

Phytochemical Constituent Analysis, Antioxidative Effect, and Anti-inflammatory Activity of *Bombax ceiba* Flowers

Steven Kuan-Hua Huang^{1,2,3,#} , Cheng-Yang Hsieh^{4,#}, Po-Wei Fu¹, Chia-Jung Lee^{4,5,6}, Gerald C. Domingo⁷, Agnes B. Alimboyoguen⁸, Po-Yu Lin¹, Lin-Chieh Hung⁹, Timothy Jen R. Roxas⁷, Kathlia A. De Castro-Cruz^{7,*} , Po-Wei Tsai^{1,*} 

¹ Department of Medical Science Industries, College of Health Sciences, Chang Jung Christian University, Tainan 711, Taiwan

² Division of Urology, Department of Surgery, Chi Mei Medical Center, Tainan 711, Taiwan

³ School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan

⁴ Ph.D. Program in Clinical Drug Development of Herbal Medicines, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan

⁵ Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei 110, Taiwan

⁶ Traditional Herbal Medicine Research Center, Taipei Medical University Hospital, Taipei 110, Taiwan

⁷ School of Chemical, Biological and Materials Engineering, and Sciences, Mapúa University, 1002 Metro Manila, Philippines

⁸ Department of Physical Science, College of Arts and Sciences, Cavite State University, Indang, Cavite, Philippines

⁹ Department of Emergency Medicine, Tri-Service General Hospital, Taipei 114, Taiwan

These authors have equally contributed to this study.

* Correspondence: powei@mail.cjcu.edu.tw (P.-W.T.); kadecastro@mapua.edu.ph (K.A.C.C.)

Scopus Author ID 56486877000

Received: 15.07.2022; Accepted: 10.09.2022; Published: 17.11.2022

Abstract: *Bombax ceiba* has been mentioned in indigenous systems of medicine due to its various ethnomedicinal applications. However, the anti-inflammatory activity of *B. ceiba* has not been thoroughly explored. Thus, this study aims to evaluate the phytochemical constituent of *B. ceiba* flowers and determine their antioxidative action and anti-inflammatory activity. The crude extracts of *B. ceiba* flower underwent colorimetric assays to measure the total polyphenol, total flavonoid, and total condensed tannin content. DPPH free radical scavenging and FRAP assay were used for the antioxidative activity. The cytotoxicity and anti-inflammatory activity of *B. ceiba* were measured through nitric oxide inhibition in LPS-treated RAW 264.7 cells. Quantitative phytochemical evaluation of *B. ceiba* flowers reveals that ethanol and water extracts have the highest polyphenol content; ethyl acetate, hexane, and acetone extracts have the highest flavonoid content, and; water extract has the highest condensed tannin content. DPPH free radical scavenging and FRAP assay indicate that ethanol ($IC_{50}=0.287\pm 0.011$ mg/mL and 1877.130 ± 20.574 mg Trolox/g) and water ($IC_{50}=0.364\pm 0.010$ and 1658.395 ± 97.228 mg Trolox/g) has the most potent antioxidative activity. All extracts are deemed nontoxic to RAW 264.7 cells, with EA extract ($IC_{50}= 101.274$ μ g/mL) having the inhibitory effect against nitric oxide. This study confirms the significant antioxidant and anti-inflammatory activity of *B. ceiba* flower. Consequently, this study provides insight for extended studies of *B. ceiba* flowers in medicinal research.

Keywords: *Bombax ceiba* flower; antioxidant; anti-inflammatory; polyphenol; flavonoid.

Abbreviations: *B. ceiba*: *Bombax ceiba*; EA: Ethyl acetate; EtOH: Ethanol; FRAP: Ferric Reducing Antioxidant Power; NO: Nitric Oxide; LPS: Lipopolysaccharide.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Bombax ceiba Linn (Bombacaceae family) is a deciduous, large tree that grows up to 40 meters with conical spines covering its entire stem. It contains pentafooliated, palmately compound leaves that are elliptic-obovate, acuminate, and glabrous, with a size of each leaflet ranging from 5-23 x 1.5-90 cm with 2 cm (in length) secondary petioles. It has cottony seeds, hence the name Red Silk Cotton Tree [1].

B. ceiba is noted in the indigenous system of medicines due to its various ethnomedicinal applications. Among its uses are recorded in traditional Ayurvedic medicine as anabolic and anti-aging (roots); treatment for arthritis (leaves); virility promoter (gum); used for wound healing, hyperpigmentation, and hemorrhagic disorders (stem); tissue regenerator and bowel regulator (roots of young *B. ceiba*); and treatment for spermatorrhea, menorrhagia, polyurea, and leucorrhea (flowers) [2–4]. Additionally, Unani traditional medicine uses *B. ceiba* as a styptic and astringent (seeds); treatment for uterine bleeding (flower). Meanwhile, Tibetan medicine uses *B. ceiba* for cardiogenic, hepatoprotective, and antibacterial activities, used explicitly for the heart, lungs, and liver (flowers) [5].

In present studies, *B. ceiba* possesses various phytoconstituents such as polysaccharides, naphthoquinones, anthraquinones, lupeol, and shamimin. Correspondingly, different studies of *B. ceiba* demonstrate anthelmintic, antihyperglycemic, hepatoprotective, immunomodulatory, anticancer, antioxidant, antidepressant, antibacterial, aphrodisiac, anti-osteoclastogenic, muscle relaxant, and locomotor [6–12].

