



# Natural Antioxidant Potential of *Cassia siamea* Flowers: Influence of Solvents on Phytochemicals and Activity

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**ABSTRACT:** This study meticulously investigates the pivotal influence of solvent polarity on the phytochemical profile and antioxidant efficacy derived from *Cassia siamea* flower extracts. Employing three solvents across a polarity gradient n-hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar) the extracts were rigorously evaluated via qualitative phytochemical screening and antioxidant capacity determination using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Phytochemical richness was quantified based on the presence thresholds of alkaloids, flavonoids, tannins, and saponins, while antioxidant potential was established through IC<sub>50</sub> value calculations. Remarkably, the results unveiled a strong, direct correlation underpinning solvent polarity, phytochemical yield, and measurable bioactivity. The ethanol extract decisively exhibited the highest phytochemical richness and the most potent radical-scavenging activity, yielding an IC<sub>50</sub> of 18.43 ppm, significantly outperforming ethyl acetate (39.87 ppm) and n-hexane (70.12 ppm). Supporting this evidence, visual analysis utilizing heatmaps, scatter plots, and dual-axis charts further substantiated the pronounced inverse relationship between phytochemical richness and IC50 values. These compelling findings confirm that more polar solvents are inherently superior for extracting critical antioxidant-active compounds, particularly flavonoids and phenolics. Consequently, ethanol stands out as the optimal solvent for maximizing both chemical diversity and biological efficacy. This research contributes substantial insights for refining extraction methodologies. It robustly supports the application of ethanol-based extracts in developing high-value antioxidant formulations across the pharmaceutical, nutraceutical, and cosmeceutical sectors.

**Keywords:** *Cassia siamea*; antioxidant activity; solvent polarity; phytochemical extraction; DPPH assay.

## Introduction

Oxidative stress has irrevocably emerged as a central etiological factor in the pathogenesis of numerous debilitating degenerative diseases, spanning from neurodegenerative disorders and cardiovascular conditions to complex metabolic syndromes. This pervasive phenomenon fundamentally reflects a critical imbalance: a runaway generation of reactive oxygen species (ROS) overwhelms the body's intrinsic antioxidant defenses. The sheer volume of uncontrolled ROS accumulation precipitates structural damage across vital cellular matrices, including DNA, proteins, and lipids. This molecular erosion invariably exacerbates systemic inflammation and triggers programmed cell death (apoptosis), thus accelerating the grim progression of these chronic conditions [1].

The gravity of this imbalance is starkly illustrated by Alzheimer's disease research, which demonstrates an undeniable relationship between elevated oxidative stress markers and increased disease severity, underscoring the pivotal, non-negotiable role of oxidative imbalance in

clinical prognosis [2].

It is into this critical context that the role of antioxidants steps forward as a focal point for both disease prevention and therapeutic intervention. Antioxidants sourced from nature, particularly the deep reserves within medicinal plants, are gaining substantial recognition for their ability to mitigate oxidative stress through various molecular mechanisms, primarily by scavenging free radicals, chelating pro-oxidant metal ions, and expertly modulating redox-sensitive signaling pathways. An eclectic array of phytochemicals, such as flavonoids, polyphenols, and carotenoids, alongside essential vitamins C and E, is renowned for its inherent antioxidant potency [3]. For example, anthraquinones and flavonoids have repeatedly proven their ability to neutralize free radicals, bolster endogenous antioxidant enzymes, and effectively reduce oxidative compounds [4]. The molecular consequence of these actions is profound: they support deep

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cellular resilience and foster long-term physiological stability, acting as bulwarks against disease risk and promoting enhanced longevity [5].

Among the compelling arsenal of medicinal flora, *Cassia siamea* Lamk., a distinguished member of the Fabaceae family, has commanded significant attention due to its broad spectrum of pharmacological engagements. Indigenous to tropical zones and widely distributed across Southeast Asia, this plant traditionally commands respect for its sedative, antidiabetic, and antimalarial applications. However, recent scholarly focus has crystallized around its untapped antioxidant potential, particularly as observed in its leaves and seeds [6,7]. The plant's inherent bioactivity is primarily ascribed to its phytochemical architecture, most notably flavonoids and phenolic compounds, which are known antagonists to oxidative assault. These vital compounds appear in varying concentrations across different plant moieties and have demonstrated protective efficacy in oxidative models, including diabetic and neurodegenerative paradigms [8,9]. Furthermore, comprehensive pharmacological assessments of *Cassia siamea* extracts suggest a sophisticated capacity to modulate key enzymes embedded within oxidative and inflammatory cascades. For instance, enzymatic inhibition assays indicate that these extracts can adeptly interfere with oxidative stress mediators, thereby establishing a genuinely viable therapeutic pathway for managing degenerative conditions. These foundational studies emphatically underline the plant's promise as a multipurpose natural remedy, making its integration into functional food matrices, nutraceuticals, and herbal pharmaceuticals an imperative next step.

