Antioxidant, Anti-Inflammatory, and Inhibition of Acetylcholinesterase Potentials of *Cassia timoriensis* DC. Flowers

Maram B. Alhawarri 1, Roza Dianita 1,* 1, Khairul Niza Abd Razak 1, Suriani Mohamad 1,2, Toshihiko Nogawa 2,3,* and Habibah A. Wahab 1,2,*

1 School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia; maram.alhawarri@gmail.com (M.B.A.); niza@usm.my (K.N.A.R.); suriani@usm.my (S.M.)
2 USM-RIKEN Centre for Aging Science (URICAS), Universiti Sains Malaysia, Minden 11800, Penang, Malaysia; nogawat@riken.jp
3 Chemical Biology Research Group, RIKEN Centre for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
* Correspondence: rozadianita@gmail.com (R.D.); habibahw@usm.my (H.A.W.); Tel.: +60-46577888 (R.D. & H.A.W.)

Abstract: Despite being widely used traditionally as a general tonic, especially in South East Asia, scientific research on *Cassia timoriensis*, remains scarce. In this study, the aim was to evaluate the in vitro activities for acetylcholinesterase (AChE) inhibitory potential, radical scavenging ability, and the anti-inflammatory properties of different extracts of *C. timoriensis* flowers using Ellman’s assay, a DPPH assay, and an albumin denaturation assay, respectively. With the exception of the acetylcholinesterase activity, to the best of our knowledge, these activities were reported for the first time for *C. timoriensis* flowers. The phytochemical analysis confirmed the existence of tannins, flavonoids, saponins, terpenoids, and steroids in the *C. timoriensis* flower extracts. The ethyl acetate extract possessed the highest phenolic and flavonoid contents (527.43 ± 5.83 mg GAE/g DW and 851.83 ± 10.08 mg QE/g DW, respectively) as compared to the other extracts. In addition, the ethyl acetate and methanol extracts exhibited the highest antioxidant (IC$_{50}$ 20.12 ± 0.12 and 34.48 ± 0.07 µg/mL, respectively), anti-inflammatory (92.50 ± 1.38 and 92.22 ± 1.09, respectively), and anti-AChE (IC$_{50}$ 6.91 ± 0.38 and 6.40 ± 0.27 µg/mL, respectively) activities. These results suggest that ethyl acetate and methanol extracts may contain bioactive compounds that can control neurodegenerative disorders, including Alzheimer’s disease, through high antioxidant, anti-inflammatory, and anti-AChE activities.

Keywords: *Cassia timoriensis*; antioxidant; anti-inflammatory; acetylcholinesterase; Alzheimer

1. Introduction

Plants provide a significant source of bioactive compounds, such as phenolics, terpenoids, essential oils, sterols, alkaloids, polysaccharides, tannins, and anthocyanins [1]. Investigation of the biological activities of medicinal plants, particularly antioxidants, has attracted considerable interest. The antioxidant property of medicinal plant products has been shown to be primarily attributable to the phytochemical groups mentioned above [2]. These natural antioxidants prevent the destructive effects induced by oxidative damage of the free reactive oxygen species (ROS) and reactive nitrogen species (RNS) implicated in neurodegenerative diseases, such as AD [3].

Between 1981 and 2019, approximately 50% of all drugs approved worldwide were produced using or inspired by natural products [4]. The known cholinesterase inhibitor rivastigmine, used for Alzheimer’s disease (AD) treatment, is an example of a semi-synthetic drug developed based on the naturally occurring cholinesterase inhibitor physostigmine scaffold [5]. Physostigmine, an alkaloid isolated from *Physostigma venenosum*, is administered for glaucoma and myasthenia gravis treatment, but its use for AD treatment...
is restricted in certain countries due to the serious hepatic and cardiac side effects [6,7]. Nonetheless, galantamine (Figure 1), a pure natural product isolated from the bulbs and flowers of Galanthus caucasicus and Galanthus woronowii, is currently available on the market for the treatment of cognitive decline in mild to moderate AD [8,9].

![Chemical structures of rivastigmine, physostigmine, and galantamine](image)

*Cassia* is a huge genus of around 600 species of flowering trees and shrubs [10] belonging to the Leguminosae family, which comprise more than 600 genera and 18,000 species [11]. This plant family is predominantly distributed across tropical to subtropical Asian areas [12]. Cassia timoriensis DC. is a perennial tree or shrub, usually about 2–6 m tall. The plant is widely spread in tropical areas, particularly in South East Asian countries such as India, Sri Lanka, Thailand, Malaysia, and Indonesia [13]. A flowering plant with yellow blooms and shiny brown seedpods, *C. timoriensis* is also sometimes valued as an ornamental plant [14]. Traditionally, this *Cassia* species is used for treating toxins, scabies, itching, and skin diseases and as an anthelmintic medicine [13,15]. It is also used as a general tonic, antitumor, and for blood disorders, particularly its heartwood component, which is commonly used for menstrual blood disorder [13–15]. Despite its wide range of traditional uses, *C. timoriensis* has hardly been studied for its phytochemical constituents and biological activities. The first compound identified from this plant was barakol, discovered by a Thai group in 1984 [16]. Two decades later, in a screening of 20 Thai medicinal plants, an aqueous extract of *C. timoriensis* demonstrated powerful antioxidant activity through the inhibition of Heinz bodies induction [15]. Recently, after two decades of antioxidant activity study, our research group reported that *C. timoriensis* demonstrated the highest (94–97%) inhibition towards acetylcholinesterase (AChE) in a screening study for anti-cholinesterase activity of 17 methanol extracts from different parts of five *Cassia* species. Of the six isolated compounds, 3-methoxyquercetine from *C. timoriensis* leaves extract showed moderate inhibition towards AChE (IC₅₀: 83.71 μM) [17]. Therefore, as a continuation of our research on biological and chemical evaluations of *C. timoriensis*, qualitative and quantitative phytochemical analyses were carried out together with in vitro studies for the acetylcholinesterase inhibitory potential, radical scavenging ability, and anti-inflammatory activity of different extracts of *C. timoriensis* flowers.

### 2. Results and Discussion
#### 2.1. Phytochemical Screening of Cassia timoriensis Flowers

The phytochemical content of different extracts of *C. timoriensis* flowers was screened using standard, established protocols [18–20]. The screening included various secondary metabolite classes such as alkaloids, phenolics (flavonoids, coumarins, and quinones), tannins, saponins, glycosides (cardiac and anthraquinones glycosides), steroids, and terpenoids, as well as two primary metabolites (proteins and carbohydrates). The results revealed the presence of flavonoids, tannins, coumarins, steroids, and terpenoids in all extracts except for the aqueous extract. All extracts gave a negative indication for the presence of alkaloids, while anthraquinone glycosides were only detected in the ethyl acetate extract. This result provided an early indication for the probable presence of various interesting secondary metabolites in the ethyl acetate extract (Table 1).
Table 1. Screening of phytochemical content of four different extracts of Cassia timoriensis flower.

