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Sequential Solvent Extraction of *Caesalpinia pulcherrima* Yellow Flowers Reveals Potent Antioxidant, Antimicrobial, and Anticancer Activities Against Various Human Cancer Cell Lines

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Abstract

While the pursuit and development of novel drugs to combat emerging diseases is ongoing, many newly characterized compounds have been discovered in different parts of traditional medicinal plants. Numerous investigations have scrutinized the phytochemical properties of the leaf, flower, seed, and bark of *Caesalpinia pulcherrima*, revealing its noteworthy attributes as an herbal remedy employed in the treatment of diverse ailments. Our research aims to re-evaluate this medicinal plant, with a focus on the yellow flower, employing a sequential solvent extraction technique. Our findings revealed that a methanolic extract exhibited the highest antioxidant capacity in 3 colorimetric antioxidant assays: DPPH, ABTS, and FRAP. Extracts from the yellow flower of *C. pulcherrima* exhibited antimicrobial resistance against a wide range of both pathogenic Gram-positive and *Acinetobacter baumannii* isolates. Additionally, these extracts manifested anticancer properties against various cancer cell lines. Our investigation marks the first inhibition of cell proliferation of the bile duct cancer cell line (KKU-213A) by this crude extract. This study contributes valuable insights to the ongoing research of potential phytochemical properties from the yellow flower of *C. pulcherrima*, providing additional information for further research on the separation and purification of crude extracts from the yellow flower of *C. pulcherrima*. Ultimately, our study presents a promising approach to specifically screening phytochemicals from *C. pulcherrima* flowers that could be developed into new antibiotic drugs or anticancer agents.

Keywords: *Caesalpinia pulcherrima*, Antimicrobial resistance, Anticancer, Antioxidant activity, Phytochemical properties

Introduction

Traditional medicine is practiced in many countries and provides alternative treatments for a number of diseases [1,2]. Traditional medicine uses different parts of plants that have a wide range of beneficial bioactive properties [3,4]. Edible flowers contain an array of bioactive compounds within their cells, including pigments, making them a sought-after dietary choice to be consumed fresh or dried [5,8].

The yellow peacock flower (*Caesalpinia pulcherrima*) has been used in Thai traditional medicine for many years [9]. Different components of *Caesalpinia* sp. have been employed for the therapeutic management of diverse human ailments including gastritis and inflammation of the intestines [10], as well

as diarrhea and dysentery (through the use of a leaf-based tea) [11]. The leaves are used to alleviate flatulence, ulcers, and hepatitis [12]. The aerial parts and leaves are used to address problems related to respiratory disorders [13]. For traditional medicine usage, the bark and leaves of this plant are used to combat fever and bronchitis [13,14] and the whole plant extract is used to treat rheumatoid arthritis in rat models [15] suggesting that different parts of *Caesalpinia* serve different purposes in traditional medicine [13]. Some of the earliest reports of this species showed that various cultures were aware of its medicinal properties worldwide. In India, it is used for its potential to stimulate menstruation and induce abortions [16,17]. Studies in India also found the leaves and flowers to have antioxidant [18] and antibacterial properties that were effective against various bacterial infections [19-21].

Additionally, extracts derived from various parts of *Caesalpinia* have been employed to investigate their anticancer properties against diverse cancer cell lines. Notably, pulcherrimin, extracted from the roots of *C. pulcherrima*, demonstrated cytotoxicity against 3 distinct cancer cell lines: MCF-7 (breast cancer cell line), HeLa, and PC-3 (prostate cancer cell line) [22]. Another root extract from *C. pulcherrima*, containing furanoditerpenoids, exhibited cytotoxic activity against human oral carcinoid cancer, human breast cancer, and small cell lung cancer [23]. The essential oil extract from the flowers of *C. peltophoroides* demonstrated cytotoxic activity against U87, HCT, and A2058 cell lines [24]. Furthermore, the aqueous extract of *C. bonduc* bark exhibited the inhibition of cell proliferation in 2 cancer cell lines: HeLa and VERO [25]. The exploration of plant extracts appears to be ceaseless, with new possibilities for compounds and phytochemical activities being discovered every single day. This study is an integral part of this ongoing process, providing comprehensive information to serve as a resource for traditional medicine. This study re-examines *C. pulcherrima* yellow flowers, focusing on their antimicrobial and anticancer properties. Using sequential extraction with hexane, ethyl acetate, methanol, and water, we reveal for the first time the flower's ability to inhibit clinical bacterial strains, and demonstrate its cytotoxicity against the bile duct cancer cell line (KKU-213A).