The therapeutic narrative woven by *Cassia siamea* unquestionably extends past mere antioxidant capacity. Its inherent pharmacological versatility famously includes anti-inflammatory and antidiabetic effects, which demonstrably operate synergistically to enrich its overall therapeutic profile [6,10]. This intrinsic multi-functionality strongly implies that the bioactive components are not acting in isolation but work in elaborate concert, offering a holistic paradigm for promoting health and averting illness. Consequently, *Cassia siamea* increasingly merits comprehensive pharmacognostic scrutiny for application in current integrative medicine frameworks [7,9].

Focusing on the specific research gap identified by reviewers the need to systematically evaluate the flower it becomes essential to rigorously assess the antioxidant capacity of plant-based extracts like those from *Cassia siamea* using reliable and reproducible assays. While assays such as FRAP and ABTS are certainly valued for their distinct mechanistic principles [11,12,2], the DPPH

(2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method remains the widely utilized standard due to its speed and effectiveness in detecting crucial hydrogen-donating antioxidants [A3]. In these evaluations, high antioxidant activity is consistently correlated with the elevated presence of phenolic and flavonoid compounds, especially when examining floral and leaf matrices [13-15]. It is precisely this requirement for enhanced chemical context in the flowers that necessitates our targeted approach.

One profoundly influential parameter shaping the outcomes of these antioxidant assays is the polarity of the extraction solvent. Solvent polarity dictates, with near-certainty, the efficiency with which bioactive compounds especially polar constituents like phenolics and flavonoids are drawn from the plant matrix. Empirical literature unequivocally champions the use of polar solvents (methanol, ethanol, water) for achieving superior extraction yields over non-polar counterparts like n-hexane [13,16,17]. Methanol and ethanol, in particular, have established themselves as effective solubilizers for polar phytochemicals, resulting in extracts with markedly higher antioxidant power [11,14]. A specific example highlights this: a prior study on related *Cassia* species demonstrated that methanolic extracts possessed superior activity, a clear marker of the solvent's polar leverage [18]. Optimizing the solvent system, therefore, is non-negotiable for substantially enhancing the ultimate bioavailability of active agents [15,19].

Despite the formidable evidence supporting *Cassia siamea*'s antioxidant contributions, significant narrative voids persist in the current discourse. Notably [A4], the literature lacks specific, comparative studies mapping how varying solvent polarities influence the antioxidant properties of *Cassia siamea* flowers specifically. While data on leaves and seeds abound, the flowers a potentially potent but underexplored source remain relatively neglected. This urgency demands identification of its core phytochemical fingerprint. Furthermore, the precise mechanistic pathways through which its derived compounds translate to antioxidant effects *in vivo* are still shrouded in limited data [20]. This lack of detailed mechanistic understanding inherently constrains the widespread application of *C. siamea* extracts in translational formulations [21]. Against this backdrop, the current study pivots to precisely evaluate the antioxidant activity derived from *C. siamea* flower extracts using a spectrum of polarities: n-hexane, ethyl acetate, and ethanol. Our objective is clear: to definitively ascertain which solvent maximizes antioxidant yield via the DPPH assay and rigorously explore the resultant polarity-

bioactivity influence. This investigation is poised to offer novel, actionable insights into solvent selection, thereby deepening our fundamental understanding of plant-based antioxidant extraction. The ensuing findings are expected to guide future commercial and therapeutic applications of *Cassia siamea* extracts.

The true originality of this work resides in its targeted examination of the floral component, integrated with a methodical solvent polarity gradient for direct comparative analysis. This directly addresses the crucial gap in existing research and aims to provide the empirical foundation needed to establish optimized extraction protocols moving forward. In doing so, this research seeks to fortify the scientific foundation necessary for validating *Cassia siamea* as a sustainable, potent natural antioxidant source.