With the increasing interest in phytochemical studies of *B. ceiba*, this study aims to conduct phytochemical screening and determine the antioxidant and anti-inflammatory activity of *B. ceiba* flower using five solvents with different polarities: ethyl acetate, hexane, acetone, ethanol, and water.

2. Materials and Methods

2.1. Preparation and extraction of plant samples.

The flowers of *B. ceiba* were collected from the campus of Chang Jung Christian University in Tainan City, Taiwan, in March 2021. The authenticity of the plants was identified and certified by Dr. Chia-Jung Lee (Ph.D. Program in Clinical Drug Development of Herbal Medicines, College of Pharmacy in Taipei Medical University, Taiwan), and stored at the Department of Medical Science Industries, Chang Jung Christian University. The voucher specimen was deposited as #CJCU-BC-001. Plant samples were air-dried for one week at ambient conditions. Following that, the samples were crushed and oven-dried until a constant weight of the sample was obtained. The dried samples were then mechanically pulverized with an osteorizer. The corresponding samples were prepared in five replicates using a 1:20 (sample: solvent) ratio of water, ethanol, acetone, ethyl acetate, and hexane as a solvent under reflux for two hours at 60°C. Subsequently, the plant extracts were obtained via vacuum filtration, followed by solvent removal with a rotary evaporator. Finally, the crude extracts were lyophilized and weighed before being utilized in the subsequent study.

2.2. *Phytochemical constituent determination.*

2.2.1. Total phenolic content determination.

The phenolic content of water, ethanol, acetone, ethyl acetate, and hexane extracts was measured by the Folin-Ciocalteu method [13]. The crude extract was diluted using ethanol solvent to a volume of 100 microliters. Subsequently, 500 microliters of Folin Ciocalteu's reagent and 400 microliters of sodium carbonate were added to the diluted samples. After that, the resulting solution was spectrophotometrically measured at 600 nm with an ELISA microplate reader. The samples were prepared in triplicates. Similarly, 10 mg of gallic acid was added to ethanol solvent to obtain the standard solution, by which 50 microliters of these were diluted to 1 milliliter. After that, the solution was serially diluted by two orders of magnitude, with gallic concentration levels ranging from 500 to 7.81 g/mL. One hundred microliters of the standard solutions were analyzed similarly to the sample to have the signals necessary to construct the calibration curves.

2.2.2. Total flavonoid content determination.

The flavonoid concentration was evaluated following the aluminum chloride colorimetric method [14]. Each crude extract was diluted with an ethanol solvent to 10 mg. Furthermore, 50 microliters of the diluted sample were treated with 2.0% AlCl₃. The resulting solution was incubated for one hour before being spectrometrically measured using an ELISA microplate reader at 430 nm. Furthermore, the standard solution was prepared by reacting 5.0 mg rutin with ethanol and diluting it to 1000 microliters. The solution was serially diluted by two orders of magnitude to generate rutin concentrations ranging between 400 to 6.25 g/mL. Then, 500 microliters of the standard solutions and the blank (ethanol) were prepared the same way as the sample. Subsequently, the absorbance readings were utilized to create the calibration plot. All of the solutions were made in triplicates.

2.2.3. Total condensed tannin content determination.

The condensed tannin concentration was determined using the methods proposed by Broadhurst and Jones [15]. Fifty microliters of crude extract were mixed with 600 microliters of Vanillin reagent and 250 microliters of ethanol. Consequently, the absorbance signals of the solution were measured using an ELISA microplate reader set at 530 nm. Similarly, 32 microliters of Catechin solution were diluted to 1 milliliter for the standard solutions. The solution was serially diluted by two orders of magnitude to produce standard solutions with Catechin concentrations ranging from 160 g/mL to 2.5 g/mL. Then, 300 microliters of the stock solution and the blank (ethanol) were handled similarly to the sample. The calibration plot was created using the absorbance signals. All of the solutions were made in triplicates.

2.3. *In vitro* antioxidant assay.

2.3.1. Free radical scavenging activity by DPPH.

The antioxidant capacity of the crude extracts was determined using the same procedures as Blois [16]. The DPPH solution was prepared by dissolving 200 microliters in ethanol solvent and then placing it in a dark container. Five milligrams of ascorbic acid were diluted using one milliliter of water to produce the standard solution. One hundred microliters

of the resulting solution were diluted to 1000 microliters with ethanol as the solvent. A serial dilution with two orders of magnitude was conducted to produce known ascorbic acid concentrations ranging from 500 to 7.80 g/mL. One hundred microliters of every standard solution were incubated for 30 mins after adding 150 microliters of the produced DPPH solution. Using an ELISA microplate reader, the obtained solutions were then examined at 517 nm. Meanwhile, the plant extracts were diluted to varying amounts, generating concentrations ranging from 2.00 mg/mL to 0.49 g/mL, which were treated identically to the reference solution. The control samples were assessed the same way as the sample, but with 100 microliters of ethanol instead of plant extracts. For the blank, 250 microliters of ethanol were utilized.

The free radical scavenging activity was reported based on the 50% inhibition activity of IC_{50} and calculated using equation (1).

$$IC_{50} = \frac{(A_{control} - A_{blank}) - (A_{sample} - A_{blank})}{(A_{control} - A_{blank})} \times 100\% \quad (1)$$

where $A_{control}$ represents the absorbance of the control, A_{sample} represents the absorbance of the sample, and A_{blank} represents the absorbance of the blank.