Material and methods

Sample preparation

The flower material was taxonomically identified as *Caesalpinia pulcherrima* according to the reference taxonomic book *Flora of Thailand, Volume 4, Part 1* [26]. Yellow flowers of *C. pulcherrima* were cleaned with distilled water and dried for 24 h in an oven at 70 °C. Once dried, the flowers were finely ground into powder and sequentially extracted with hexane, ethyl acetate, methanol, and water. The powdered flower was initially mixed with hexane at a 1:10 ratio and shaken at 200 rpm for 16 h. The resulting extract was filtered through Whatman filter paper, and the clear filtrate was collected. The residual powder was extracted under the same conditions using ethyl acetate, methanol, and water. The extracts obtained from hexane, ethyl acetate, and methanol were concentrated using a Buchi R-134 rotary evaporator, while the water extract was dried with a freeze-dryer. This sequential extraction method was designed to isolate the various phytochemicals in the yellow flowers of *C. pulcherrima* based on their chemical polarity.

Total phenolic contents

To assess total phenolic content, the *C. pulcherrima* flower extract from various solvent extractions was dissolved in 70 % ethanol and kept at 4 °C for 1 h with periodic shaking. The total phenolic content (TPC) was then determined using a 96-well microplate format, following a previously established method [27] with some adjustments. Each reaction involved adding 20 µL of the extracts, followed by 100 µL of 10 % Folin-Ciocalteu reagent (Sisco Research Laboratories, India) and 80 µL of 7.5 % Na_2CO_3. The mixture was then incubated at room temperature for 1 h. 70 % ethanol served as the blank, and various concentrations of a gallic acid standard (Merck Co., Germany) were used to generate a standard curve. TPC was determined by measuring the absorbance at 765 nm and expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Antioxidant activities

DPPH free radical scavenging activity

To assess the free radical scavenging capacity, the *C. pulcherrima* yellow flower extract from various solvent extractions was dissolved in 70 % ethanol and

shaken continuously for 1 h at 4 °C. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was conducted using a 96-well microplate format [28]. Each reaction involved adding 190 µL of DPPH solution to 10 µL of extract samples. 70 % ethanol was used as the control, and various concentrations of Trolox were used to generate a standard curve. The total reaction volume of 200 µL was incubated at room temperature for 1 h. Absorbance was measured at 517 nm, and the percentage of DPPH inhibition was calculated using the following equation:

$$Inhibition\ of\ DPPH\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \quad (1)$$

The DPPH scavenging ability was measured as the Trolox Equivalent Antioxidant Capacity (TEAC) and reported in milligrams of Trolox equivalents per gram of dry weight (mg Trolox/g DW).

ABTS free radical scavenging activity

A working $ABTS^+$ solution was prepared by reacting 40 mM potassium persulfate ($K_2S_2O_8$) with 7 mM ABTS and incubating it for 16 h at 4 °C. A resulting solution was then diluted with 50 mM phosphate buffer (pH 7.4) until an absorbance reached approximately 0.7 at 734 nm [29]. Extract samples from various solvent extractions were dissolved in 50 mM phosphate buffer (pH 7.4). The ABTS free radical scavenging assay was performed using a 96-well microplate spectrophotometer. For each reaction, 20 µL of the sample was mixed with 180 µL of the diluted $ABTS^+$ solution. Fifty mM phosphate buffer (pH 7.4) served as the blank, and Trolox standards at different concentrations were used to create a standard curve. The mixtures were incubated at 30 °C for 6 min, and the absorbance at 734 nm was then measured. The percentage inhibition of the $ABTS^+$ radical was determined using the following equation:

$$\%\ inhibition = [\frac{(A_{control} - A_{sample})}{A_{control}} \times 100] \quad (2)$$

The ABTS scavenging activity was measured as the TEAC and reported in milligrams of Trolox equivalents per gram of dry weight (mg Trolox/g DW).

Ferric reducing antioxidant power (FRAP)

The FRAP assay was conducted following the previous study with some modifications [30]. The FRAP reagent was prepared by mixing 10 mL of 300 mM acetate buffer (pH 3.6) with 1 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 mL of 20 mM $FeCl_3$. Each reaction was carried out in a 96-well plate by adding 150 µL of FRAP reagent and 20 µL of extract. Trolox served as the standard. The reaction mixtures were incubated in the dark at 25 °C for 10 min, and the absorbance was then recorded at 593 nm. The reducing capacity was determined and expressed as milligrams of Trolox equivalents per gram of dry weight (mg Trolox/g DW).

