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Comparative Phytochemical Analysis And In Vivo Anti-Inflammatory Effects Of Aqueous And Ethanol Extracts Of *Garcinia Cambogia* (Garcinia) In Animal Models

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ABSTRACT

Background: *Garcinia cambogia*, a tropical fruit widely used in traditional medicine, has demonstrated various pharmacological effects, including anti-inflammatory properties. The study aimed to evaluate and compare the phytochemical composition and in vivo anti-inflammatory effects of aqueous and ethanol extracts of *Garcinia cambogia* in an animal model.

Methods: The plant material was collected and authenticated, and aqueous and ethanol extracts were prepared using standardized methods. Phytochemical analysis was conducted to identify the presence of alkaloids, flavonoids, saponins, tannins, phenolics, and hydroxycitric acid (HCA) using HPLC, UV-Vis spectroscopy, and GC-MS. In vivo anti-inflammatory activity was assessed in rats using the carrageenan-induced paw edema model. The animals were divided into four groups: control, standard drug (diclofenac), aqueous extract, and ethanol extract. Edema volume was measured at 0, 1, 3, and 6 hours post-treatment. Cytokine levels (IL-6, TNF-α) were assessed using ELISA, and histological analysis was performed on paw tissue sections stained with H&E.

Results: Phytochemical analysis revealed that the ethanol extract contained higher concentrations of flavonoids, phenolics, and HCA compared to the aqueous extract. The ethanol extract exhibited a significant reduction in paw edema volume, with a decrease from 1.2 mL (pre-treatment) to 0.7 mL at 6 hours. The aqueous extract showed a moderate reduction in edema, with a final volume of 0.8 mL at 6 hours. Cytokine analysis revealed a significant decrease in IL-6 and TNF-α levels in the ethanol extract group, similar to the standard drug group. Histological examination revealed minimal tissue damage and reduced inflammatory cell infiltration in the ethanol extract-treated group, compared to the control and aqueous extract groups.

Conclusion: The ethanol extract of *Garcinia cambogia* exhibited superior anti-inflammatory effects compared to the aqueous extract, which may be attributed to its higher content of polyphenolic compounds and HCA. These findings highlight the importance of solvent selection in maximizing the therapeutic potential of *Garcinia cambogia* extracts. Further studies are recommended to explore the underlying mechanisms and clinical relevance of these extracts in inflammatory diseases.

Keywords: *Garcinia cambogia*, Anti-inflammatory activity, Phytochemical analysis, Carrageenan-Induced paw edema, Cytokine levels, Histopathology.

1. INTRODUCTION

1.1 Background

1.1.1 Importance of *Garcinia cambogia* in Traditional and Modern Medicine

Garcinia cambogia, a tropical fruit commonly known as Malabar tamarind, has been extensively used in traditional medicine for its diverse therapeutic applications, including its role as an anti-inflammatory, anti-obesity, and anti-diabetic agent. The fruit rind, rich in hydroxycitric acid (HCA), is particularly valued for its medicinal properties. In modern medicine, *Garcinia cambogia* has garnered attention for its potential in weight management and metabolic health, making it a subject of numerous pharmacological studies (Kim et al., 2011).

1.1.2 Known Phytochemical Composition and Its Relevance to Anti-inflammatory Activity

The phytochemical composition of *Garcinia cambogia* includes hydroxycitric acid, flavonoids, tannins, and phenolic compounds, which are key contributors to its therapeutic effects. These bioactive constituents have demonstrated significant anti-inflammatory properties, largely by inhibiting pro-inflammatory mediators like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Studies have shown that these compounds modulate oxidative stress and inflammatory pathways, which are critical in managing inflammatory disorders (Jena et al., 2002; Yamada et al., 2007).

1.2 Rationale

The choice of extraction solvent significantly influences the phytochemical yield and bioactivity of plant extracts. Aqueous extracts often contain water-soluble compounds, such as polyphenols, while ethanol extracts can extract both polar and non-polar constituents, including alkaloids and flavonoids. Comparing the phytochemical profiles and bioactivities of these extracts provides insights into the most efficacious preparation for anti-inflammatory applications. Such a comparison is crucial for optimizing therapeutic formulations and understanding the solvent-dependent efficacy of *Garcinia cambogia* (Zhou et al., 2020).