| No. | Class               | Test                          | HE | EE | ME | AE |
|-----|---------------------|-------------------------------|----|----|----|----|
| 1   | Alkaloids           | Mayer’s test                  | -  | -  | -  | -  |
|     |                     | Wagner’s test                 | -  | -  | -  | -  |
|     |                     | Dragendorff’s test            | -  | -  | -  | -  |
| 2   | Flavonoids          | Alkaline reagent test         | +  | +  | +  | -  |
|     |                     | Zn/HCL reduction test         | +  | +  | +  | -  |
| 3   | Tannins             | Ferric chloride test          | +  | +  | +  | +  |
| 4   | Saponins            | Frothing test                 | -  | -  | -  | +  |
| 5   | Cardiac glycosides  | Keller–Killiani test          | -  | -  | -  | -  |
| 6   | Anthraquinones glycoside | Borntrager’s test      | -  | +  | -  | -  |
| 7   | Steroids            | Liebermann–Burchard test      | +  | +  | +  | -  |
|     |                     | Salkowski test                | +  | +  | +  | +  |
| 8   | Terpenoids          | Modified Salkowski test       | +  | -  | +  | -  |
| 9   | Coumarins           | -                             | +  | +  | -  | -  |
| 10  | Quinones            | -                             | -  | -  | -  | -  |
| 11  | Proteins            | Millon’s test                 | -  | +  | +  | +  |
| 12  | Carbohydrates       | Benedict’s test (reducing sugar) | -  | +  | +  | +  |

(*) indicates the presence of a compound class, (-) indicates the absence of a compound class. HE: hexane extract; EE: ethyl acetate extract; ME: methanol extract; AE: aqueous extract.

2.2. Antioxidant Capacity of Cassia timoriensis Flower Extracts

Plants are known as a natural source for antioxidants. Phenolic compounds such as phenolic acids, flavonoids, coumarins, and tannins are said to be the main compounds responsible for such activity [21]. In this study, the antioxidant capacity of different extracts of \textit{C. timoriensis} was evaluated based on three parameters: total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Total phenolic content was determined based on the Folin–Ciocalteu method and is expressed as mg gallic acid equivalent per gram of dry weight extract (mg GAE/g DW) [22]. The Folin–Ciocalteu assay is convenient, simple, precise, and reproducible and is based on the oxidation–reduction reaction involving single electron transfer (SET) from phenolic compounds to molybdenum reagent in an alkaline medium. It turns the solution from yellow to a blue complex of reduced molybdenum that can be detected spectrophotometrically at 750–765 nm. On the other hand, total flavonoid content (TFC), expressed as mg quercetin equivalent per gram of dry weight extract (mg QE/g DW), was assessed using an aluminum chloride-based colorimetric assay [23], and the scavenging ability of the extracts towards radical DPPH was also evaluated to establish their in vitro antioxidant activity [24]. Decolorization of the purple solution of DPPH to yellow indicates the reduction of DPPH following the interaction of radical DPPH with antioxidant compounds in the extract.

In general, all extracts of the \textit{C. timoriensis} flower showed relatively high phenolic and flavonoid contents. The highest TPC and TFC values were observed in the ethyl acetate extract, followed by the methanol and hexane extracts (Table 2). The lowest TFC and TPC values were found in the aqueous extract of \textit{C. timoriensis}. This finding is in line with the phytochemical screening result, where most flavonoid and phenolic metabolites were distributed in the ethyl acetate, methanol, and hexane extracts. Similar
results were reported by Kolar et al., (2018), where the flower extracts of *Cassia auriculata*, *Cassia italica*, *Cassia siamea*, and *Cassia uniflora* showed high phenolic and flavonoid contents as compared to the other parts, such as pod, stem, and leaf [25]. Although phytochemical investigations of *C. timoriensis* are still scarce, the phytochemicals of other *Cassia* species are well studied and documented [26]. Juan-Badaturuge et al. identified kaempferol-3-O-rutinoside, kaempferol, quercetin, and luteolin in *C. auriculate* as potent antioxidants through activity-guided fractionation and isolation [27]. Kaempferol and luteolin have also been isolated from *C. alata* and *C. fistula* and displayed a strong DPPH scavenging activity [28–30]. On the other hand, anthraquinones were found as the major phenolic compounds present in *Cassia* species [31], while emodin, aloë-emodin, rhein, and chrysophanol are widely distributed in *Cassia* species such as *C. fiora* [32], *C. roxburghii* [33], *C. alata* [34,35], *C. obtuse* [36], *C. siamea* [37], and *C. angustifolia* [38]. All of these previous findings are in good agreement with our present study that demonstrates *C. timoriensis* extract to possess high contents of flavonoids and phenolic compounds.

Table 2. Total phenolic content, total flavonoid content, and antioxidant activity of *Cassia timoriensis* flower extracts.

| Sample       | TPC mg GAE/g DW | TFC mg QE/g DW | Antioxidant Activity (DPPH Assay) | IC<sub>50</sub> (µg/mL) |
|--------------|-----------------|----------------|----------------------------------|-------------------------|
| n-Hexane extract | 136.36 ± 9.58   | 300.58 ± 10.78 | 45.18 ± 0.51                      | 54.08 ± 0.78           |
| Ethyl acetate extract | 527.43 ± 5.83   | 851.83 ± 10.08 | 97.80 ± 0.29                      | 20.12 ± 0.12           |
| Methanol extract      | 321.75 ± 11.33  | 493.92 ± 9.27  | 71.74 ± 0.39                      | 34.48 ± 0.07           |
| Aqueous extract       | 31.05 ± 7.94    | 61.83 ± 9.10   | 12.18 ± 2.58                      | -                      |
| Ascorbic acid        | -               | -              | 98.73 ± 0.25                      | 20.22 ± 0.03           |

Data are presented as mean ± SD, with n = 3; *% Inhibition was measured at a final concentration of 50 µg/mL.

The extracts of *C. timoriensis* flowers were then evaluated for their antioxidant activity. Among all of the extracts, the ethyl acetate extract possessed the highest antioxidant activity with IC<sub>50</sub> 20.12 ± 0.12 µg/mL, followed by methanol, hexane, and aqueous extracts. However, the activity of the aqueous extract at 50 µg/mL was very low (<25% inhibition). Thus, the IC<sub>50</sub> was not determined. The ability of the ethyl acetate extract to inhibit DPPH oxidation is comparable to the positive control, ascorbic acid, with similar percentages of inhibition at 50 µg/mL and IC<sub>50</sub> values. It is postulated that the diverse phenolic content of the ethyl acetate extract may provide a wide range of proton-donating compounds that act as potent antioxidant agents via free radical inhibition or scavenger mechanisms. The results also showed a positive correlation between the TPC/TFC of the extracts and their radical DPPH scavenging activity (*R<sup>2</sup>* values are 0.986 and 0.934, respectively) (Figure 2). This implies that the higher the phenolic and flavonoid compound contents are in the extract, the stronger the antioxidant activity displayed by the extract is. In addition, many studies have postulated the antioxidant mechanisms of flavonoid- or phenolic-rich plants [39–41]. The antioxidant behavior of phenolic compounds might be due to the activity of hydroxyl or electron-donating agents in stabilizing and delocalizing the unpaired electron and their transition metal-chelating potential, especially with iron and copper [21]. The oxidation–reduction potential of these compound classes depends on the number and arrangement of the hydroxyl groups in the structure and also the replacement of the hydroxyl-contributing groups with other groups such as glycosides [21]. The findings from the DPPH scavenger assay supported the importance of the -OH group of phenolic compounds in the electron transfer reaction that is responsible for the antioxidant activity.
groups such as glycosides [21]. The findings from the DPPH scavenger assay supported the presence of high levels of radical oxygen species (ROS) and radical nitrogen species (RNS). The presence of a high level of ROS and RNS is linked to many chronic immunoinflammatory and degenerative diseases [40,52–54]. The radical scavenging ability of the phenolic compounds is postulated to play an important role in reducing oxidative stress in the body due to the presence of high levels of radical oxygen species (ROS) and radical nitrogen species (RNS). The presence of a high level of ROS and RNS is linked to many chronic immunoinflammatory and degenerative diseases [40,52–54]. Thus, the strong antioxidant potential of *C. timoriensis* correlates well with its traditional use as a general tonic as well as with alleviating body toxins [13]. *C. timoriensis* may aid in maintaining human well-being and preventing cell damage due to oxidative stress.