Determination of antimicrobial activity

The antimicrobial properties of *C. pulcherrima* yellow flower extract were investigated using the following **13 Gram-positive bacteria**: *Bacillus cereus* ATCC11778, *Bacillus subtilis* 7988 (Clinical isolate), *Bacillus subtilis* ATCC6051, *Enterococcus faecalis* 4232 (Clinical isolates), *Enterococcus faecalis* 3532 (Clinical isolates), *Listeria monocytogenes* (Clinical isolates), *Staphylococcus agalactiae* (Clinical isolates), *Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC29213, Methicillin Resistant *Staphylococcus aureus* (Clinical isolate), *Staphylococcus epidermitis* 35984, *Streptococcus mutans* ATCC19615 and *Streptococcus pyogenes* ATCC49619 and **9 Gram-negative bacteria**: *Acinetobacter baumannii* ATCC19606, multidrug-resistant *Acinetobacter baumannii* (MDR), *Escherichia coli* ATCC25922, *Escherichia coli* O157: H7, *Klebsiella pneumoniae* ATCC70063, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* serotype Typhi (Clinical isolates), *Shigella enteritis* (Clinical isolates) and *Vibrio cholerae* (Clinical isolates).

Screening for antibacterial activities

Both Gram-positive were cultured at 37 °C on nutrient agar (NA; Gibco, Thermo Fisher Scientific, Inc.) and Gram-negative bacteria were cultured on Luria-Bertani (LB) agar (Gibco, Thermo Fisher Scientific, Inc.) under the same condition. Before the screening, the cultures were grown overnight in Muller Hilton broth (MHB; Gibco, Thermo Fisher Scientific,

Inc.) at 37 °C and then diluted to approximately 1×10^7 colony-forming units (CFU/mL) for all tests.

Antimicrobial activity by overlay spotted assay

For screening antimicrobial activity [31], bacterial cultures grown overnight were diluted into approximately 1×10^7 CFU/mL and then swabbed onto MH agar plates in three different directions. Each *C. pulcherrima* extract was prepared at a concentration of 100 mg/mL in DMSO and then sterilized through a 0.22 sterile hydrophilic PTFE filter. To assess antimicrobial activity, 10 µL of the highest concentration of each extract (100 mg/mL) was spotted onto swabbed MH agar plates. All plates were incubated overnight at 37 °C. The bacterial lawn was examined for clear spots to determine the sensitivity of bacteria to the extracts.

The overlay spotted assay was used to determine MICs by adapting the methodology from Sirisarn *et al.* [31]. In brief, bacterial cultures were grown and diluted into approximately 1×10^7 CFU/mL. The diluted bacterial cultures were swabbed onto MH agar plates in 3 different directions. Four extracts underwent a 2-fold dilution starting from 100 to 0.098 mg/mL. To apply the extracts, the amount of 10 µL of each concentration of all extracts together with 10 µg/mL of ampicillin and gentamycin as positive control specialized for Gram-positive and Gram-negative bacteria respectively was spotted onto the MH agar plates which were previously swabbed. All plates were incubated at 37 °C. After overnight incubation, the clear spots were observed to ascertain the concentration as MICs of each extract, defined as the concentration at which a clear spot appeared on the bacterial lawn. All tests were conducted in triplicate.

Anticancer

Cell culture

In this study, four human cancer cell lines (KKU-213A, A549, HT-29, and MCF-7) were used to investigate the anticancer activity of the four *C. pulcherrima* extracts. Two normal cell lines (MMNK-1 and HDF) were included in the study for comparative analysis. KKU-213A, a human cholangiocarcinoma (CCA) cell line and the immortalized human cholangiocyte cell line (MMNK-1) were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan). All cell lines were cultured in a humidified incubator at 37 °C with 5 % CO_2 in high-glucose Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Thermo Fisher Scientific, Inc.) supplemented with heat-inactivated 10 % fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc.) and 1 % antibiotics (100 IU penicillin and 100 µg/mL streptomycin; Gibco, Thermo Fisher) in a humidified incubator at 37 °C and 5 % CO_2. Logarithmically growing cells were used in all subsequent experiments.

Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine the cytotoxic effects of *C. pulcherrima* extracts. Briefly, 3×10^3 cells per well were plated onto 96-well plates for 24 h and then grown in either DMEM with 10 % FBS as control or DMEM containing different concentrations of *C. pulcherrima* extracts (62.5, 125, 250, 500, 750, 1,000 µg/mL). The plates were then incubated for another 72 h. MTT solution (0.5 mg/mL) was added to each well and incubated for 2 h. Excess MTT solution was then removed and formazan crystals were solubilized with 100 µL of DMSO. After recording the absorbance of the reactions at 540 nm with a microplate reader (TECAN, SPARK), the viability rate of cells in each treatment group was calculated relative to the control group, which was defined as 100 % cell viability.