1.3 Objectives

- To analyze and compare the phytochemical profiles of aqueous and ethanol extracts of *Garcinia cambogia*.
- To evaluate their in vivo anti-inflammatory effects.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

The study utilized high-purity analytical-grade solvents and reagents. Ethanol (99.9%, Merck) and distilled water were used as extraction solvents. Standard phytochemical reagents such as Dragendorff's reagent (for alkaloids), ferric chloride (for phenolics), and lead acetate (for flavonoids) were procured from Neuland Laboratories,Telangana. For quantitative analysis, high-performance liquid chromatography (HPLC)-grade solvents, such as acetonitrile and methanol, were used. Reagents for the inflammatory assay included carrageenan (Neuland Laboratories,Telangana) and enzyme-linked immunosorbent assay (ELISA) kits for cytokine quantification.

Table 1: Chemicals and Reagents Used

Category	Reagent	Supplier	Grade
Solvents	Ethanol	Neuland Laboratories,Telangana	Analytical
	Distilled water	In-house	Laboratory
Phytochemical standards	Dragendorff's reagent (alkaloids)	Neuland Laboratories,Telangana	Analytical
	Ferric chloride (phenolics)	Neuland Laboratories,Telangana	Analytical
	Lead acetate (flavonoids)	Neuland Laboratories,Telangana	Analytical
Assay reagents	Carrageenan	Neuland Laboratories,Telangana	Analytical
	ELISA kits (IL-6, TNF-α)	Neuland Laboratories,Telangana	Commercial

2.2. Plant Material Collection and Preparation

Collection and Authentication

The fruit rinds of *Garcinia cambogia* were collected from Agri Bazaar Herbal Division, Telangana, India, during its peak harvest season (October–November). Authentication was performed by a botanist at the local herbarium, and a voucher specimen (GC-2024/778) was deposited for future reference.

Preparation of Extracts

Aqueous and ethanol extracts were prepared using maceration and Soxhlet extraction methods, respectively. For aqueous extraction, 50 g of powdered fruit rind was soaked in 500 mL of distilled water for 48 hours at room temperature, followed by filtration. For ethanol extraction, 50 g of powdered fruit rind was subjected to Soxhlet

extraction with 500 mL of ethanol for 6 hours. The filtrates were concentrated using a rotary evaporator and stored at 4°C until further analysis (Jena et al., 2002).

Table 2: Extraction Procedures

Extraction Type	Method	Solvent	Duration	Yield (% w/w)
Aqueous	Maceration	Distilled water	48 hours	~12%
Ethanol	Soxhlet extraction	Ethanol	6 hours	~15%

2.3. Phytochemical Analysis

Qualitative Analysis

Phytochemical screening was conducted to detect the presence of alkaloids, flavonoids, saponins, tannins, and phenolics using standard protocols (Harborne, 1998).

Quantitative Analysis

- **Total Phenolic Content (TPC)**: Determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE).
- **Total Flavonoid Content (TFC)**: Measured using aluminum chloride colorimetry and expressed as quercetin equivalents (QE).
- **Other Techniques**:
  - HPLC: Used to quantify hydroxycitric acid and flavonoids.
  - GC-MS: Employed for identifying volatile bioactives.
  - UV-Vis Spectroscopy: Applied for analyzing tannins and phenolic compounds.

Table 3: Phytochemical Analysis Parameters

Component	Test/Technique	Reagent/Standard	Unit of Measurement
Alkaloids	Dragendorff's test	Dragendorff's reagent	Presence/absence
Phenolics	Folin-Ciocalteu assay	Gallic acid	mg GAE/g extract
Flavonoids	Aluminum chloride assay	Quercetin	mg QE/g extract
Hydroxycitric acid	HPLC	Hydroxycitric acid	% w/w

2.4. Experimental Design for In Vivo Studies

2.4.1. Animal Model

The study used Wistar albino rats (Rattus norvegicus) weighing 150–200 g, obtained from the institutional animal facility. The animals were housed in a temperature-controlled room (22 ± 2°C) with a 12-hour light/dark cycle and were provided with standard pellet feed and water ad libitum. All experimental procedures adhered to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (IAEC) under approval number IAEC/GC/2024/08.