2.3.2. Ferric reduction antioxidation power (FRAP) assay.

The crude extracts' ferric reduction antioxidation power was assessed using the techniques described by Benzie and Strain [17]. The crude extracts were diluted in a 1.0 microliter of ethanol:ddH₂O (2:3) mixture at a 10 mg/mL concentration. Fifty microliters of the resulting solution were treated with 1450 FRAP reagent microliters, which were then evaluated at 593 nm with an ELISA microplate reader. Similarly, 10 mg of Trolox was dissolved in 2.0 mL of ethanol and 3.0 mL of ddH₂O to produce the standard solution. The solution was then diluted with 500 microliters of ethanol:ddH₂O (2:3) solution. The solution was serially diluted by a factor of two to generate known Trolox concentrations ranging from 1000 to 15.63 g/mL. Then, comparable treatments to those used on the samples were carried out. The calibration curve was created using the absorbance signals.

2.4. *In vitro* anti-inflammatory assay.

2.4.1. MTT Assay.

RAW 264.7 (4×10^5 cells) were placed in a 96-well plate and incubated overnight. Then, different sample doses were added to the cells and treated with 500 ng/mL LPS. The resulting solution was incubated again for another 24 h. After which, five mg/mL of the MTT solution was added to the solution, then incubated for another four hours. After the incubation period, the medium was removed, then isopropanol was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm.

2.4.2. Determination of LPS-stimulated NO production.

The removed medium treated with LPS and incubated for 24 h in MTT assay was placed in a 96-well plate. The nitric oxide (NO) was produced due to the addition of LPS into the RAW 264.7 cells. Subsequently, the Griess reagent was added into the medium, followed by the absorbance measurement at 530 nm. The results were reported in %NO inhibition.

$$\% \text{ NO Inhibition} = 1 - \frac{T}{C} \tag{2}$$

where *T* and *C* correspond to the mean optical density of LPS-treated cells with and without samples.

2.5. Statistical analysis

The statistical results were analyzed using IBM SPSS Statistics 28.0 and ClustVis. Principal component analysis (PCA) was used to determine the relationship between variables, and Factor analysis to determine the correlation between each variable and the group features.

3. Results and Discussion

3.1. Phytochemical constituent determination.

The phytochemical content of the *B. ceiba* flowers is evaluated by determining its total polyphenol, flavonoid, and condensed tannin content based on five solvents with different polarities: water, ethanol, acetone, ethyl acetate, and hexane.

Table 1 suggests that extracts from polar protic solvents (water and ethanol) contain the highest polyphenol content, followed by polar aprotic solvents (acetone and ethyl acetate) and nonpolar solvent extracts (hexane). Interestingly, the trend for the polyphenol content of *B. ceiba* flowers complements the solvent’s polarity, showing that the highest polyphenol content is extracted from highly polar solvents, which declines with decreasing solvent's polarity. Notably, the concentrations of polyphenol for *B. ceiba* flowers expressed as mg gallic acid equivalent per gram of extract are 166.458±5.595 (ethanol), 165.597±2.881 (water), 48.377±1.517 (acetone), 22.815±0.866 (ethyl acetate), and 19.967±0.574 (hexane). In contrast, the total flavonoid content of *B. ceiba* flowers obtained the highest concentration from the extracts of polar aprotic solvents (ethyl acetate and acetone) and nonpolar (hexane) solvents, followed by polar protic solvents (water and acetone). Specifically, the concentrations of flavonoid in *B. ceiba* flowers, in mg rutin equivalent per gram of extracts, are 392.329±4.876 (ethyl acetate), 315.486±9.688 (hexane), 277.708±21.418 (acetone), 188.63±13.155 (water), and 182.406±8.103 (ethanol). Furthermore, the total condensed tannin content of *B. ceiba* flowers acquired the highest concentration in water extract, followed by the four remaining extracts (ethanol, ethyl acetate, acetone, and hexane) with nearly equal concentrations. In particular, the total condensed tannin concentration of *B. ceiba* flowers, expressed as mg Catechin equivalent per gram of extract, is 530.605±15.779 (water), 322.647±12.830 (ethyl acetate), 298.089±5.934 (ethanol), 276.666±5.504 (acetone), and 265.694±3.263 (hexane).

Table 1. Phytochemical content analysis of *Bombax ceiba* flower extracts.

	Sample Extract	Total polyphenol content analysis (mg/g)	Total flavonoid content analysis (mg/g)	Total condensed tannin content analysis(mg/g)
<i>Bombax ceiba</i> flower	Water	165.597±2.881	188.630±13.155	530.605±15.779
	EtOH	166.458±5.595	182.406±8.103	298.089±5.934
	Acetone	48.377±1.517	277.708±21.418	276.666±5.504
	EA	22.815±0.866	392.329±4.876	322.647±12.830
	Hexane	19.967±0.574	315.486±9.688	265.694±3.263