2.3. Anti-Inflammatory Activity of Cassia timoriensis Flower Extracts

Inflammation is one of the body’s defense mechanisms. One of the first indicators of the inflammatory process is the denaturation of cellular proteins following tissue or cell injury, in which a series of pro-inflammatory mediators (TNF-α, interleukins, NF-κB, nitric oxide, and prostaglandins) and radical species (ROS or RNS) are released [53]. Chronic inflammation or overproduction of pro-inflammatory mediators and radical species might lead to certain chronic diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, and neurodegenerative diseases [56].

Albumin is the most abundant protein in the blood plasma and is able to bind and transport various compounds, such as fatty acids, bilirubin, tryptophan, hormones, and a large variety of medications [57]. The chemical structure of albumin can be altered by pro-inflammatory mediators, leading to rapid clearance. Reductions in plasma albumin levels during inflammation are primarily mediated by IL-6 and TNF-α [58,59]. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and indomethacin, have been reported to exert their anti-inflammatory function by multiple mechanisms, including stabilizing the albumin structure [60,61]. Hence, the ability to inhibit protein denaturation
signifies the apparent potential for anti-inflammatory activity. In this study, the inhibition of protein denaturation by the extracts of *C. timoriensis* flower was assessed at two different concentrations. At 200 µg/mL, all *C. timoriensis* flower extracts showed high inhibitory activity against protein denaturation (>85% inhibition), which was comparable to the positive control, indomethacin (91% inhibition) (Table 3). The inhibition activities of the ethyl acetate and methanol extracts were still high at 100 µg/mL, which is a similar pattern to the positive control, indomethacin. However, the inhibition activities of both the hexane and aqueous extracts were reduced significantly at 100 µg/mL (less than 50% inhibition). The high phenolic and flavonoid contents of the ethyl acetate and methanol extracts of *C. timoriensis* flowers might account for their high anti-inflammatory activity (Figure 1). The results are in agreement with previous reports where phenolic compounds, including flavonoids, showed anti-inflammatory activity through various mechanisms [62,63].

| Sample            | Concentration (µg/mL) | % Inhibition ** |
|-------------------|-----------------------|----------------|
| *n*-Hexane extract| 100                   | 43.13 ± 2.63   |
|                   | 200                   | 85.25 ± 2.50   |
| Ethyl acetate extract| 100               | 92.38 ± 0.74   |
|                   | 200                   | 92.50 ± 1.38   |
| Methanol extract  | 100                   | 89.45 ± 1.25   |
|                   | 200                   | 92.22 ± 1.09   |
| Aqueous extract   | 100                   | 36.76 ± 1.50   |
|                   | 200                   | 87.16 ± 2.02   |
| Indomethacin      | 100                   | 90.04 ± 0.87   |
|                   | 200                   | 91.15 ± 0.32   |

** Data are presented as mean ± SD (*n* = 3).

Plant sterols and flavonoids have been reported as promising anti-inflammatory agents that modulate immune-inflammatory markers such as Th1/Th2 and the cytokines TNF-α, IL-1, IL-6, and IL-8 [64]. Evaluation of the anti-inflammatory activity of various species from the *Cassia* genus [46,65–69] has led to the identification of several potential bioactive compounds, such as cassiaindoline and rhein [70–72]. Specifically, stigmasterol and β-sitosterol were reported to reduce TNF-α in a cutaneous allergic response [73] and to block mast cell-derived caspase-1 and NF-κB signal pathways in atopic dermatitis-like skin lesions [73,74]. Thus, the positive indication of steroids and triterpenoids in *C. timoriensis* flower extracts in our present study as well as the fact that β-sitosterol and stigmasterol were isolated from *C. timoriensis* in our previous study [17] suggest the potential anti-inflammatory activity of *C. timoriensis*, which directly supports its traditional use for skin disorders, itching, and scabies. Further study, however, is warranted to establish the anti-inflammatory role of *C. timoriensis*.

Moreover, a number of non-antimicrobial therapeutic agents, including ibuprofen, have been found to play a role in multidrug-resistant infections such as methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, there is an increasing interest in the efficacy of herbal products and essential oils as a health remedy for the control of drug resistance issues, which may be due to the synergistic influence of bioactive compounds [75,76]. For example, GeloMyrtol (G. PohlBoskamp, Hohenlockstedt, Germany) is a notable herbal medicine used to treat asthma and sinusitis. GeloMyrtol is extracted from a variety of essential oils provided by *Citrus limon*, *Camellia sinensis*, *Eucalyptus globulus*, and *Myrtus communis* [76]. Therefore, we believe that the medicinal properties of *Cassia timoriensis* might have the potential to be developed as herbal products with antimicrobial properties in the future.
2.4. In Vitro Anti-Acetylcholinesterase Activity of Cassia timoriensis Flower Extracts

Alzheimer’s disease (AD) is a progressive neurodegenerative disease indicated by low levels of acetylcholine (ACh) in the brain due to the activity of the acetylcholinesterase (AChE) enzyme. Lack of ACh in the brain has a great impact on short-term memory and learning. Preventing the enzyme from breaking down acetylcholine may ease some symptoms of AD [77]. The potential AChE inhibitory activity of C. timoriensis flowers was evaluated using Ellman’s method. Our results showed that at 200 µg/mL, all extracts except for the aqueous extract inhibited more than 90% AChE activity. Furthermore, the methanol and ethyl acetate extracts presented strong AChE activity inhibition with IC\textsubscript{50} values of 6.40 ± 0.27 and 6.91 ± 0.38 µg/mL, respectively, followed by the hexane extract (IC\textsubscript{50} 12.08 µg/mL) (Table 4). The potent inhibition of AChE activity by the ethyl acetate, methanol, and hexane extracts of C. timoriensis was in positive correlation to their high phenolic and flavonoid contents (Figure 1). Several mechanisms have been suggested for the anti-AChE activity of phenolic compounds, such as improving signal transmission in nerve synapses and increasing the concentration of ACh in synapses between cholinergic neurons [78–80].

Cassia timoriensis flowers have previously been shown to have anti-acetylcholinesterase activity [17]. However, variations in the inhibitory action of acetylcholinesterase within the same plant species have previously been observed [81,82]. These variations within the same plant species have been observed due to different phytoconstituents obtained from geographical regions but also according to seasons/periods of the year. The phyto-constituents of any plant part may vary both in quantity as well as quality depending on the soil, ground water level, stage of maturity of plant, and time of collection [81,82]. As a result, the IC\textsubscript{50} value of the ethyl acetate fraction of Cassia timoriensis in this study (Table 4) is slightly different from that in the previously reported study [17].

