inhibition = [(control OD−sample OD) / (control OD)] ×100

2.3.2. Hydroxyl radical scavenging activity

The reaction mixture contained 0.8 ml of phosphate buffer solution (50 mmol L^{-1}, pH 7.4), 0.2 ml of sample of different concentrations (20, 40, 60, 80 and 100 µg/ml), 0.2 ml of EDTA (1.04 mmol L^{-1}), 0.2 ml of $FeCl_3$(1 mmol L^{-1})

and 0.2 ml of 2-deoxyribose (60 mmol L^{-1}). The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 ml of ascorbic acid (2 mmol L^{-1}) and 0.2 ml of H_2O_2 (10 mmol L^{-1}). After incubation at 37 °C for 1 h, 2 ml of cold thiobarbituric acid (10 g L^{-1}) was added to the reaction mixture followed by 2 ml of HCl (25%). The mixture was heated at 100 °C for 15 minutes and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation on hydroxyl radicals. The scavenging percentage was calculated according to the following formula:

Scavenging effect (%) = [(control OD−sample OD)/ (control OD)] ×100

2.4. Anti-arthritic activity

The anti-arthritic effect of the methanol extracts of *C. halicacabum* seeds was evaluated using a protein denaturation assay with bovine serum albumin (BSA) as the standard protein.The reaction mixture (0.5 ml) contained 0.45 mlof 5 % aqueous solution of BSA and 0.05 mlof different concentrations (12.5, 25, 50, 100, 200 µg/ml) of the sample (CH-M) and aspirin (reference drug). The pH of each solution was adjusted to 6.3 using 1 N HCl. The samples were incubated at 37 °C for 20 minutes followed by heating at 57°C for 30 minutes. Then phosphate buffer (2.5 ml) was added and absorbanceat 660 nm was measured using a spectrophotometer. For the test control 0.05 ml distilled water was used instead of the sample while the product control lacked BSA (Chandra et al., 2012). The percentage inhibition of protein denaturation was calculatedusing the following formula:

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{Abs TestSolution} - \text{Abs Product Control}}{\text{Abs Test Control}} \times 100 \right]$$

2.5. Anti- inflammatory assays

RAW 264.7 cellsprocured from National Centre for Cell Sciences (NCCS), Pune, India were maintained in Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA) supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml) and Amphotericin B (2.5µg/ml).The cells, upon attaining 60% confluency,were activated using 1 µL lipopolysaccharide (LPS: 1µg/ml). LPS-stimulated RAW cells were exposed todifferent concentrations (25,50,100 µg/ml) of sample solution and incubated for 24 h. After the incubation, the cells were lysed and anti-inflammatory assays were performed(Walker et al.,2010).

2.5.1. Cyclooxygenase (COX) activity

The COX activity was assayed following the method of Walker and Gierse (2010). Briefly, 100µL cell lysate was incubated with Tris-HCl buffer (pH 8) containing glutathione (5 mM/L) and haemoglobin (5 mM/L) for 1 minutes at 25°C. The reaction was initiated by the addition of 200 mM/L arachidonic acid followed by incubation at 37°C for 20 minutes and terminated by the addition 200 µL of 10% trichloroacetic acid in 1 N hydrochloric acid. The reaction mixture was centrifuged at 1000 × g for 3 minutes, 200 µl of 1% thiobarbituric acid was added, and the tubes were incubated at 100°C for 20 minutes. After cooling, the tubes were centrifuged for 3 minutes and COX activity was determined by measuring the absorbance at 632 nm. Thepercentage inhibition of COX activity was determined using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{(\text{Absorbance of control})} \times 100$$

2.5.2. Lipoxygenase (LOX) activity

Lipoxygenaseactivity was assessed as described by Axelrod et al. (1981). Briefly, the reaction mixture (2 ml final volume) contained Tris-HCl (pH 7.4), 50 µl of cell lysate and 10 mM sodium linoleate (200 µl). The LOX activity was measured as an increase in absorbanceat 234 nm(Agilent Cary 60), corresponding to the formation of 5-hydroxyeicosatetraenoic acid. The percentage inhibition was calculated as