A previous study reported the phytochemical content of *B. ceiba* flowers having alkaloids, cardiac glycosides, carbohydrates, flavonoids, phenols, phlobatannin, proteins,

saponin, sterols, tannins, terpenoids, and quinones. Moreover, a qualitative study of *B. ceiba* flowers in ethanol extracts indicates the presence of alkaloids, cardiac glycosides, carbohydrates, flavonoids, phenols, saponins, tannin, terpenoids, and quinones. Detailed exploration of the ethanol extracts of *B. ceiba* flowers obtained flavonol glycosides, flavones, flavanone, xanthone glycosides, anthocyanidin, 9-norneolignan and its glycosides, and lactone derivatives [18–21]. Furthermore, the flower pigment of *B. ceiba* contains mainly cyanidin glucosides isolated from alcoholic extracts [20,22]. Likewise, a qualitative screening in the flower petals of ethanol extracts of *B. ceiba* confirms the presence of cardiac glycosides, saponins glycosides, and flavonol glycosides. Hence, the considerable amount of polyphenols in the ethanol extracts is attributed to the significant number of glycosides (cyanidin glucosides, cardiac glycosides, saponins glycosides, flavonol glycosides), flavonoids, xanthenes, anthocyanidin, and lignans in the ethanol extracts of *B. ceiba* flowers.

Meanwhile, the water extracts of *B. ceiba* flower exhibit the highest condensed tannin content (530.605 ± 15.779 mg/g) with significant polyphenol content (165.597 ± 2.881 mg/g). In this regard, qualitative analysis of water extracts of *B. ceiba* flower shows the presence of carbohydrates, triterpenoid sterols, glycosides, flavonoids, alkaloids, quinones, saponin, and tannins [23]. The considerable number of polyphenols in water extracts of *B. ceiba* is primarily due to the significant number of highly polar compounds such as glycosides. Additionally, the considerable amount of condensed tannin in water extract (530.605 ± 15.779 mg/g) suggests that the tannins exist as oligomeric flavonoids (2-10 flavonoid monomers) rather than polymeric flavonoids or large polymers [24,25].

On the other hand, the extracts from polar aprotic solvents and nonpolar solvents exhibit the highest flavonoid content, predominated by ethyl acetate extract (392.329 ± 4.876 mg/g) followed by hexane (315.486 ± 9.688 mg/g) and acetone (277.708 ± 21.418) extracts. Thus, it can be deduced that the *B. ceiba* flower contains less polar flavonoids or flavonoid aglycones [26]. Consequently, a detailed analysis of the ethyl acetate extract of *B. ceiba* flower reveals the presence of flavonol, syringin, and a significant number of phenolic acids [21]. This is further supported by the quantitative analysis of *B. ceiba*, indicating the presence of rutin, a flavonol compound [27]. Therefore, the considerable concentration of flavonoids in the *B. ceiba* flowers is attributed to its high concentration of less polar flavonoids.

Moreover, the significant number of flavonoids in hexane extracts may suggest the presence of flavonol in the *B. ceiba* flower. This is supported by a study reporting the presence of two flavonols in the hexane extracts, quercetin, and kaempferol, extracted in *B. ceiba* flowers [28,29]. These findings are comparable to acetone extract, wherein a study shows high solubility of flavonol, particularly quercetin, in acetone solvent [30].

In summary, the considerable amount of phenols in the water and ethanol extract with relatively low flavonoid content suggest that *B. ceiba* flower contains a significant number of glycosides (i.e., cardiac glycosides, saponin glycosides, flavonol glycosides, cyanidin glucosides), including anthocyanidins, lignans, and xanthenes. Moreover, the considerable tannin content in water extract indicates that the condensed tannin exists as oligomeric flavonoids. Meanwhile, the relatively high flavonoid content in polar aprotic and nonpolar extracts suggests the presence of less polar flavonoids, such as flavonol.

3.2. *In vitro* antioxidation activity.

The antioxidative effect of *B. ceiba* flowers is evaluated using DPPH free radical scavenging activity and ferric reducing/antioxidant power (FRAP) assay.

Table 2 suggests the scavenging activity of *B. ceiba* flower extracts having ascorbic acid as the reference drug. Consequently, the ethanol extract exhibits the most potent scavenging activity, followed by water extract with a relatively small difference. However, acetone extract shows mild scavenging activity, while ethyl acetate and hexane extracts display undetectable scavenging activity using the DPPH assay. Specifically, the computed IC₅₀ (mg/mL) are: 0.068±0.001 (ascorbic acid), 0.287±0.011 (ethanol), 0.364±0.010 (water), and 2.534±0.062 (acetone).

Table 2. Antioxidant activity of *Bombax ceiba* flower extracts.

Sample Extract	DPPH assay IC ₅₀ (mg/mL)	FRAP assay (mg trolox/g extract)
Water	0.364±0.009	1658.395±97.228
EtOH	0.287±0.011	1877.130±20.574
EA	NA	395.437±3.294
Acetone	2.534±0.062	831.005±53.019
Hexane	NA	1354.068±42.828

Additionally, the antioxidative effect of *B. ceiba* flowers using ferric reduction/antioxidant power (FRAP) assay shows the highest reducing power for polar protic solvents; ethanol extracts followed by water extracts by a relatively small difference. This trend is succeeded by hexane extract, which shows considerable reducing power, followed by acetone and ethanol extracts. In this study, the antioxidant activity of *B. ceiba* flowers using FRAP assay, expressed as mg Trolox per g of extracts, are 1877.130±20.574 (ethanol), 1658.395±97.228 (water), 1354.068±42.828 (hexane), 831.005±53.019 (acetone), and 395.437±3.294 (ethyl acetate).