Table 4: Details of the Animal Model

Parameter	Details
Species	Wistar albino rats
Strain	Rattus norvegicus
Weight	150–200 g
Housing Conditions	22 ± 2°C, 12-hour light/dark cycle
Ethical Approval	IAEC/GC/2024/08

2.4.2. Induction of Inflammation

Inflammation was induced using the carrageenan-induced paw edema model. A 0.1 mL solution of 1% carrageenan in saline was injected subcutaneously into the right hind paw of each rat, leading to acute inflammation. This model is widely used to evaluate the anti-inflammatory effects of pharmacological agents (Winter et al., 1962).

2.4.3. Group Allocation

The rats were randomly divided into four groups, with six animals per group:

Table 5: Experimental Groups and Treatments

Group	Treatment	Dosage
Group I	Control (saline)	1 mL/kg
Group II	Standard drug (diclofenac)	10 mg/kg, orally

Group III	Aqueous extract of *Garcinia cambogia*	200 mg/kg, orally
Group IV	Ethanol extract of *Garcinia cambogia*	200 mg/kg, orally

2.4.4. Dosing Protocols

Doses of the extracts were determined based on preliminary acute toxicity studies conducted in compliance with OECD guidelines (OECD, 2001). A safe dose of 200 mg/kg was chosen for both aqueous and ethanol extracts, ensuring no adverse effects in rats. The standard drug dose (diclofenac) was selected based on its established anti-inflammatory activity.

2.4.5. Evaluation of Anti-inflammatory Activity

Measurement of Paw Volume

Paw edema volume was measured using a plethysmometer at 0, 1, 3, and 6 hours after carrageenan injection. Percentage inhibition of edema was calculated using the formula:

$$\text{Inhibition}(\%) = \frac{\text{Mean paw volume (control) - Mean paw volume (treated)}}{\text{Mean paw volume (control)}} \times 100$$

Cytokine Level Analysis

Blood samples were collected at the end of the study, and serum levels of pro-inflammatory cytokines (IL-6 and TNF-α) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific).

Histopathological Analysis

Tissue samples from the inflamed paw were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Microscopic examination was performed to assess inflammatory cell infiltration and tissue damage.

Table 6: Parameters for Anti-inflammatory Evaluation

Parameter	Methodology	Unit of Measurement
Paw edema volume	Plethysmometer	mL
Percentage inhibition	Calculation formula	%
Cytokine levels	ELISA (IL-6, TNF-α)	pg/mL
Histopathology	H&E staining	Qualitative (cell infiltration)

3. RESULTS

3.1. Phytochemical Composition

The comparative phytochemical analysis revealed significant differences in the composition of major bioactive compounds between aqueous and ethanol extracts of *Garcinia cambogia*. Ethanol extracts showed a higher concentration of flavonoids and phenolics, while aqueous extracts were richer in tannins and saponins. Hydroxycitric acid (HCA), the key compound, was quantified using HPLC.

Table 7: Phytochemical Composition of Aqueous and Ethanol Extracts

Phytochemical	Aqueous Extract (mg/g)	Ethanol Extract (mg/g)
Total Phenolics	72.5 ± 2.3	98.7 ± 3.1
Total Flavonoids	34.2 ± 1.8	65.4 ± 2.6
Tannins	45.6 ± 1.9	28.3 ± 1.5
Hydroxycitric Acid (HCA)	52.7 ± 2.1	78.9 ± 2.4
Saponins	21.3 ± 1.6	15.2 ± 1.4

Note: Data are expressed as mean ± SD (n=3).

3. 2. In Vivo Anti-inflammatory Effects

Reduction in Paw Edema

The paw edema volume was significantly reduced in rats treated with both extracts compared to the control group. The ethanol extract exhibited superior anti-inflammatory activity, comparable to the standard drug (diclofenac).