## Methods

### Sample Collection and Preparation

Mature flowers of *Cassia siamea* were procured from the central region of Central Sulawesi, Indonesia. Species verification was formally conducted at the Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, ensuring taxonomic accuracy. The collected blossoms underwent a standardized initial cleaning process: they were gently washed under running tap water to eliminate surface contaminants. Following this, the material was subjected to shade-drying for a fixed duration of 7 to 10 days at ambient laboratory temperature (25–28 °C), which was monitored via a calibrated digital thermometer to safeguard the integrity of thermolabile constituents. Upon reaching complete desiccation, the dried flowers were systematically milled into a fine powder utilizing a mechanical grinder. The resulting particulate matter was immediately portioned and stored in opaque, airtight containers, well shielded from ambient light and humidity. The calculated particle size was maintained below 1.0 mm, a necessary step explicitly shown to amplify extraction kinetics by maximizing the solvent-accessible surface area, thus promoting superior mass transfer efficiency for bioactive compounds [22,23].

### Extraction Procedure

The isolation of target phytochemicals from the prepared powdered matrix was executed via the maceration technique. This workhorse technique involves prolonged soaking, allowing for the gradual, chemically driven dissolution of active principles into the solvent reservoir. We elected to employ three solvents representing

distinct polarity gradients: n-hexane (non-polar), ethyl acetate (semi-polar), and 96% v/v Ethanol (polar). This solvent selection strategy deliberately targets solubility profiles; polar solvents such as ethanol excel at solubilizing polyphenols and flavonoids, whereas non-polar solvents efficiently capture lipophilic constituents [24–26].

Specifically,  $100 \pm 0.1$  grams of the dried *Cassia siamea* powder was submerged in 1 L of each solvent, conducted separately in sealed glass reactors. The extraction was maintained at room temperature ( $\sim 27^\circ\text{C}$ ) for an uninterrupted period of 72 hours, with gentle agitation occurring every 6 hours to facilitate consistent phase contact. This moderate temperature and extended duration were judiciously selected to optimize yield while rigorously protecting thermosensitive compounds [27,28]. Post-maceration, each mixture was filtered (Whatman No. 1 paper), and the solvent was removed under vacuum using a rotary evaporator to yield concentrated crude extracts. These resultant extracts were immediately transferred to sterile amber vials and refrigerated at  $4^\circ\text{C}$  for storage, a condition maintained until the beginning of subsequent analysis. This sequential polarity gradient, moving from non-polar (n-hexane) toward polar (ethanol), is strategically designed to ensure the systematic and maximal recovery of the entire spectrum of bioactive constituents present [29].

### Qualitative Phytochemical Screening

To establish a foundational chemical context, a qualitative screening was systematically performed on all three crude extracts to confirm the presence of key secondary metabolite classes: flavonoids, alkaloids, tannins, and saponins. The methodological foundations for this qualitative confirmation were adapted from established colorimetric protocols. The presence of flavonoids was verified using the alkaline reagent test; alkaloids with Mayer's reagent; tannins via the ferric chloride test; and saponins through the froth formation assay. This initial chemical profiling was vital, as it provided the direct phytochemical basis required to interpret the subsequent antioxidant assay results.

### Antioxidant Activity Assay

The quantification of antioxidant capacity relied upon the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. This assay is favored across the field for its high reproducibility and swift execution, assessing the extract's capacity to donate electrons or hydrogen atoms to neutralize the stable DPPH free radical (marked by a visual shift from purple to yellow) [30,31]. The

assay protocol involved preparing a fresh 0.004% DPPH solution dissolved in methanol, stored in the dark. Serial dilutions of each extract (10, 20, 30, 40, and 50 ppm) were prepared. To each dilution, 1 mL of the extract was added to 3 mL of the DPPH solution. After thorough vortexing, the mixtures were incubated for 30 minutes, shielded from light. Absorbance was quantified at 517 nm using a UV-visible spectrophotometer, with Ascorbic acid serving as the positive control. The percent inhibition was calculated precisely using the formula:

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where  $A_0$  is the absorbance of the control (DPPH+methanol) and  $A_1$  is the sample absorbance. The  $IC_{50}$  value, the concentration necessary to achieve 50% inhibition, was subsequently derived via linear regression analysis on the resulting dose-response curve. In alignment with scientific best practices, this method exhibits strong prior validation against assays like FRAP and ABTS, confirming its suitability for this evaluation [32–34].