Accordingly, polyphenols as an antioxidant can mitigate the production of oxidative free radicals via single-electron transfer or hydrogen-atom transfer [31]. The DPPH free radical scavenging activity is based on the hydrogen-atom transfer, wherein the phenolic antioxidants donate their hydrogen atom to quench the free radical species. The resulting phenolic antioxidants in their radical form are more stable than the free radicals [31,32]. Phytochemical screening suggests that the ethanol and water extracts, having the highest polyphenol contents, contain a significant number of phenolic glycosides, flavonoids, and xanthone. More specifically, a previous study reveals that two quercetin glycosides (rutin and isoquercitrin) and xanthone (mangiferin) are the primary polyphenols exhibiting an intense scavenging activity in the *B. ceiba* flower [21]. The insignificant scavenging activity of acetone, hexane, and ethyl acetate corresponds to their minuscule number of polyphenols based on the phytochemical screening.

Meanwhile, the ferric reducing antioxidant power (FRAP) assay is based on single-electron transfer in mitigating the free radicals. The reducing power of phenolic antioxidants reduces the ferric (Fe³⁺) salt to the ferrous (Fe²⁺) complex. Similar to the DPPH assay, the quercetin glycosides and xanthone are the primary compounds attributed to the significant reducing power of *B. ceiba* flower in ethanol and water extracts, specifically of rutin and mangiferin [21]. The reducing power of the *B. ceiba* flower complements the polyphenol content of *B. ceiba* flower extracts.

3.3. *In vitro* anti-inflammatory activity.

The cytotoxicity and anti-inflammatory activity of *B. ceiba* flower extracts are assessed via the degree of inhibition of the extracts against LPS-induced nitric oxide (NO) in RAW

264.7 cells. Table 3 reveals that the extracts from polar aprotic and nonpolar solvents exhibit an inhibitory effect against NO predominated, no inhibition is observed in water, ethanol, acetone, and hexane extracts. In particular, the EA extract of *B. ceiba* flower computed IC₅₀ 101.274 µg/mL. Further, all extracts of *B. ceiba* flower manifest non-toxicity levels in RAW 264.7 cells, as observed in Table 3.

Table 3. NO inhibitory activity and cell viability of *B. ceiba* flower extract on RAW 264.7 cells.

Sample	NO inhibition (IC ₅₀ µg/mL)	Cell viability	
<i>Bombax ceiba</i> flower	Water	-	Non-toxicity
	EtOH	-	Non-toxicity
	Acetone	-	Non-toxicity
	EA	101.274	Non-toxicity
	Hexane	-	Non-toxicity

An array of inflammatory mediators and pro-inflammatory cytokines are released by RAW 264.7 cells that have been stimulated by LPS, which is a complex process that leads to inflammation [33–35]. Pro-inflammatory cytokines stimulate inducible nitric oxide synthase (iNOS), producing nitric oxide at very high concentrations exceeding the physiological nitric oxide production by a thousand-fold [36]. As a result, nitric oxide can react with superoxide at a high rate (exceeding the superoxide dismutase activity for superoxide), forming peroxynitrite, which can oxidize lipids, iron-sulfur centers, and sulfhydryl groups [37]. Accordingly, there have been no approved iNOS inhibitors for human use [38]. In line with this, the present study shows that the hexane, acetone, and ethyl acetate extracts of *B. ceiba* flower manifest anti-inflammatory activity by nitric oxide inhibition. Phytochemical screening suggests that the extracts contain a significant amount of flavonoids, such as quercetin and kaempferol, which previous studies suggest inhibits inducible nitric oxide synthase and reduces the nitrite concentration in the cell [29,39,40]. Similar studies have shown the anti-inflammatory role of flavonoids, primarily quercetin and kaempferol, in inhibiting nitric oxide synthase and have demonstrated potential treatment for COVID-19 [41–43].

3.4. Correlation of *B. ceiba* extracts on Antioxidant Activity, Total Phenolic Content, total flavonoid content, and total condensed tannins content

Principal component analysis through IBM SPSS was used to determine the relationships between the antioxidant activity (DPPH and FRAP), total phenolic content (TPC), total flavonoid content (TFC), and total condensed tannins content (TCTC) of the five extraction methods using different solvents (i.e., water, ethanol, acetone, ethyl acetate, and hexane). The software uses the fed data to perform factor and principal component analysis. Shown in Figure 1 is the generated plot of the PCA outputted by the software. Principal component 1 captures 62.539% of the total variance, whereas principal component 2 captures 22.214% of the total variance. Based on the rotated component matrix shown in Table 3, PC1 shows that there are large positive associations with total polyphenolic content (TPC), total condensed tannin content (TCTC), and antioxidant activity (FRAP). In contrast, PC2 shows large positive associations between total flavonoid content (TFC) and antioxidant activity (DPPH). The PCA plot (Figure 1) and the correlation matrix (Table 3) affirm these positive associations. Analysis of the correlation matrix shows that there is a positive correlation between total condensed tannins content (TCTC), total polyphenol content (TFC), and

antioxidant activity (FRAP), as well as between total phenolic content (TPC) and antioxidant activity (DPPH). The spatial representations in PC1 also show that TPC, TCTC, and FRAP, are closely related, as shown by their proximity in both the x and y-axis. For the analysis of principal component 2, it is observed that FRAP was highly correlated to TFC, as given by their proximity. Thus, it can be speculated that the total phenolic content is what drives the ferric-reducing antioxidant power of the *B. ceiba* flower extracts using different solvents through their capacity to carry out ferric-reducing antioxidant power as determined by the FRAP assay. Total flavonoid content is the main determinant of the antioxidant radical scavenging activities of the different solvent-extracted flowers through the hydrogen atom transfer mechanism using the DPPH assay.