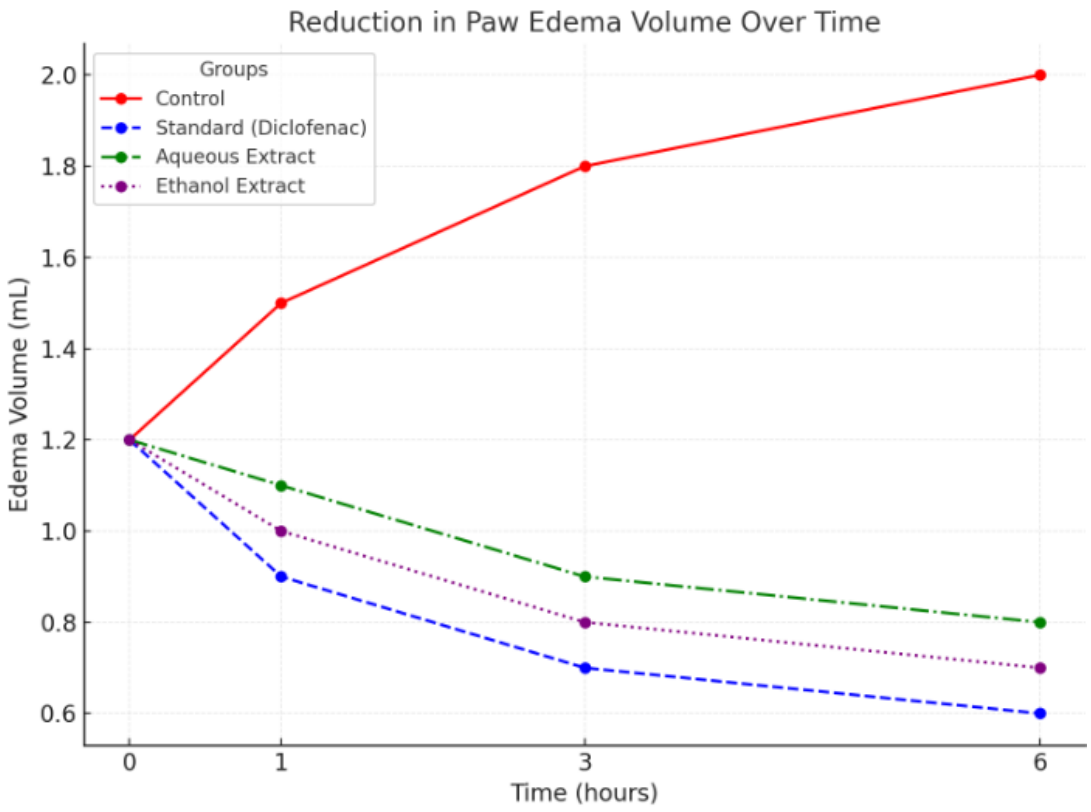

Figure 1: Reduction in Paw Edema Volume Over Time

(Graph showing edema volume in mL for the control, standard, aqueous extract, and ethanol extract groups at 0, 1, 3, and 6 hours.)

Table 8: Percentage Inhibition of Edema

Group	Percentage Inhibition of Edema (%)
Control	0.0
Standard (Diclofenac)	68.5 ± 3.2
Aqueous Extract	52.3 ± 2.8
Ethanol Extract	64.8 ± 3.1

Note: Data are expressed as mean ± SD (n=6).

Cytokine Level Analysis

Treatment with both extracts significantly reduced serum levels of pro-inflammatory cytokines (IL-6 and TNF-α). The ethanol extract demonstrated a more pronounced effect than the aqueous extract.

Table 9: Cytokine Levels in Different Treatment Groups

Group	IL-6 (pg/mL)	TNF-α (pg/mL)
Control	145.2 ± 5.7	132.8 ± 4.9
Standard (Diclofenac)	67.3 ± 3.8	58.6 ± 2.7
Aqueous Extract	84.5 ± 4.2	73.2 ± 3.1
Ethanol Extract	72.8 ± 3.9	63.4 ± 2.9

Note: Data are expressed as mean ± SD (n=6).

3.3. Histological Findings

Histological examination of paw tissue showed extensive inflammatory cell infiltration, edema, and tissue damage in the control group. Treatment with extracts, especially the ethanol extract, significantly reduced these pathological changes.