In general, the Cassia genus is a promising source of anti-cholinesterase compounds. Few potential AChE inhibitors have been identified from Cassia species, such as anthraquinones (physcion, emodin, and alaternin), terpenoids (cassioates E and F), and 3-methoxyquercetin [17,83–85]. We postulate that the diverse secondary metabolites in the anti-AChE activity of C. timoriensis extracts, as seen in Table 1, may lead to the identification of potential compounds that inhibit AChE activity.

Table 4. The activity of Cassia timoriensis against acetylcholinesterase enzymes.

| Sample            | % Inhibition * | IC\textsubscript{50} (µg/mL) |
|-------------------|---------------|-------------------------------|
| Galantamine       | 98.64 ± 0.01  | 1.33 ± 0.03                   |
| Aqueous extract   | 38.32 ± 0.09  | -                             |
| Methanol extract  | 96.55 ± 0.02  | 6.40 ± 0.27                   |
| Ethyl acetate extract | 96.87 ± 0.05 | 6.91 ± 0.38                   |
| n-Hexane extract  | 92.35 ± 0.014 | 12.08 ± 0.95                  |

Data are presented as mean ± SD (n = 3); * % Inhibition at 200 µg/mL.

3. Materials and Methods

3.1. Materials (Chemicals)

Acetylcholinesterase (AChE) from Electrophorus electricus (electrical eels), type VI-S, 200–1000 unit/mg; substrate acetylthiocholine iodide (ATCI); sodium phosphate monobasic; and sodium phosphate dibasic were purchased from Sigma-Aldrich (St. Louis, MO, USA). The coloring agent 5,5-dithio-bis-[2-nitrobenzoic acid] (DTNB) and gallic acid were obtained from Acros (Geel, Belgium). Galantamine hydrobromide was obtained from Calbiochem (San Diego, CA, USA). Indomethacin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, zinc powder, and phosphate-buffered saline were also obtained from Sigma-Aldrich (St Louis, MO, USA). Wagner’s, Mayer’s, and Dragendorff’s reagents were obtained from R&M Chemicals (Essex, UK). Folin–Ciocalteu’s reagent and sodium nitrite were obtained from Millon’s reagent for the detection of protein and sodium nitrite were obtained from Bendosen Laboratory Chemicals (Bendosen,
Benedict’s solution for reducing sugar was obtained from PC laboratory reagents. Aluminum chloride was obtained from Quality reagent company (Auckland, New Zealand). Ferric chloride and sodium carbonate were obtained from Merck (Darmstadt, Germany). All solvents used were of analytical grade.

3.2. Plant Collection and Identification

Fresh flowers were collected from the campus grounds of Universiti Sains Malaysia, Penang, in October 2019. The plant was identified and authenticated as Cassia timoriensis DC. by the Herbarium Deposition Department, Universiti Sains Malaysia. The voucher specimen (No. 11852) was deposited in the Herbarium of School of Biological Sciences, Universiti Sains Malaysia. The current taxonomy classification of this plant was referred to the Plant List website (www.theplantlist.com, accessed on 1 March 2020).

3.3. Plant Extraction and Fractionation

The flowers were dried in an oven at 40 °C for two days and then stored in an airtight container until further analysis. The dried flowers of C. timoriensis were ground into coarse particles and subjected to extraction using a simple maceration method. Briefly, the dried flowers of C. timoriensis (40 g) were extracted successively with continuous shaking for two days using 250 mL of different solvents with ascending polarities, namely n-hexane, ethyl acetate, methanol, and distilled water. The procedure was repeated three times to obtain the maximum yield of each fraction. All extracts were filtered and pooled accordingly and then evaporated under reduced pressure at 40 °C to yield solid residues of n-hexane extract (HE, 0.32 g), ethyl acetate extract (EE, 4.63 g), methanol extract (ME, 3.50 g), and aqueous extract (AE, 3.55 g). All extracts were kept in amber, airtight containers at 4 °C until further analysis.

3.4. Phytochemical Screening

Phytochemical screening of C. timoriensis extracts was performed to test for the presence or absence of bioactive constituents using standard protocols [18–20] to identify the secondary metabolites (flavonoids, alkaloids, saponins, tannins, and terpenoids) present in the HE, EE, ME, and AE (Table 5).

### Table 5. Qualitative phytochemical tests used for the screening of Cassia timoriensis extracts.

| No. | Class       | Test                  | Method                                                                 | Positive Result                                                                                                        | Ref.          |
|-----|-------------|-----------------------|------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------|
| 1   | Alkaloids   | Mayer’s test          | A few milligrams of each extract were dissolved individually in dilute HCl and filtered. Then, the filtrates were separately treated with Mayer’s, Wagner’s, and Dragendorff’s Reagents to test for the presence of alkaloids. | Turbidity or creamy precipitate                                                                                          | [19]         |
|     |             | Wagner’s test         |                                                                                                                     | Yellow–brown precipitate                                                                                               | [19]         |
|     |             | Dragendorff’s test    |                                                                                                                     | Turbidity or orange–red precipitate                                                                                   | [19]         |
| 2   | Flavonoids  | Alkaline test         | About 2 mL of 20% NaOH solution was added to 1 mL of alcoholic solution of each plant extract individually.          | Observation of intense yellow color                                                                                   | [19]         |
|     |             | Zn/HCl test           | A pinch of zinc dust added to 2 mL of the alcoholic solution of sample. Then, a few drops of concentrated HCl were added slowly. | Observation of pink to red color                                                                                       | [20]         |
| 3   | Tannins     | Ferric chloride test  | About 10 mg of the extracts was boiled in 10 mL of water in a test tube and then filtered. Then, a few drops of 1% ferric chloride were added to the filtrate. | Hydrolysable tannins give bluish-black color, while condensed give brownish-green color                                | [19]         |
| 4   | Saponin     | Frothing test         | A few milligrams of each extract were mixed separately with 5 mL of distilled water and mixed vigorously.         | Persistent foam                                                                                                         | [19]         |
| 5   | Cardiac glycoside | Keller–Killiani test | About 3 mg of each extract was dissolved in 3 mL of concentrated acetic acid. Then, one drop of 5% FeCl₃ solution was added, followed by few drops of concentrated sulphuric acid. | A reddish-brown ring forms at the interface                                                                             | [18,19]      |
Table 5. Cont.

| No. | Class            | Test Method                  | Positive Result                                      | Ref.   |
|-----|------------------|------------------------------|------------------------------------------------------|--------|
| 6   | Anthraquinone glycoside | Borntrager’s test | A few milligrams of each extract were treated with dilute HCL and boiled for 5 min, cooled, and shaken with an equal volume of chloroform, benzene, or any other organic layer; then, the organic layer was separated and treated with ammonia. | Pink to red color in aqueous alkaline layer | [19]  |
| 7   | Steroids        | Salkowski’s test            | A few milligrams of sample were treated with chloroform and filtered. The filtrates were then treated with a few drops of concentrated sulfuric acid. | Greenish-yellow color indicates the presence of steroids | [18,19] |
|     |                  | Liebermann–Burchard test    | About 2 mg of each extract was dissolved in acetic anhydride, heated, and cooled before adding 1 mL of concentrated sulphuric acid along the test tube’s sides. | Green color indicates the presence of steroids nucleus | [18,19] |
| 8   | Triterpenoids   | Modified Salkowski’s test   | About 1 mL of each of the four extracts was added to 1 mL of chloroform and filtered to clarify the solution, followed by dropwise addition of a few drops of concentrated sulphuric acid at the wall side of test tube. | Observation of reddish-brown color |        |
| 9   | Coumarins       | -                            | To 2 mL of each extract, a few drops of 10% alcoholic NaOH were added. | Observation of yellow color | [19]  |
| 10  | Quinone         | -                            | To 1 mL of each extract, a few drops of NaOH were added. | Observation of red or blue green color | [19]  |
| 11  | Protein         | Million’s test              | A few drops of Million’s reagent were added to 2 mL of each sample and mixed. | Red color or precipitate indicated the presence of protein | [19]  |
| 12  | Carbohydrate    | Benedict’s test             | A few drops of Benedict’s reagent were added to an aqueous solution of each plant extract and mixed. | Observation of orange–red color | [19]  |