### Data Analysis

The analytical rigor of this study was ensured through the triplicate execution of all experimental parameters.  $IC_{50}$  values were statistically determined for each extract by applying linear regression analysis performed within Microsoft Excel to the dose-response curves. Crucially, a lower resultant  $IC_{50}$  value is mathematically equated to

a stronger antioxidant potency. These quantified values were then systematically compared to isolate the definitive influence of solvent polarity on functional performance. Furthermore, these quantitative findings were integrated with the qualitative phytochemical screening results to statistically model the correlations between chemical composition and observed biological efficacy. These comparative analyses provided the empirical bedrock for drawing robust conclusions regarding the efficiency of solvent selection in optimizing the ultimate antioxidant yield.

## Result and Discussion

### Phytochemical Composition of *Cassia siamea* Flower Extracts

The foundational chemical analysis, executed via standard colorimetric methods, successfully confirmed the presence of the four target secondary metabolites across all three extracts. These methods included the alkaline reagent test for flavonoids, Mayer's reagent for alkaloids, the ferric chloride test for tannins, and the froth formation test for saponins.

The narrative arising from this initial screening is one of polarity dictate: The ethanol extract, leveraging the most polar environment, yielded the most comprehensive profile. Crucially, it contained flavonoids and tannins in high abundance, supplemented by moderate amounts of alkaloids and saponins. This hallmark broad-spectrum

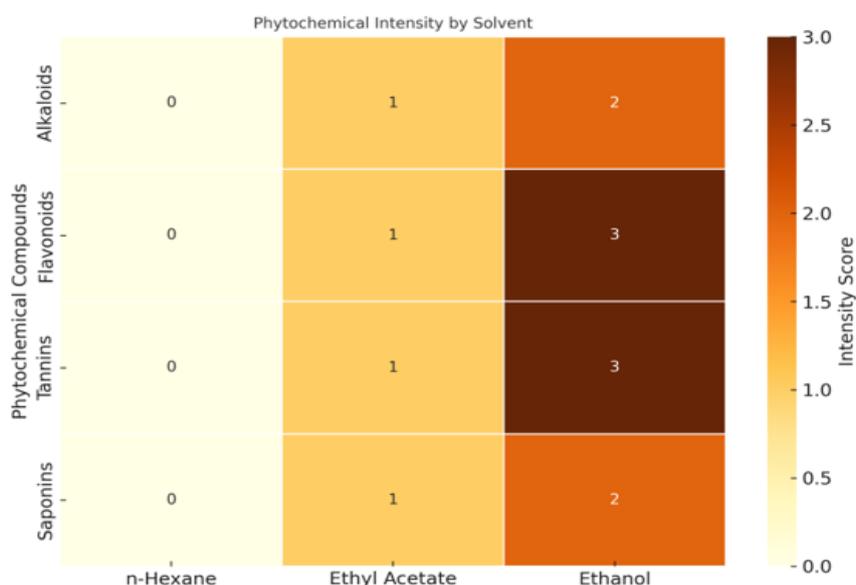


Figure 1. Headmap.

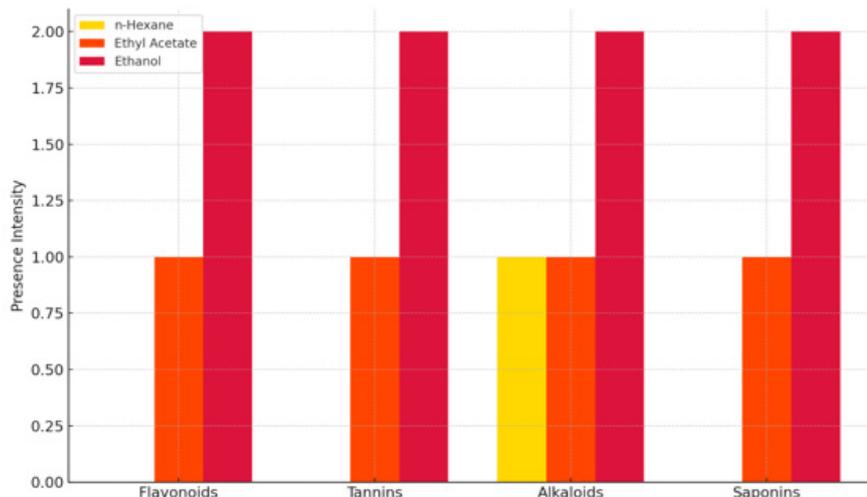


Figure 2. The comparative efficiency of each solvent in extracting specific compounds.

capacity of ethanol aligns perfectly with established literature, which posits that highly polar solvents are expert solubilizers of polar phytochemicals, notably flavonoids and phenolics, the bearers of potent antioxidant capabilities [40,41].