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{(\text{Absorbance of control})} \times 100$$

3. Result:

3.1. Antioxidant Activity

A 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay was performed to assess the free-radical scavenging activity of the extract. The results indicated the antioxidant activity of the sample (CH-M) compared to the standard at various concentrations. The IC_{50} values suggested that the standard exhibited stronger antioxidant activity than the sample (Table 1).Hydroxyl radical scavenging activity ofthe seeds of *C. halicacabum* methanol extracts exhibited an increase in activity when compared to the standard. However, not much variation was observed in the IC_{50} value between the two radical scavenging assays. A dose dependent increase in activity was observed(Table 2).

Table 1: Antioxidant activity of the methanol extracts of *C. halicacabum*seeds and standard at different concentrations - DPPH assay

Concentration µg/ ml	Sample	
	CH -M	Standard
20	33.99 ± 0.546	36.69 ± 0.299
30	47.425 ± 0.146	58.442 ± 0.113
40	56.427 ± 0.14	64.967 ± 3.078
IC $_{50}$ Value	34.503	27.619

CH -M*:Cardiospermum halicacabum* Methanol extract; Values are mean ± standard deviation of three replicates

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Table 2: Antioxidant activity of the seeds of *C. halicacabum* methanol extracts and Standard at different Concentrations - Hydroxyl radical scavenging assay

Concentration µg/ ml	Sample	
	CH-M	Standard
20	88.393 ± 0.058	67.527 ± 0.017
30	66.804 ± 0.143	56.506 ± 0.012
40	42.526 ± 0.115	19.3 ± 0.033
IC $_{50}$ Value	36.936	29.078

CH -M*:Cardiospermum halicacabum*Methanol extract; Values are mean ± standard deviation of three replicates

3.2. Anti-arthritic Activity

The anti-arthritic activity of the extract was evaluated using a protein denaturation assay with bovine serum albumin (BSA) as the standard protein and aspirin as a reference drug. The percentage inhibition of protein denaturation was calculated and the dose-dependent changes in protein denaturation was compared to that of the reference drug.Our results showed that the percentage of inhibition for CH-M increased with increase in concentration, indicating a dose-dependent response (Table 3)

Table 3. Anti-arthriticactivityof the seedsof *C. halicacabum* methanol extracts and Aspirin Standard at various concentrations

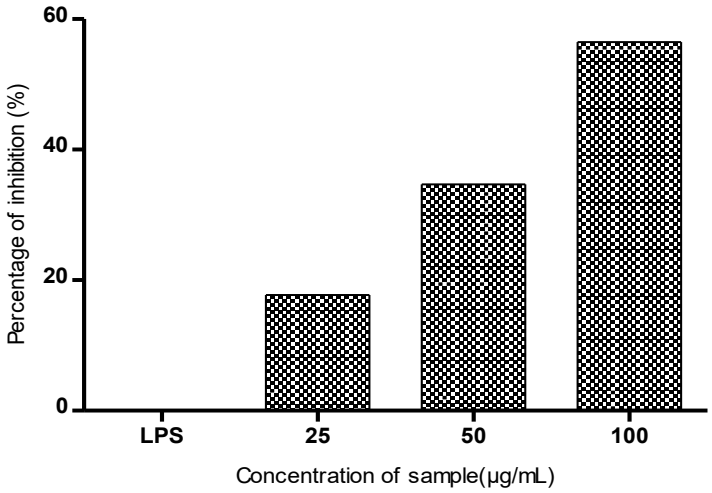
Sample	Percentage of inhibition (%)				
	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
CH-Methanol	45.014 ± 1.066	53.846 ± 0.697	62.393 ± 0.697	74.074 ± 1.065	78.633 ± 3.041
Std (Aspirin)	52.201 ± 0.513	59.119 ± 0.5135	69.601 ± 0.296	78.616 ± 0	82.336 ± 1.452

3.3.Anti-inflammatory activity

3.3.1 Cyclooxygenase (COX) activity

The anti- inflammatory activity of the samplewas assessed on LPS-stimulated RAW cellsexposed to different concentrationsof the extract. Our results showed that COX activity was inhibited in a dose-dependent manner withmild(17.74%) at25µg/ml, moderate(34.68%)at 50 µg/ml and maximum (56.45%) inhibition at100 µg/ml (Figure - 1).