Table 4. Rotated component matrix (loadings plot). Rotation method: Varimax with Kaiser normalization.

	Components	
	1	2
Total Polyphenol (TPC)	0.892	-0.363
Total Condensed Tannins (TCTC)	0.853	-
FRAP	0.752	-0.306
Total Flavonoid (TFC)	0.710	0.678
DPPH	-	0.955

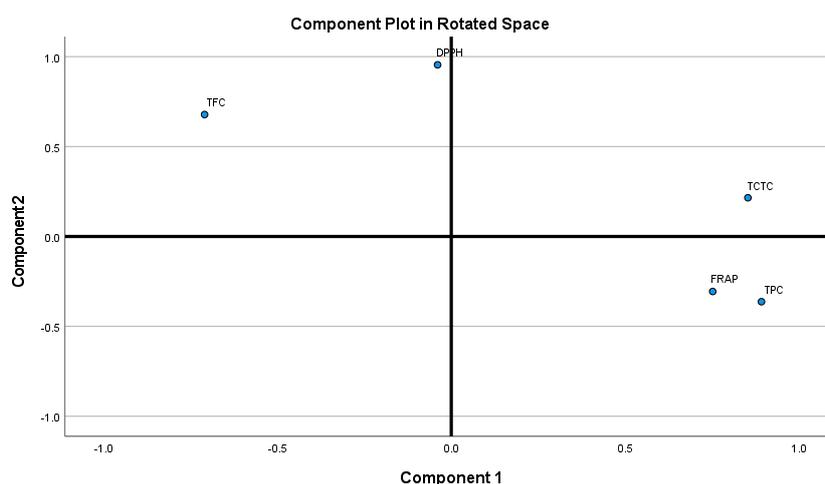


Figure 1. Principal component analysis plot generated from the IBM SPSS program. The x- and y-axis show principal components 1 and 2 that explain 62.539% and 22.214% of the total variance, respectively. N=5 data points.

4. Conclusions

The present study provides quantitative data regarding the phytochemical content (i.e., polyphenol, flavonoid, condensed tannin) of *B. ceiba* flower extracts and demonstrates its antioxidative effect and anti-inflammatory activity via nitric oxide inhibition. In particular, the ethanol extract displays the highest polyphenol content and high antioxidant activity than other extracts. Meanwhile, the anti-inflammatory activity of *B. ceiba* flower can be manifested in ethyl acetate ($IC_{50}=101.274 \mu\text{g/mL}$). Therefore, extensive study regarding the bioactive compound involved in the anti-inflammatory of *B. ceiba* flower and *in vivo* study should be conducted to determine the feasibility of *B. ceiba* as a medicinal source.

Funding

The authors are thankful to the Office of Research and Development, Chang Jung Christian University, Taiwan, for funding this research grant.