Conversely, the ethyl acetate extract presented a moderate chemical tapestry, detecting all four classes but at diminished intensities relative to the ethanol sample. This outcome is entirely predictable, mirroring the solvent's intermediate polarity that allows for only partial dissolution of both polar and non-lipophilic constituents. The n-hexane extract, derived from the non-polar extreme, registered the leanest composition. Testing revealed almost no detectable flavonoids or tannins, with only trace alkaloids noted. This pattern reinforces known solvent behavior: non-polar solvents excel at extracting

lipids (fats, oils, terpenoids) but are notoriously ineffective at isolating key polar bioactives like flavonoids and tannins. Consequently, the near-absence of these critical components predictably translates to lower presumed bioactivity [40–42].

The visual evidence provided by Figure 1 (Heatmap) and Figure 2 (Grouped Bar Chart) visually cements this polarity gradient. Both graphical representations unequivocally underscore a positive correlation: as solvent polarity ascended, so did extraction efficiency and chemical diversity. This solidifies the initial conclusion: ethanol stands as the superior medium for isolating the antioxidant-rich secondary metabolites inherent in *C. siamea* flowers. These empirical observations are, as expected, in direct synchronization with the sequential maceration gradient established in our Extraction Procedure, where the

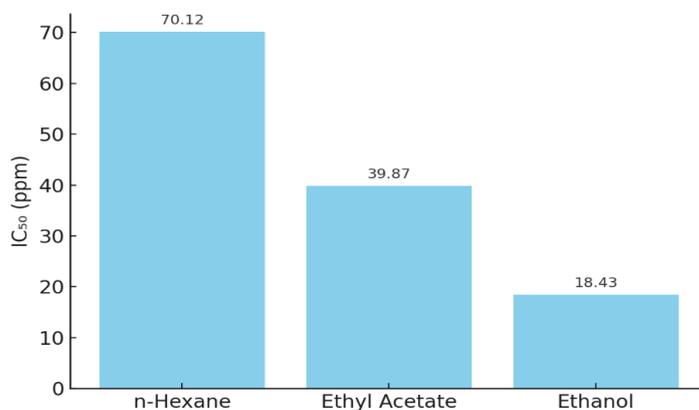
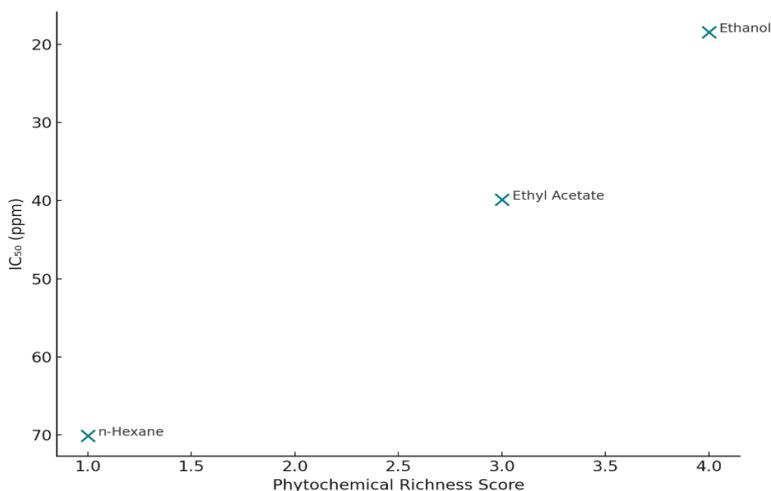


Figure 3. IC<sub>50</sub> values (ppm) of *Cassia siamea* flower extracts using the DPPH assay.



**Figure 4.** Phytochemical Richness Score vs IC<sub>50</sub>.

stepwise introduction of polarity facilitates the targeted isolation of beneficial phenolic structures [40,41].