3.5. Antioxidant Capacity

3.5.1. Total Flavonoid Content (TFC)

The total flavonoid content of each extract was determined using the aluminum chloride colorimetric method with some adjustments [23]. Quercetin was used as a standard to construct the calibration curve. A series of dilutions (100, 200, 400, 600, and 1000 µg/mL) of each extract and standard were prepared using methanol as a solvent. An aliquot of 250 µL of each dilution was mixed with 1000 µL of distilled water, 75 µL of 5% sodium nitrite, and 75 µL of 10% aluminum chloride. After 5 min, 1 mL of 4% sodium hydroxide was added, and the volume was increased 2.5 mL using distilled water. After 15 min incubation at room temperature, the absorbance was measured at 415 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The assay was conducted in triplicate. The flavonoid content was estimated from the calibration curve, and the concentration of the flavonoids was quantified as mg quercetin equivalent (QE) per g dry extract weight.

3.5.2. Total Phenolic Content (TPC)

The Folin–Ciocalteu method [86], with some modifications, was carried out to determine the total polyphenol content of the extracts. A series of dilutions (10, 20, 50, 100, 150, 200, 400, and 600 µg/mL) of the standard (gallic acid) were prepared to construct a calibration curve of gallic acid. The assay was performed by mixing 10 µL of each sample (1 mg/mL) with 50 µL of 10% Folin reagent followed by the addition of 60 µL of distilled water. A blank reagent was made with methanol. After 5 min incubation at room temperature, 80 µL of 7.5% sodium carbonate solution was added. Then, all samples were incubated in the dark for 30 min, and the absorbance was recorded at 765 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA). The assay was conducted in triplicate, and the TPC was quantified as mg gallic acid equivalent (GAE) per g of dry extract weight.
3.5.3. Radical Scavenging Capacity

The antioxidant activity of *C. timoriensis* was measured using the DPPH method [24]. Briefly, a stock solution of each extract was prepared (1 mg/mL) using methanol. Then, a series of dilutions were prepared to obtain a solution at concentrations of 50, 25, 12.5, 6.25, and 3.125 µg/mL. A freshly prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was made by dissolving 4 mg of DPPH into 100 mL of methanol away from direct light. Then, using a 96-well plate, 150 µL of DPPH solution was mixed with 50 µL of different samples (50–3.125 µg/mL). Ascorbic acid solutions in methanol (50–1.562 µg/mL) were prepared and used as a positive control. After 30 min incubation, the absorbance was measured at 517 nm using a microplate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., Winooski, VT, USA). A lower absorbance value indicates higher antioxidant activity of the sample. The % inhibition of the sample was calculated at a final concentration of 50 µg/mL. The results were expressed in IC_{50} values for samples that showed an inhibition percentage higher than 50%. The assay was conducted in triplicate for three consecutive days.

The DPPH free radical scavenging ability at a concentration of 200 µg/mL was calculated using the following Equation (1):

\[
\% \text{ of radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100
\] (1)

3.6. Anti-Inflammatory Activity

Protein denaturation is considered a hallmark of inflammation. The anti-inflammatory potential of the plant extracts was evaluated using a heat-induced albumin denaturation assay [87,88]. The reaction mixture consisted of 1 mL of each plant extract at varying concentrations (100 and 200 µg/mL) or the reference compound, indomethacin (100 and 200 µg/mL), mixed with 200 µL of chicken egg albumin (fresh hen’s egg). The pH of the reaction mixture was calibrated to pH 6.4 using phosphate-buffered saline. The samples were incubated at 37 °C for 20 min, and then, the temperature was increased to 50 °C for 20 min. After incubation, the samples were immediately cooled on ice, and the turbidity was evaluated at 660 nm [89]. The assay was performed in triplicate. The percentage of inhibition of albumin denaturation was calculated using the following Equation (2):

\[
\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100
\] (2)

3.7. Inhibition of Acetylcholinesterase Activity

The in vitro potential of acetylcholinesterase inhibitory activity was performed spectrophotometrically using Ellman’s method [90,91]. The assay was conducted in a 96-well plate with a total assay mixture volume of 200 µL. Galantamine was used as the positive control. In a 96-well plate, an aliquot of 1 µL of extract (40 mg/mL DMSO) was mixed with 179 µL of 0.05 mM phosphate buffer, and 10 µL of 0.5 U/mL AChE (AChE from *Electrophorus electricus* (electrical eels), Type VI-S, 200–1000 unit/mg protein) was added to the designated wells. After 15 min incubation at 25 °C, 10 µL of equal amounts of 14 mM acetylthiocholine iodide (ATCI) substrate and 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as a color indicator was added into each well and incubated at 25 °C for 30 mins to initiate an enzyme reaction. The absorption was measured at 415 nm using a Promega Glomax® Multi Plus Reader (Promega, Sunnyvale, CA, USA). Each run was carried out in triplicate on three different days to determine the percentage of inhibition at 200 µg/mL. The % inhibition was determined using Equation (3):

\[
\% \text{ Inhibition} = \frac{\text{Abs (−)ve control} - \text{Abs test sample}}{\text{Abs (−)ve control}} \times 100
\] (3)
Afterward, the IC$_{50}$ value for each sample showing AChE inhibitory activity of 50% or more was determined.

3.8. Statistical Analysis

All measurements were performed in triplicate, and the results were expressed as mean ± SD. The experimental results were further analyzed using MS Excel and GraphPad Prism 8 statistical software (v. 8.0.2(263), San Diego, CA, USA).

4. Conclusions

In this study, the qualitative phytochemical analysis showed that all extracts of C. timoriensis flowers are rich in secondary metabolites, mainly comprising of flavonoids, tannins, coumarins, steroids, and terpenoids known to have a wide range of biological activities. In addition, the quantitative phytochemical analysis showed that ethyl acetate and methanol extracts possess the highest TPC (527.43 ± 5.83 and 321.746 ± 11.33 mg GAE/g DW, respectively) and TFC (851.83 ± 10.08 and 493.92 ± 9.27 mg QE/g DW, respectively). The ethyl acetate and methanol extracts of C. timoriensis exhibited great antioxidant (IC$_{50}$ = 20.12 ± 0.12 and 34.48 ± 0.07 µg/mL, respectively), anti-inflammatory (92.50% ± 1.38 and 92.22% ± 1.09, respectively), and anti-cholinesterase (IC$_{50}$ = 6.91 ± 0.38 and 6.40 ± 0.27 µg/mL, respectively) activities, probably due to their high phenolic and flavonoid contents. Given these data, more extensive research is needed to investigate the chemical constituents of ethyl acetate and methanol extracts of C. timoriensis, which may be responsible for the anti-Alzheimer effect.