Acknowledgments

The authors are thankful to the Office of Research and Development, Chang Jung Christian University, Taiwan, for funding this research grant.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Chaudhary, P. H.; Tawar, M. G. Pharmacognostic and Phytopharmacological Overview on Bombax Ceiba. *Syst. Rev. Pharm.* **2018**, *10*, 20–25, <https://doi.org/10.5530/srp.2019.1.4>.
2. Fatima, S.; Siddiqui, A.; Khan, A. Mochrus (Bombax Ceiba Linn.): A Comprehensive Review on Pharmacology Phytochemistry, and Ethnomedicinal Uses. *TANG Humanit. Med.* **2019**, *9*, 41–45, <https://doi.org/10.5667/TANG.2019.0019>.
3. Ernst, E. Major Herbs of Ayurveda. *Focus Altern. Complement. Ther.* **2010**, *8*, 98–98, <https://doi.org/10.1111/j.2042-7166.2003.tb05765.x>.
4. Rajput, R. T. Ethanomedicine and Pharmacology of Semal (Bombax Ceiba L.)- A Indian Medicinal Plant: A Review. *Agric. Rev.* **2021**, *43*, 145–153, <https://doi.org/10.18805/ag.R-1942>.
5. Rani, S.; Rahman, K.; Sultana, A. Ethnomedicinal and Pharmacological Activities of Mochrus (Bombax Ceiba Linn.): An Overview. *TANG Humanit. Med.* **2016**, *6*, 21–29, <https://doi.org/10.5667/TANG.2015.0025>.
6. Minsakorn, S.; Watthanadirek, A.; Poolsawat, N.; Puttarak, P.; Chawengkirttikul, R.; Anuracpreeda, P. The Anthelmintic Potentials of Medicinal Plant Extracts and an Isolated Compound (Rutin, C₂₇H₃₀O₁₆) from Terminalia Catappa L. against Gastrothylax Crumenifer. *Vet. Parasitol.* **2021**, *291*, 109385, <https://doi.org/10.1016/j.vetpar.2021.109385>.
7. Aly, O.; R. Elias, T.; N. Ashour Mervet H. Agaibiyi, M.; I. Rasheed, W.; N. Yassen, N.; Diab, Y. Antidiabetic and Hepatoprotective Activities of Bombax Ceiba Extract in Obese Rats with Metabolic Syndrome. *PLANT Arch.* **2021**, *21*, 748–756, <https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.113>.
8. Sharma, N.; Kispotta, S.; Mazumder, P. Immunomodulatory and Anticancer Activity of Bombax Ceiba Linn Leaf Extract. *Asian Pac. J. Trop. Biomed.* **2020**, *10*, 426, <https://doi.org/10.4103/2221-1691.290134>.
9. Diab, K.; El-Shenawy, R.; Helmy, N.; El-Toumy, S. Polyphenol Content, Antioxidant, Cytotoxic, and Genotoxic Activities of Bombax Ceiba Flowers in Liver Cancer Cells Huh7. *Asian Pac. J. Cancer Prev.* **2022**, *23*, 1345–1350, <https://doi.org/10.31557/APJCP.2022.23.4.1345>.
10. Chauahn, prashant; Monika. Formulation And Evaluation of Antibacterial Gel Containing Ethanol Extract of Thorns of Bombax Ceiba. *Int. J. Pharm. Sci. Res.* **2022**, *13*, 4199–4204. [https://doi.org/10.13040/IJPSR.0975-8232.13\(10\).4199-04](https://doi.org/10.13040/IJPSR.0975-8232.13(10).4199-04).
11. Chaudhary, H. P.; Rai, P. D.; Deore, S. L.; Farooqui, I. A.; Khadabadi, S. S. Comparative Aphrodisiac Activity of Formulated Tablet of Bombax Ceiba Linn. Extract with Sildenafil Citrate Tablet in Male Mice. *Research & Review: A Journal of Pharmacology.* **2015**, *5*, 19–26. <https://pharmajournals.stmjournals.in/index.php/RRJoP/article/view/570>.
12. Nguyen, H. D.; Le, T. H.; Duong, T. T. Screening For Vietnamese Plant Extracts with Potential Benefit for Anti-Osteoclastogenesis. *Vietnam J. Sci. Technol.* **2021**, *59*, 507, <https://doi.org/10.15625/2525-2518/59/4/15825>.
13. Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158, <https://www.ajevonline.org/content/16/3/144>.
14. Chang, C.-C.; Yang, M.-H.; Wen, H.-M.; Chern, J.-C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colometric Methods. *J. Food Drug Anal.* **2020**, *10*, <https://doi.org/10.38212/2224-6614.2748>.
15. Broadhurst, R. B.; Jones, W. T. Analysis of Condensed Tannins Using Acidified Vanillin. *J. Sci. Food Agric.* **1978**, *29*, 788–794, <https://doi.org/10.1002/jsfa.2740290908>.
16. Blois, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* **1958**, *181*, 1199–1200, <https://doi.org/10.1038/1811199a0>.
17. Benzie, I. F. F.; Strain, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76, <https://doi.org/10.1006/abio.1996.0292>.