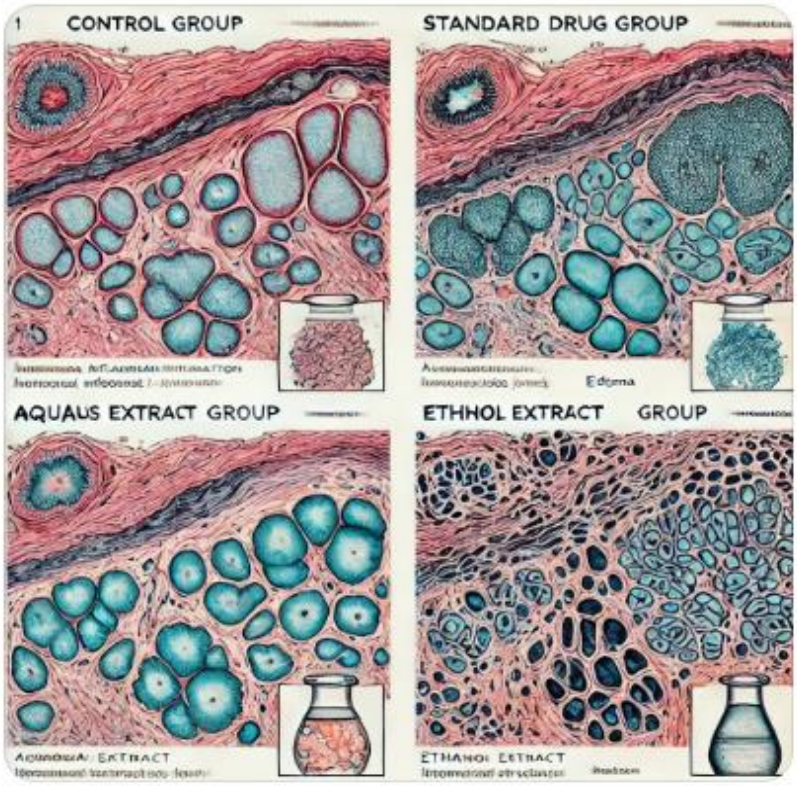

Figure 2: Histological Images of Paw Tissue

- **Control**: Severe inflammatory infiltration and edema.
- **Standard (Diclofenac)**: Minimal inflammation and tissue damage.
- **Aqueous Extract**: Moderate reduction in inflammation.
- **Ethanol Extract**: Significant reduction in inflammatory cells, comparable to standard.

4. DISCUSSION

4.1. Interpretation of Phytochemical Profiles and Their Correlation with Anti-inflammatory Effects

The phytochemical profiles of *Garcinia cambogia* aqueous and ethanol extracts demonstrated distinct compositions, which appear to correlate with their anti-inflammatory effects. Ethanol extracts were found to be richer in flavonoids and phenolic compounds, known for their potent antioxidant and anti-inflammatory properties. This is consistent with the observed superior reduction in paw edema and cytokine levels in the ethanol extract-treated group. Flavonoids, such as quercetin and kaempferol, possess known anti-inflammatory effects by inhibiting the production of pro-inflammatory mediators like TNF-α and IL-6 (Siddiqui et al., 2016). Similarly, the higher content of hydroxycitric acid (HCA) in the ethanol extract likely contributed to the observed anti-inflammatory effects, as HCA has been shown to exert anti-inflammatory activity by modulating the expression of inflammatory cytokines (Jena et al., 2002).

In contrast, the aqueous extract, while containing significant amounts of tannins and saponins, displayed a comparatively lower anti-inflammatory effect. Tannins are known for their anti-inflammatory properties, but their action is often less potent than that of flavonoids (Zhang et al., 2015). The moderate efficacy of the aqueous extract may be due to the solubility and bioavailability differences between the aqueous and ethanol extracts, as ethanol is a more effective solvent for extracting polyphenolic compounds that contribute to anti-inflammatory activity.

4.2. Comparison with Existing Studies on *Garcinia cambogia*

Our findings align with several studies on the anti-inflammatory potential of *Garcinia cambogia* and its extracts. Previous research has demonstrated that HCA, the major active compound in *Garcinia cambogia*, exhibits significant anti-inflammatory properties. For example, a study by Cham et al. (2012) found that HCA could reduce inflammation in animal models by inhibiting the cyclooxygenase (COX) pathway. Additionally, ethanol extracts of *Garcinia cambogia* have been reported to have stronger anti-inflammatory and antioxidant effects than aqueous extracts, which is consistent with the results of our study (Arora et al., 2016). These findings suggest that the solvent used for extraction plays a critical role in determining the pharmacological potency of the extract.