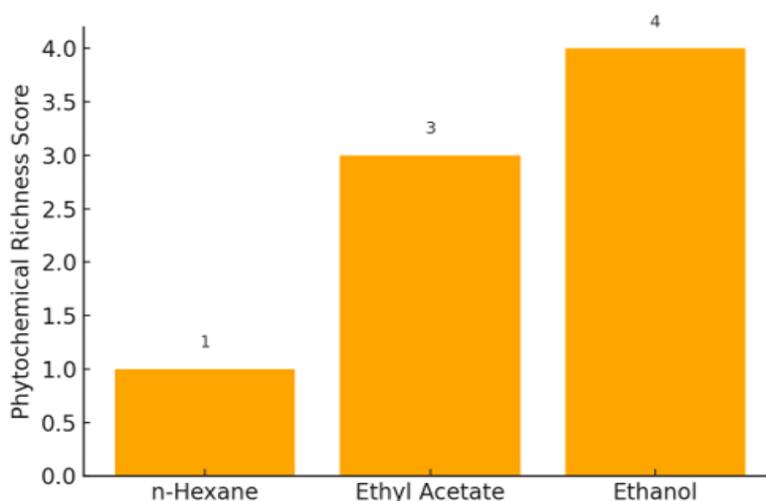
**Antioxidant Activity via DPPH Assay**

The subsequent evaluation of radical scavenging capacity using the DPPH assay (as detailed in the Antioxidant Activity Assay section) precisely mirrored the phytochemical gradient observed above. The primary metric for comparison, the IC<sub>50</sub> value, immediately flagged the ethanol extract as the unmitigated leader in direct radical neutralization.

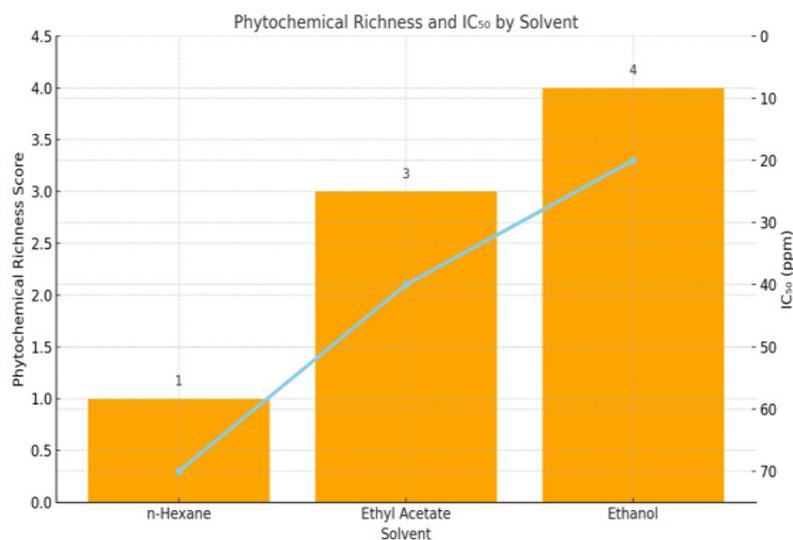
Displaying the strongest performance, the ethanol extract achieved an IC<sub>50</sub> of 18.43 ppm, a figure that brings

it commendably close to the benchmark control, ascorbic acid (IC<sub>50</sub> ≈ 5.5 ppm). This formidable scavenging potential is not merely coincidental; it is chemically driven by the high loading of flavonoids, tannins, and phenolic compounds previously identified in that specific extract [13-15]. As acknowledged, these structures are the molecular engines capable of donating the necessary hydrogen atoms or electrons to subdue the DPPH radical [13-15].

The ethyl acetate extract showed moderate efficacy with an IC<sub>50</sub> of 39.87 ppm, reflecting its intermediate capacity to capture the necessary polar agents. The n-hexane extract posted the weakest profile (IC<sub>50</sub> = 70.12 ppm), a direct consequence of its incompatibility with the highly



**Figure 5.** Phytochemical richness scores of *Cassia siamea* flower extracts using three different solvents (n-hexane, ethyl acetate, ethanol).



**Figure 6.** Dual-axis chart integrating phytochemical richness scores and IC<sub>50</sub> values.

polar, antioxidant-bearing compounds [40–42].