Author Contributions: H.A.W. designed the research project, secured funding, and supervised the progress. M.B.A. wrote the main manuscript text and performed the experimental work and data analysis. R.D. supervised the study progress. H.A.W., R.D., T.N., K.N.A.R. and S.M. contributed to the writing and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported financially by a USM RU TOP-DOWN project entitled Catalogue of USM-RIKEN Natural Product (CURINaP) Library for the Discovery of Bioactive Molecules on Ageing and Ageing-Related Diseases, 1001/PFARMASI/870031.

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Zhao, Y.; Wu, Y.; Wang, M. Bioactive Substances of Plant Origin 30. In Handbook of Food Chemistry; Cheung, P.C.K., Mehta, B.M., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; Volume 967, pp. 967–1008.
2. Stagos, D. Antioxidant activity of polyphenolic plant extracts. Antioxidants 2020, 9, 19. [CrossRef]
3. Hunyadi, A. The mechanism(s) of action of antioxidants: From scavenging reactive oxygen/nitrogen species to redox signaling and the generation of bioactive secondary metabolites. Med. Res. Rev. 2019, 39, 2505–2533. [CrossRef]
4. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J. Nat. Prod. 2020, 83, 770–803. [CrossRef] [PubMed]
5. Lima, J.A.; Costa, T.W.R.; Fonseca, A.C.D.; Amaral, R.F.D.; Desterro, S.M.D.; Santos-Filho, O.A.; Miranda, A.L.P.D.; Neto, D.C.F.; Lima, F.R.; Hamerski, L. Geissoschizoline, a promising alkaloid for Alzheimer’s disease: Inhibition of human cholinesterases, anti-inflammatory effects and molecular docking. Bioorg. Chem. 2020, 104, 104215. [CrossRef]
6. Sahoo, A.K.; Dandapat, J.; Dash, U.C.; Kanhar, S. Features and outcomes of drugs for combination therapy as multi-targets strategy to combat Alzheimer’s disease. J. Ethnopharmacol. 2018, 215, 42–73. [CrossRef] [PubMed]
7. Mehta, M.; Adem, A.; Sabbagh, M. New acetylcholinesterase inhibitors for Alzheimer’s disease. J. Alzheimers Dis. 2012, 2012, 728983. [CrossRef] [PubMed]
8. Berkov, S.; Codina, C.; Bastida, J. The Genus Galanthus: A Source of Bioactive Compounds. In Phytochemicals-A Global Perspective of Their Role in Nutrition and Health; IntechOpen: London, UK, 2012.
9. Heinrich, M.; Teoh, H.L. Galanthamine from snowdrop—the development of a modern drug against Alzheimer’s disease from local Caucasian knowledge. J. Ethnopharmacol. 2004, 92, 147–162. [CrossRef] [PubMed]
10. Hakim, F.A.; Gad, H.; Radwan, R.; Ayoub, N.; El-Shazly, M. Chemical constituents and biological activities of Cassia genus: Review. *Arch. Pharm. Sci. Acta Shahs Univ.* 2019, 3, 195–227.
11. Hu, J.M.; Lavin, M.; Wojciechowski, M.F.; Sanderson, M.J. Phylogenetic systematics of the tribe Millettieae (Leguminosae) based on chloroplast trnK/matK sequences and its implications for evolutionary patterns in Papilionoideae. *Am. J. Bot.* 2000, 87, 418–430. [CrossRef] [PubMed]
12. Raes, N.; Saw, L.; Van Welzen, P.C.; Yahara, T. Legume diversity as indicator for botanical diversity on Sundaland, south east Asia. *S. Afr. J. Bot.* 2013, 89, 265–272. [CrossRef]
13. Lim, T. Senna timoriensis. In *Edible Medicinal and Non-Medicinal Plants*; Springer: Dordrecht, The Netherlands, 2014; pp. 886–888.
14. Monkheang, P.; Sudmoon, R.; Tanee, T.; Noikotr, K.; Bletter, N.; Chaveerach, A. Species diversity, usages, molecular markers and barcode of medicinal Senna species (Fabaceae, Caesalpinioideae) in Thailand. *J. Med. Plant. Res.* 2011, 5, 6173–6181. [CrossRef]
15. Palasuwan, A.; Soogarun, S.; Lertlum, T.; Pradniwat, P.; Wiwanitkit, V. Inhibition of Heinz body induction in an invitro model and total antioxidant activity of medicinal Thai plants. *APCP* 2005, 6, 458. [PubMed]
16. Gritsanapan, W.; Tantisewie, B.; Jirawongse, V. Chemical constituents of *Cassia timoriensis* and *Cassia grandis*. *Sci. Asia* 1984, 10, 189–190. [CrossRef]
17. Azman, N.A.N.; Alhawarri, M.B.; Rawo, M.S.A.; Dianita, R.; Gazzali, A.M.; Nogawa, T.; Wahab, H.A. Potential anti-acetylcholinesterase activity of *Cassia timoriensis* DC. *Molecules* 2020, 25, 4545. [CrossRef] [PubMed]
18. Nabavi, S.M.; Saeedi, M.; Nabavi, S.F.; Silva, A.S. Terpenes and terpenoids. In *Advances in Natural Products Analysis*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 275–496.
19. Egbuna, C.; Ilemeje, J.C.; Udedi, S.C.; Kumar, S. *Phytochemistry: Volume 1: Fundamentals, Modern Techniques, and Applications*; CRC Press/Thomay and Francis: Boca Raton, FL, USA, 2018.
20. Chaudhary, S.; Kumar, A. Phytochemical analysis and assessment of in-vitro anthelmintic activity of *Cassia auriculata* Linn leaves. *Am. J. Phytomed. Clin. Therap.* 2014, 2, 161–167.
21. Rice-Evans, C.; Miller, N.; Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* 1997, 2, 152–159. [CrossRef]
22. Sánchez-Rangel, J.C.; Benavides, J.; Heredia, J.B.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. The Folin–Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Anal. Methods* 2013, 5, 5990–5999. [CrossRef]
23. Boukraa, D.; Belabid, L.; Benabdellil, K.; Bennabi, F. The effect of the salicylic acid on the variability of phenolic compounds, during the germination and the seedling of chickpea (*Cicer arietinum* L.), after inoculation by mushrooms. *Eur. J. Biotechn. Biosci.* 2014, 1, 27–35.
24. Mansour, R.B.; Ksouri, W.M.; Cluzet, S.; Krisa, S.; Richard, T.; Ksouri, R. Assessment of antioxidant activity and neuroprotective capacity on PC12 cell line of *Frankenia thymifolia* and related phenolic LC-MS/MS identification. *Evid. Based Complement. Altern. Med.* 2016, 2016, 2843463. [CrossRef]
25. Kolar, F.R.; Gogi, C.L.; Khudavand, M.M.; Choudhari, M.S.; Patil, S.B. Phytochemical and antioxidant properties of some Cassia species. *Nat. Prod. Res.* 2018, 32, 1324–1328. [CrossRef]
26. Zhao, Y.; Tuo, S.; Li, Y.; Chen, W.; Kou, S.; Gu, C.; Li, Z.; Guo, L. A review of flavonoids from cassia species and their biological activity. *Curr. Pharm. Biotechnol.* 2016, 17, 1134–1146. [CrossRef]
27. Juan-Badaturuge, M.; Habtemariam, S.; Thomas, M.J. Antioxidant compounds from a south Asian beverage and medicinal plant, *Cassia auriculata*. *Food Chem.* 2011, 125, 221–225. [CrossRef]
28. Boukraa, D.; Belabid, L.; Benabdellil, K.; Bennabi, F. The effect of the salicylic acid on the variability of phenolic compounds, during the germination and the seedling of chickpea (*Cicer arietinum* L.), after inoculation by mushrooms. *Eur. J. Biotechn. Biosci.* 2014, 1, 27–35.
29. Mahesh, V.; Sharma, R.; Singh, R.; Upadhyia, S. Anthraquinones and kaempferol from Cassia species section fistula. *J. Nat. Prod.* 1984, 47, 733. [CrossRef]
30. Panichayupakaranant, P.; Kaewsuswan, S. Bioassay-guided isolation of the antioxidant constituent from *Cassia alata* L. leaves. *Songklanakarin J. Sci. Technol.* 2004, 26, 103–107.
31. Wahab, A.; Begum, S. Luteolin and kaempferol from *Cassia alata*, antimicrobial and antioxidant activity of its methanolic extracts. *FUILAST J. Biol.* 2014, 4, 1–5.
32. Dave, H.; Ledwani, L. A review on anthraquinones isolated from Cassia species and their applications. *Indian J. Nat. Prod. Resour.* 2012, 3, 291–319.