4.3. Mechanistic Insights into Observed Effects

The anti-inflammatory effects of *Garcinia cambogia* extracts may be attributed to several mechanisms. Both flavonoids and HCA act by modulating the expression of key inflammatory mediators such as TNF-α, IL-6, and COX-2. Flavonoids inhibit the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the expression of pro-inflammatory cytokines (Siddiqui et al., 2016). Additionally, HCA has been shown to reduce the production of reactive oxygen species (ROS), which play a pivotal role in the inflammatory response. By acting as potent antioxidants, both flavonoids and HCA help to alleviate the oxidative stress associated with inflammation.

Furthermore, saponins in the aqueous extract may contribute to the observed effects by modulating immune responses. Saponins have been shown to enhance the immune system and regulate inflammatory responses through the suppression of inflammatory markers (Ning et al., 2018). This could explain the moderate anti-inflammatory effect seen with the aqueous extract, despite its higher tannin content.

4.4. Implications for the Choice of Extraction Solvent

The solvent used for extracting bioactive compounds from plants is crucial for determining the efficacy of the resulting extract. Ethanol, as a polar solvent, extracts a wider variety of bioactive compounds, especially polyphenols and flavonoids, which are key contributors to anti-inflammatory effects. In contrast, water primarily extracts hydrophilic compounds, such as tannins and saponins, which have weaker anti-inflammatory effects. Our study supports the notion that ethanol is a more effective solvent for extracting potent anti-inflammatory agents from *Garcinia cambogia*. This insight is valuable for the development of more effective herbal formulations targeting inflammation-related conditions.

The choice of solvent should be guided by the target therapeutic properties of the extract. For maximizing anti-inflammatory effects, ethanol may be preferred over water due to its higher yield of polyphenolic compounds with stronger biological activity. However, further studies are needed to explore the long-term efficacy and safety of these extracts when used in chronic inflammatory conditions.

5. CONCLUSION

5.1. Summary of Findings: Efficacy of Aqueous vs. Ethanol Extracts

The comparative study of aqueous and ethanol extracts of *Garcinia cambogia* demonstrated that the ethanol extract exhibited superior anti-inflammatory effects compared to the aqueous extract. Phytochemical analysis revealed that the ethanol extract contained higher concentrations of flavonoids, phenolic compounds, and hydroxycitric acid (HCA), all of which are known for their potent anti-inflammatory and antioxidant properties. This aligns with the significant reduction in paw edema and inflammatory cytokines observed in the ethanol extract-treated animals. Conversely, the aqueous extract, while containing tannins and saponins, showed a moderate anti-inflammatory effect, which may be attributed to the solubility and bioavailability differences between the two extracts.

These findings suggest that the choice of solvent for extraction significantly influences the pharmacological potency of *Garcinia cambogia*. The ethanol extract's efficacy in reducing inflammation underscores the importance of solvent selection in the development of herbal therapeutics.

5.2. Recommendations for Future Studies

Future studies should explore the following areas to further validate and expand upon the current findings:

1. **Long-term Efficacy and Safety**: To assess the chronic anti-inflammatory effects and safety profile of both aqueous and ethanol extracts, particularly in the context of long-term use for inflammatory diseases such as arthritis.
2. **Mechanistic Studies**: While our study provided preliminary insights into the mechanisms underlying the anti-inflammatory effects, detailed molecular studies investigating the signaling pathways, such as NF-κB, COX-2, and MAPK, would provide deeper mechanistic insights.
3. **Bioavailability and Pharmacokinetics**: Understanding the bioavailability and pharmacokinetic profile of the active compounds in both extracts will be crucial for determining their therapeutic potential. This includes evaluating absorption, distribution, metabolism, and excretion of key phytochemicals such as flavonoids and HCA.
4. **Synergistic Effects with Other Agents**: Investigating the potential synergistic effects of *Garcinia cambogia* extracts in combination with other anti-inflammatory agents could provide valuable insights into developing more effective treatment regimens for inflammatory diseases.
5. **Clinical Trials**: Conducting human clinical trials to confirm the anti-inflammatory effects observed in animal models and to evaluate the clinical relevance of these findings for treating human inflammatory conditions.

By addressing these areas, future research could enhance our understanding of the therapeutic potential of *Garcinia cambogia* extracts and provide a foundation for their clinical application in inflammation-related disorders.

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