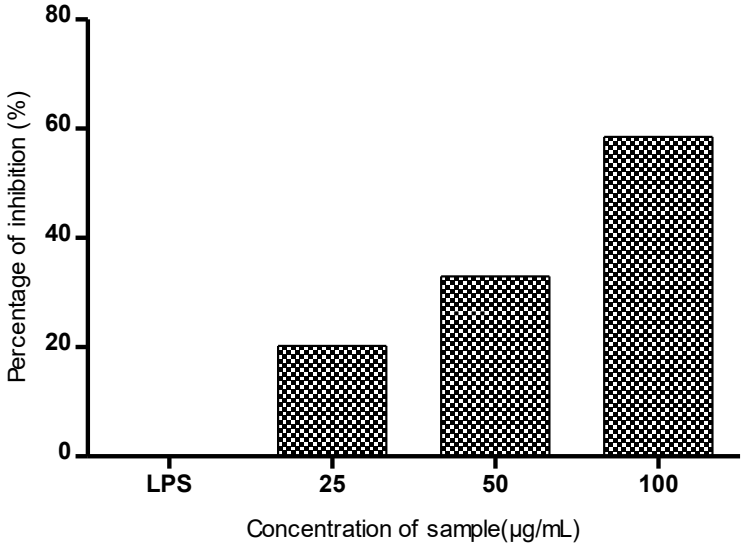
Figure 1: Inhibitory activity of the *C. halicacabum*seed extract against Cyclooxygenase (COX) enzyme



3.3.2. Lipoxygenase (LOX) activity

The anti- inflammatory activity of the samplewas further analysed via LOX activity on LPS-stimulated RAW cellsexposed to different concentrationsof the extract. The sample exhibited a clear dose response relationship in its anti-inflammatory activity, with inhibition increasing 20.21 % at 25 µg/ml to 32.98 % at 50 µg/ml and reaching 58.51% at 100 µg/ml (Figure-2).

Figure 2: Inhibitory activity of the *C. halicacabum*seed extract against Lipoxygenase (LOX) enzyme



4. Discussion

Plants are a rich source of traditional medicines derived from different parts of the plant. The phytochemicals like terpenoids, flavonoids, saponins, phenols and alkaloids present in the extract of these plants has helped in the development of drugs due to their therapeutic potential (Patwardhan et al., 2004). In the present study the antioxidant, anti-arthritic and anti-inflammatory potential of *Cardiospermum halicacabum* seeds has been investigated.

Antioxidants act as crucial free radical scavengers by donating electrons to neutralize reactive oxygen species (ROS) and other free radicals, thus stabilizing their unpaired electrons and preventing oxidative damage to cellular lipids, proteins and DNA. This function is particularly relevant in pharmacology, as oxidative stress plays a central role in the pathophysiology of numerous chronic diseases, including cancer, cardiovascular diseases, neurodegenerative disorders such as Alzheimer's and Parkinson's and inflammatory conditions (Valko et al., 2007). In order to protect the human body from oxidative damage, chemicals that suppress or scavenge ROS / RNS are highly sought for (Kuriakose and Kurup, 2010). Due to their capacity to counteract free radicals or their effects, antioxidants are micronutrients that have gained significant attention in recent years (Vadlapudi and Naidu, 2010).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay is a widely used method for assessing the antioxidant capacity of plant extracts, based on their ability to donate hydrogen atoms and neutralize free radicals (Brand-Williams et al., 1995). In the present study, the methanolic extract of *Cardiospermum halicacabum* seeds (CH-M) demonstrated dose-dependent antioxidant activity, as evidenced by increasing DPPH radical inhibition with rising concentrations of the extract. However, across all tested concentrations (20, 30, and 40 µg/ml), the scavenging activity of the CH-M extract was consistently lower than that of the standard antioxidant (presumably ascorbic acid or another synthetic reference compound). Interestingly, the extract exhibited comparatively higher hydroxyl radical scavenging activity than the standard, suggesting that the differential response between the two assays may be attributed to the distinct nature of the reactive species, variations in the mechanisms of radical neutralization, assay sensitivity or other experimental parameters. The IC_{50} values, representing the concentration required to inhibit 50% of free radicals, further reinforce this observation. The IC50 values for the CH-M extract were 34.503 µg/ml and 36.936 µg/ml (from two independent datasets), whereas the corresponding values for the standard antioxidant were 27.619 µg/ml and 29.078 µg/ml, respectively. Since lower IC_{50} values indicate greater antioxidant efficacy, the standard compound demonstrated superior radical scavenging potential compared to the CH-M extract.This suggests the presence of effective phytochemicals in the methanol extract, such as flavonoids, phenolics, and other polyphenols, which are known contributors to antioxidant effects.These findings align with earlier studies reporting the antioxidant potential of *C. halicacabum* leaf and seed extracts due to the presence of flavonoids, saponins and fatty acids (Dowlath et al., 2020; Elangovan et al., 2022). While the extract does not surpass the standard antioxidant, its significant scavenging capacity supports its potential use in natural antioxidant formulations or as a complementary therapy for oxidative stress–related disorders.