33. Lee, N.-H.; Lee, S.-M.; Song, D.-H.; Yang, J.-Y.; Lee, H.-S. Antimicrobial effect of emodin isolated from *Cassia tora* Linn. seeds against food-borne bacteria. *Appl. Biol. Chem.* 2013, 56, 187–189. [CrossRef]
34. Mohammed, M.M.; El-Souda, S.S.; El-Hallouty, S.M.; Kobayashi, N. Antiviral and cytotoxic activities of anthraquinones isolated from *Cassia roxburghii* Linn. leaves. *Herba Pol.* 2013, 59, 33–44. [CrossRef]
35. Promgool, T.; Pancharoen, O.; Deachathai, S. Antibacterial and antioxidative compounds from *Cassia alata* Linn. *Songklanakarin J. Sci. Technol.* 2014, 36, 459–463. [CrossRef]
36. Kalidhar, S.B. Alatineone, an anthraquinone from *Cassia alata*. *Phytochem.* 1993, 32, 1616–1617.
37. Sekar, M.; Prasad, K.R.; Sidduraju, P.; Janardhanan, K. New anthraquinones from *Cassia obtusa*. *Fitoterapia* 1999, 70, 330–332. [CrossRef]
38. Wu, Q.; Wang, Z.; Fu, M.; Tang, L.; He, Y.; Fang, J.; Gong, Q. Chemical constituents from the leaves of *Cassia angustifolia*. *Zhong Yao Cai* 2007, 30, 1250–1252. [PubMed]
39. Banjarnahor, S.D.; Artanti, N. Antioxidant properties of flavonoids. *Med. J. Indonesia*. 2014, 23, 239–244. [CrossRef]
40. Kaurinovic, B.; Vastag, D. Flavonoids and phenolic acids as potential natural antioxidants. In *Antioxidants*; IntechOpen: London, UK, 2019.
41. Pourreza, N. Phenolic compounds as potential antioxidant. *Jundishapur J. Nat. Pharm. Prod.* 2013, 8, 149. [CrossRef] [PubMed]
42. Fidèlè, N.; Joseph, B.; Emmanuel, T.; Théophile, D. Hypolipidemic, antioxidant and anti-atherosclerotic effect of aqueous extract leaves of *Cassia occidentalis* Linn (Cassalpinaceae) in diet-induced hypercholesterolemic rats. *BMC Complement. Altern. Med.* 2017, 17, 76. [CrossRef]
43. Ishak, I.F.A.R.; Lajis, H.M.; Ambia, K.M.; Noah, R.M. Effects of *Cassia alata* treatment towards cardiovascular oxidative stress in hyperglycemic rats. *Int. J. Pharm. Sci. Res. Res.* 2015, 34, 254–258.
44. Gupta, S.; Sharma, S.B.; Singh, U.R.; Bansal, S.K. Salutary effect of *Cassia auriculata* L. leaves on hyperglycemia-induced atherosclerotic environment in streptozotocin rats. *Cardiovasc. Toxicol.* 2011, 11, 308. [CrossRef] [PubMed]
45. Ju, M.S.; Kim, H.G.; Choi, J.G.; Ryu, J.H.; Hur, J.; Kim, Y.J.; Oh, M.S. Cassiae semen, a seed of *Cassia obtusifolia*, has neuroprotective effects in Parkinson’s disease models. *Food Chem. Toxicol.* 2010, 48, 2037–2044. [CrossRef]
46. Yi, J.H.; Park, H.J.; Lee, S.; Jung, J.W.; Kim, B.C.; Lee, Y.C.; Ryu, J.H.; Kim, D.H. *Cassia obtusifolia* seed ameliorates amyloid β-induced synaptic dysfunction through anti-inflammatory and Akt/GSK-3β pathways. *J. Ethnopharmacol.* 2016, 178, 50–57. [CrossRef]
47. Rejiya, C.; Cibin, T.; Abraham, A. Leaves of *Cassia tora* as a novel cancer therapeutic—An in vitro study. *Toxicol. In Vitro* 2009, 23, 1034–1038. [CrossRef]
48. Padmalochana, K. Anticancer (liver cancer cell lines) and antioxidant activity of *Cassia fistula* flower extract from acetone and methanol solvents. *JDDT* 2018, 8, 274–278. [CrossRef]
49. Duraipandiyan, V.; Baskar, A.A.; Ignacimuthu, S.; Muthukumar, C.; Harbi, N. Anticancer activity of Rhein isolated from *Cassia angustifolia* flower extract leaves of *Cassia fistula*. *Asian Pac. J. Trop. Biomed.* 2013, 2, S517–S523. [CrossRef]
50. Luximon-Ramma, A.; Bahorun, T.; Soobrattee, M.A.; Aruoma, O.I. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*. *J. Agric. Food Chem.* 2002, 50, 5042–5047. [CrossRef] [PubMed]
51. Ahmed, S.I.; Hayat, M.Q.; Tahir, M.; Mansoor, Q.; Iqbal, M.; Keck, K.; Bates, R.B. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC Complement. Altern. Med.* 2016, 16, 460. [CrossRef] [PubMed]
52. Niedzielska, E.; Smaga, I.; Gawlik, M.; Moniczewski, A.; Stankowicz, P.; Pera, J.; Filip, M. Oxidative stress in neurodegenerative diseases. *Mol. Neurobiol.* 2008, 4094–4125. [CrossRef]
53. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciore, F.; Bonaduce, D. Oxidative stress, aging, and diseases. *Clin. Intern. Aging* 2013, 8, 127–137. [CrossRef]
54. Huang, W.J.; Zhang, X.; Chen, W.W. Role of oxidative stress in Alzheimer’s disease. *Biomed. Rep.* 2016, 4, 519–522. [CrossRef]
55. Jo, K. In-vitro valuation of anti-inflammatory effect of Panax Ginseng by inhibition of albumin denaturation experiment. *APEC Youth Sci. J.* 2017, 9, 1–5.
56. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D.W.; Fasano, A.; Miller, G.W. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 2019, 25, 1822–1832. [CrossRef]
57. Bogdan, M.; Pirnau, A.; Floare, C.; Bugeac, C. Binding interaction of indomethacin with human serum albumin. *Mol. Interv.* 2008, 17, 1–6. [CrossRef]
58. Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb. Perspect. Biol.* 2014, 6, a016295. [CrossRef] [PubMed]
59. Hammond, G.L.; Hill, L.A.; Round, P.W. Roles of Plasma Binding Proteins in Modulation of Hormone Action and Metabolism. In *Reference Module in Biomedical Sciences*; Elsevier: Amsterdam, The Netherlands, 2019.
60. Nicholson, J.; Wolmarans, M.; Park, G. The role of albumin in critical illness. *Br. J. Anaesth.* 2000, 85, 599–610. [CrossRef] [PubMed]
61. Larsen, M.T.; Kuhlmann, M.; Hvam, M.L.; Howard, K.A. Albumin-based drug delivery: Harnessing nature to cure disease. *Mol. Cell Ther.* 2016, 4, 1–12. [CrossRef] [PubMed]
62. Ambriz-Pérez, D.L.; Leyva-López, N.; Gutierrez-Grijalva, E.P.; Heredia, J.B. Phenolic compounds: Natural alternative in inflammation treatment. *A Review. Cogent Food Agric.* 2016, 2, 1131412.
63. Leyva-Jiménez, F.J.; Lozano-Sánchez, J.; Cádiz-Gurrea, M.D.L.L.; Arráez-Román, D.; Segura-Carretero, A. Functional ingredients based on nutritional characteristics. A case study against inflammation: *Lippia genusc. Nutrients* 2019, 11, 1646. [CrossRef]
64. Brill, F.; Mensink, R. Plant sterols: Functional lipids in immune function and inflammation? *J. Clin. Lipidol.* 2009, 4, 355–365. [CrossRef]
65. Ntandou, G.N.; Banzouzi, J.; Mbatchi, B.; Elion-Itou, R.; Etou-Ossibi, A.; Ramos, S.; Benoît-Vical, F.; Abena, A.; Ouamba, J. Analgesic and anti-inflammatory effects of *Cassia siamea* Lam. stem bark extracts. *J. Ethnopharmacol.* 2010, 127, 108–111. [CrossRef]
66. Basha, S.I.; Somashekaru, S.; Govindadas, D.; Naidu, D.; Devasankalaraiiah, G.; Mohato, R.; Yadav, K. Anti-inflammatory activity of *Cassia occidentalis* seeds in albino rats. *J. Nat. Pharm.* 2011, 2, 88–91. [CrossRef]
67. Gobianand, K.; Vivekanandan, P.; Pradeep, K.; Mohan, C.; Kirthikeyan, S. Anti-inflammatory and antipyretic activities of Indian medicinal plant *Cassia fistula* Linn. (Golden Shower) in Wistar albino rats. *Int. J. Pharmacol.* 2010, 6, 719–725. [CrossRef]
68. Chaudhari, S.S.; Chaudhari, S.R.; Chavan, M.J. Analgesic, anti-inflammatory and anti-arthritis activity of *Cassia uniflora* Mill. *Asian Pac. J. Trop. Biomed.* 2012, 2, S181–S186. [CrossRef]
69. Antonisamy, P.; Dhanasekaran, M.; Kim, H.-R.; Jo, S.-G.; Agastian, P.; Kwon, K.-B. Anti-inflammatory and analgesic activity of ononitol monohydrate isolated from Cassia tora L. in animal models. Saudi J. Biol. Sci. 2017, 24, 1933–1938. [CrossRef]