18. Wu, J.; Zhang, X.-H.; Zhang, S.-W.; Xuan, L.-J. Three Novel Compounds from the Flowers Of Bombax Malabaricum. *Helv. Chim. Acta* **2008**, *91*, 136–143, <https://doi.org/10.1002/hlca.200890004>.
19. Gopal, H.; Gupta, R. K. Chemical Constituents of Salmalia Malabarica Schott and Endl. Flowers. *J. Pharm. Sci.* **1972**, *61*, 807–808, <https://doi.org/10.1002/jps.2600610534>.
20. Niranjana, G.; Gupta, P. Anthocyanins from the Flowers of Bombax Malabaricum. *Planta Med.* **1973**, *24*, 196–199, <https://doi.org/10.1055/s-0028-1099488>.
21. Zhang, Y.-B.; Wu, P.; Zhang, X.-L.; Xia, C.; Li, G.-Q.; Ye, W.-C.; Wang, G.-C.; Li, Y.-L. Phenolic Compounds from the Flowers of Bombax Malabaricum and Their Antioxidant and Antiviral Activities. *Molecules* **2015**, *20*, 19947–19957, <https://doi.org/10.3390/molecules201119660>.
22. Scogin, R. Reproductive Phytochemistry of Bombacaceae. *Aliso* **1986**, *11*, 377–385, <https://doi.org/10.5642/aliso.19861103.09>.
23. Wanjari, M. M.; Gangoria, R.; Dey, Y. N.; Gaidhani, S. N.; Pandey, N. K.; Jadhav, A. D. Hepatoprotective and Antioxidant Activity of Bombax Ceiba Flowers against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. *Hepatoma Res.* **2016**, *2*, 144, <https://doi.org/10.20517/2394-5079.2015.55>.
24. Bennick, A. Interaction of Plant Polyphenols with Salivary Proteins. *Crit. Rev. Oral Biol. Med.* **2002**, *13*, 184–196, <https://doi.org/10.1177/154411130201300208>.
25. de Hoyos-Martínez, P. L.; Merle, J.; Labidi, J.; Charrier – El Bouhtoury, F. Tannins Extraction: A Key Point for Their Valorization and Cleaner Production. *J. Clean. Prod.* **2019**, *206*, 1138–1155, <https://doi.org/10.1016/j.jclepro.2018.09.243>.
26. Ferreira, O.; Pinho, S. P. Solubility of Flavonoids in Pure Solvents. *Ind. Eng. Chem. Res.* **2012**, *51*, 6586–6590, <https://doi.org/10.1021/ie300211e>.
27. Revealing the Phytochemical Profile and Antioxidant Potential of a Medicinal Folklore: Bombax Ceiba L. *J. Bioresour. Manag.* **2017**, *4*, <https://doi.org/10.35691/JBM.7102.0074>.
28. Joshi, K. R.; Devkota, H. P.; Yahara, S. Chemical Analysis of Flowers of Bombax Ceiba from Nepal. *Nat. Prod. Commun.* **2013**, *8*, 1934578X1300800, <https://doi.org/10.1177/1934578X1300800508>.
29. Sharma, S.; Vig, A. P. Preliminary Phytochemical Screening and *In vitro* Antioxidant Activities of Parkinsonia Aculeata Linn. *BioMed Res. Int.* **2014**, *2014*, 1–8, <https://doi.org/10.1155/2014/756184>.
30. Chebil, L.; Humeau, C.; Anthoni, J.; Dehez, F.; Engasser, J.-M.; Ghoul, M. Solubility of Flavonoids in Organic Solvents. *J. Chem. Eng. Data* **2007**, *52*, 1552–1556, <https://doi.org/10.1021/je7001094>.
31. Amarowicz, R.; Pegg, R. B. Natural Antioxidants of Plant Origin. In *Advances in Food and Nutrition Research*; Elsevier, **2019**; *90*, 1–81, <https://doi.org/10.1016/bs.afnr.2019.02.011>.
32. Huang, D.; Ou, B.; Prior, R. L. The Chemistry behind Antioxidant Capacity Assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856, <https://doi.org/10.1021/jf030723c>.
33. Zhou, M.; Tang, Y.; Liao, L.; Liu, M.; Deng, Y.; Zhao, X.; Li, Y. Phillygenin Inhibited LPS-Induced RAW 264.7 Cell Inflammation by NF-KB Pathway. *Eur. J. Pharmacol.* **2021**, *899*, 174043, <https://doi.org/10.1016/j.ejphar.2021.174043>.
34. Wang, L.; Gu, J.; Zong, M.; Zhang, Q.; Li, H.; Li, D.; Mou, X.; Liu, P.; Liu, Y.; Qiu, F.; Zhao, F. Anti-Inflammatory Action of Physalin A by Blocking the Activation of NF-KB Signaling Pathway. *J. Ethnopharmacol.* **2021**, *267*, 113490, <https://doi.org/10.1016/j.jep.2020.113490>.
35. Yang, L.; Cao, L.; Li, C.; Li, X.; Wang, J.; Chen, H.; He, J. Hostaflavone A from Hosta Plantaginea (Lam.) Asch. Blocked NF-KB/INOS/COX-2/MAPKs/Akt Signaling Pathways in LPS-Induced RAW 264.7 Macrophages. *J. Ethnopharmacol.* **2022**, *282*, 114605, <https://doi.org/10.1016/j.jep.2021.114605>.
36. Sharma, J. N.; Al-Omran, A.; Parvathy, S. S. Role of Nitric Oxide in Inflammatory Diseases. *Inflammopharmacology* **2007**, *15*, 252–259, <https://doi.org/10.1007/s10787-007-0013-x>.
37. Pfeilschifter, J. Therapeutic Strategies For The Inhibition Of Inducible Nitric Oxide Synthase—Potential For A Novel Class Of Anti-Inflammatory Agents. *Cell Biol. Int.* **1996**, *20*, 51–58, <https://doi.org/10.1006/cbir.1996.0008>.
38. Cinelli, M. A.; Do, H. T.; Miley, G. P.; Silverman, R. B. Inducible Nitric Oxide Synthase: Regulation, Structure, and Inhibition. *Med. Res. Rev.* **2020**, *40*, 158–189, <https://doi.org/10.1002/med.21599>.
39. Al-Khayri, J. M.; Sahana, G. R.; Nagella, P.; Joseph, B. V.; Alessa, F. M.; Al-Mssallem, M. Q. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules* **2022**, *27*, 2901, <https://doi.org/10.3390/molecules27092901>.
40. Yu, J. S.; Lim, S. H.; Lee, S. R.; Choi, C.-I.; Kim, K. H. Antioxidant and Anti-Inflammatory Effects of White Mulberry (Morus Alba L.) Fruits on Lipopolysaccharide-Stimulated RAW 264.7 Macrophages. *Molecules* **2021**, *26*, 920, <https://doi.org/10.3390/molecules26040920>.
41. Levita, J.; Alfiana, A.; Bernadette, M.; Fajri Nuwa, R.; Sumiwi, S. A.; Saptarini, N. M.; Amien, S. Potential of Ageratum Conyzoides in Inhibiting Nitric Oxide Synthase. *Pak. J. Biol. Sci.* **2021**, *24*, 840–846, <https://doi.org/10.3923/pjbs.2021.840.846>.
42. Chansiw, N.; Champakam, S.; Chusri, P.; Pangjit, K.; Srichairatanakool, S. Quercetin-Rich Ethanolic Extract of Polygonum Odoratum Var Pakphai Leaves Decreased Gene Expression and Secretion of Pro-Inflammatory Mediators in Lipopolysaccharide-Induced Murine RAW264.7 Macrophages. *Molecules* **2022**, *27*, 3657, <https://doi.org/10.3390/molecules27123657>.

43. Khazdair, M.; Anaeigoudari, A.; Agbor, G. Anti-Viral and Anti-Inflammatory Effects of Kaempferol and Quercetin and COVID-2019: A Scoping Review. *Asian Pac. J. Trop. Biomed.* **2021**, *11*, 327, <https://doi.org/10.4103/2221-1691.319567>.