Methanol extracts of *C. halicacabum* exhibited a dose-dependent anti-arthritic effect, with inhibition increasing from 45.01% at 12.5 µg/ml to 78.63% at 200 µg/ml. This trend supports its potential as a therapeutic agent. The effect may involve suppression of pro-inflammatory pathways, possibly via cytokine modulation or COX inhibition (Zhang et al., 2015; Patel et al., 2020). The enhanced response at higher concentrations may reflect increased bioavailability or synergism among active constituents, a phenomenon often reported in plant-based therapies (Sharma et al., 2019).

COX inhibition by the sample followed a clear dose-response pattern,with increasing concentrations of the sample correlating with greater percentage inhibition. This suggests strong anti-inflammatory potential, possibly via suppression of prostaglandin synthesis, key mediators of inflammation (Smith et al., 2019). The results align with previous findings on COX-inhibitory phytochemicals (Liu et al., 2020) and are comparable to NSAIDs at similar concentrations (O'Neill et al., 2021). These results underscore the therapeutic promise of the extract, though in vivo validation is necessary.

The assessment of lipoxygenase (LOX) activity in the sample demonstrated a clear dose-response relationship in its anti-inflammatory effects. Specifically, the percentage of inhibition increased from 20.21% at 25 µg/ml to 32.98% at 50 µg/ml, ultimately reaching 58.51% at 100 µg/ml. This trend highlighted the potential of the sample as an effective anti-inflammatory agent, particularly at higher concentrations. LOX enzymes were crucial in the metabolism of arachidonic acid, leading to the production of leukotrienes, which were potent mediators of inflammation (Samuelson et al., 2020). The inhibition of LOX activity, as observed in this study, suggests that the sample may effectively reduce leukotriene synthesis, thereby mitigating inflammatory responses. This aligns with findings that various plant extracts demonstrated significant LOX inhibitory activity, contributing to their overall anti-inflammatory properties (Choudhury et al., 2021). Agarwal et al.(2019) indicated that certain phytochemicals act synergistically to enhance

their inhibitory effects on inflammatory pathways, including LOX. The significant dose-dependent inhibition of LOX activity observed in this study reinforces the sample's potential as an anti-inflammatory agent. Further investigations were warranted to elucidate the specific active compounds responsible for this activity and to evaluate their safety and efficacy in vivo.

5. CONCLUSION

The methanolic extract of *Cardiospermum halicacabum* seed extract exhibited antioxidant, anti-arthritic, and anti-inflammatory activities. Its antioxidant potential was confirmed by dose-dependent free radical scavenging in DPPH and hydroxyl assays. The extract also stabilized proteins and inhibited COX and LOX enzyme indicating its anti-arthritic and anti-inflammatory activity which is due to its bioactive phytochemicals. These results support its traditional use and potential as a natural therapeutic agent.

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