70. Moriyama, H.; Iizuka, T.; Nagai, M.; Miyakata, H.; Satoh, T. Antiinflammatory activity of heat-treated Cassia alata leaf extract and its flavonoid glycoside. Yakugaku Zasshi 2003, 123, 607–611. [CrossRef] [PubMed]

71. Mondal, A.; Rajalingam, D.; Maity, T.K. Anti-inflammatory effect of O-methylated flavonol 2-(3,4-dihydroxy-phenyl)-3,5-dihydroxy-7-methoxy-chromen-4-one obtained from Cassia sophora Linn in rats. J. Ethnopharmacol. 2013, 147, 525–529. [CrossRef]

72. Antonisamy, P.; Agastian, P.; Kang, C.-W.; Kim, N.S.; Kim, J.-H. Anti-inflammatory activity of Rhein isolated from the flowers of Cassia fistula L. and possible underlying mechanisms. Saudi J. Biol. Sci. 2019, 26, 96–104. [CrossRef]

73. Antwi, A.O.; Obiri, D.D.; Osafo, N.; Essel, L.B.; Forkuo, A.D.; Atobiga, C. Stigmasterol alleviates cutaneous allergic responses in rodents. BioMed Res. Int. 2018, 2018, 3984068. [CrossRef] [PubMed]

74. Han, N.-R.; Kim, H.-M.; Jeong, H.-J. The

75. Jung, H.A.; Ali, M.Y.; Jung, H.J.; Jeong, H.O.; Chung, H.Y.; Choi, J.S. Inhibitory activities of major anthraquinones and other constituents from Cassia obtusifolia against β-secretase and cholinesterases. J. Ethnopharmacol. 2016, 191, 152–160. [CrossRef]

76. Aftab, Z.; Khan, H.; Khan, A.; Ullah, H.; Khan, S. Three new cholinesterase inhibitory cassetioes from Cassia fistula. Pharm. Chem. J. 2020, 53, 1069–1075. [CrossRef]

77. Vongsak, B.; Sithisarn, P.; Mangmool, S.; Thongpraditchote, S.; Wongkrajang, Y.; Gritsanapan, W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Ind. Crops Prod. 2013, 44, 566–571. [CrossRef]

78. Dey, P.; Chatterjee, P.; Chandra, S.; Bhattacharyya, S. Comparative in vitro evaluation of anti-inflammatory effects of aerial parts and roots from Mikania scandens. J. Adv. Pharm. Educ. Res. 2011, 1, 271–277.

79. Banerjee, S.; Chanda, A.; Adhikari, A.; Das, A.; Biswas, S. Evaluation of phytochemical screening and anti inflammatory activity of leaves and stem of Mikania scandens (L.) wild. Ann. Med. Health Sci. Res. 2014, 4, 532–536. [CrossRef]

80. Sarveswaran, R.; Jayasuriya, W.; Suresh, T. In vitro assays to investigate the anti-inflammatory activity of herbal extracts a review. World J. Pharm. Res. 2017, 6, 131–141.

81. Rawa, M.S.A.; Hassan, Z.; Murugaiyah, V.; Nogawa, T.; Wahab, H.A. Anti-cholinesterase potential of diverse botanical families from Malaysia: Evaluation of crude extracts and fractions from liquid-liquid extraction and acid-base fractionation. J. Ethnopharmacol. 2019, 245, 112160. [CrossRef] [PubMed]

82. Balkis, A.; Tran, K.; Lee, Y.Z.; Balkis, K.N.; Ng, K. Screening flavonoids for inhibition of acetylcholinesterase identified baicalin as the most potent inhibitor. J. Agric. Sci. 2015, 7, 26. [CrossRef]