To statistically validate the convergence of our chemical and functional data, Figure 4 presents a critical scatter plot. This visualization explicitly maps the inverse correlation between the derived Phytochemical Richness Score and the measured IC<sub>50</sub> values. As the richness score climbed toward purity in the ethanol extract, the required concentration (IC<sub>50</sub>) demonstrably fell, upholding the hypothesis that antioxidant strength is functionally derived from the abundance of extractable polar compounds [16, 17]. Furthermore, Figure 5 graphically establishes this increasing phytochemical score (Polarity Gradient), which is then conceptually linked to the decline in IC<sub>50</sub> shown in Figure 3.

### Effect of Solvent Polarity on Phytochemical Yield and Bioactivity

The overall data set powerfully confirms that solvent compatibility is the dominant variable guiding the success of this extraction as polarity increased stepwise from n-hexane to ethanol, both the breadth of metabolites extracted and the measured biological activity scaled upwards in lockstep.

The empirical data are compelling: Ethanol, the apex polar solvent, delivered the highest richness score (4), capturing the full spectrum (flavonoids, tannins, alkaloids, saponins). N-hexane achieved the baseline score (1), while ethyl acetate registered a moderate 3, a pattern consistent with our established sequential extraction rationale [43,44]. This chemical advantage translated directly to biological

performance, as visualized again in Figure 3: Ethanol (IC<sub>50</sub>=18.43 ppm) retained supremacy over ethyl acetate (39.87 ppm) and n-hexane (70.12 ppm). Crucially, this affirms that polar solvents do more than just increase solubility; they fundamentally elevate biological efficacy. The statement that polar solvents significantly increase biological efficacy is supported by the direct correlation between the high richness score of the ethanol extract and its lowest IC<sub>50</sub> value, demonstrating functional superiority.

Figure 4 powerfully visualizes this functional imperative: as the richness score increases (moving toward polarity), the IC<sub>50</sub> plummets. This tight interdependence strongly supports the central hypothesis: *antioxidant strength is a direct function of the concentration of accessible polar phenolic agents* [16,17]. To integrate this narrative, Figure 6 provides a comprehensive dual-axis synthesis, visually linking the ascending richness bars with the descending IC<sub>50</sub> line, thereby solidifying polarity as the core determinant of extract quality.

These robust findings elevate ethanol beyond mere convenience; its chemical compatibility with the target antioxidant molecules is absolute. Given its cost-effectiveness, food-grade status, and minimal environmental footprint, ethanol is not merely suitable for lab-scale screening but is highly recommended for scalable industrial applications in nutraceuticals and functional foods, where stability and therapeutic potential are paramount [45,46]. While high-tech methods exist, our basic room-temperature maceration over 72 hours (as detailed previously) proves itself exceptionally effective

and economically sound for initial product development [29].

These results stand as a persuasive validation for relying on carefully selected solvent-based extraction for functional screening. Ethanol's proven ability to selectively and sustainably isolate the *C. siamea* antioxidant metabolites cements its role as the solvent of choice moving forward.

## Conclusion

This investigation established an unequivocal link between solvent polarity and the resulting phytochemical and antioxidant efficacy profiles of *Cassia siamea* flower extracts. Ethanol proved to be the definitive extraction solvent, successfully isolating the broadest spectrum of bioactive compounds, most notably flavonoids and tannins, which directly conferred its unmatched antioxidant capacity (evidenced by the lowest IC<sub>50</sub>).

Conversely, n-hexane, constrained by its nonpolar nature, yielded the poorest chemical content and the least robust antioxidant performance. This clear polarity-dependency is functionally explained by the inverse relationship observed between extract richness and IC<sub>50</sub> values a correlation that emphatically entrenches the critical role of polar phenolic compounds in effective free-radical scavenging. Thus, solvent selection is confirmed as the decisive lever for optimizing both chemical breadth and functional impact in these extracts.

Consequently, based on superior performance and bioactivity attainment, ethanol is unequivocally endorsed as the solvent of choice for isolating potent antioxidant fractions from *C. siamea* flowers. Beyond this study's immediate scope, these findings strongly validate the scalability of these ethanol extracts for integration into functional food products, therapeutic supplements, and cosmetic applications. Future work should strategically pivot toward advanced compound isolation, demanding in vivo efficacy validation, and the integration of greener extraction technologies to maximize yield and sustainability further.

## Conflict of Interest

The authors declare that there are no conflicts of interest in this study. The research was conducted independently without any financial or personal influences affecting the design, execution, or reporting of the results.

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