GC-MS analysis

Gas chromatography (GC) coupled with triple quadrupole mass spectrometry (MS; Agilent 7000D, Agilent-7890) was used to analyze four extracts obtained from hexane, ethyl acetate, methanol, and water (aqueous) solvent extractions. The less polar extracts, hexane and ethyl acetate, were separated using an Agilent 19091-433 HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm; maximum temperature 340 °C). In contrast, the more polar extracts, methanol and aqueous, were separated using an Agilent 19091N-113I HP-INNOWax capillary column (30 m × 0.25 mm × 0.25 µm; maximum temperature 340 °C). Ultra-high purity helium was used as the carrier gas at a constant flow rate of 1 mL/min, with the ionizing energy set to 70 eV. The oven temperature was programmed to increase from 60 to 300 °C at a rate of 3 °C/min. Crude extract samples were diluted with ethanol at a 1:10 ratio

(v/v). Data were acquired in full-scan mode in the 35 - 550 amu range. Individual chromatogram peaks were identified using the Wiley10 and National Institute of Standards and Technology (NIST) version 17 databases.

Statistical analysis

Five replicates were conducted in each experiment unless otherwise specified. Normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was tested using the Bartlett test. Analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test were employed for comparative analysis. Statistically significant differences were indicated when the *p*-value was less than 0.05. All data are reported as mean ± standard deviation (SD).

Results and discussion

Total phenolic contents

Phenolics make up the vast majority of secondary metabolic compounds. The hydroxyl group is the key component of phenolic compounds and is fundamental to their biochemical properties. The hydroxyl group can initiate antioxidant activity by donating hydrogen and lone-pair electrons to neutralize free radicals and initiate antimicrobial activity by destabilizing the bacterial cell wall or cell membrane. Among the four extracts, the methanol extract (298.76 ± 14.88 mg GAE g^{-1} DW) had the highest TPC followed by the aqueous (85.03 ± 3.55 mg GAE g^{-1} DW), ethyl acetate (54.42 ± 2.49 mg GAE g^{-1} DW) and hexane (34.16 ± 1.32 mg GAE g^{-1} DW) extracts (**Figure 1(A)**).

A previous investigation of the TPC in various parts of *C. pulcherrima* revealed that a methanol leaf extract had slightly higher TPC than flower and seed methanol [32]. Conversely, another study employing methanol extraction demonstrated higher TPC levels in flower extract than in leaf extract [33]. In the present study, the methanol extract of *C. pulcherrima* flower exhibited the highest TPC, and could therefore be a reservoir of bioactive compounds with pharmaceutical applications.

In vitro antioxidant activity

We used the three most widely used colorimetric assays, DPPH, ABTS and FRAP, to investigate the antioxidant capacity of the *C. pulcherrima* flower extracts.

DPPH free radical scavenging activity

The DPPH assay is a widely used method for evaluating plant extracts' free radical scavenging activity. In the present study, the methanol extract of *C. pulcherrima* flowers exhibited the highest DPPH scavenging activity, with a value of 8.98 ± 1.21 mg TEAC/g DW, followed by the aqueous extract at 3.33 ± 0.75 mg TEAC/g DW. In contrast, the ethyl acetate and hexane extracts demonstrated relatively low activity, yielding 0.83 ± 0.05 and 0.28 ± 0.12 mg TEAC/g DW, respectively (**Figure 2(B)**). Interestingly, another study reported that the ethyl acetate extract of *C. pulcherrima* flowers showed the highest DPPH scavenging activity (as indicated by a low IC_{50}), followed by the methanol, aqueous, and petroleum ether extracts [34]. Additionally, a separate DPPH assay revealed that the seed extract of *C. pulcherrima* exhibited the strongest antioxidant activity, followed by the flower and leaf extracts [32], a trend that paralleled the total phenolic content (TPC), as seen in the present study. Collectively, these findings suggest that higher phenolic content correlates with greater antioxidant activity.

ABTS radical scavenging activity

The ABTS assay, similar to the DPPH assay, quantitatively evaluates the single electron transfer mechanism responsible for the reduction of $ABTS^{\bullet+}$ to ABTS. A distinct advantage of the ABTS assay is its ability to assess antioxidant activity under a wide range of pH conditions. In this study, we measured antioxidant capacity at the physiological pH of 7.4. The methanol extract exhibited the highest ABTS scavenging activity (311.36 ± 44.50 mg TEAC/g DW), followed by the aqueous extract (63.96 ± 2.40 mg TEAC/g DW), ethyl acetate extract (31.38 ± 1.73 mmol TEAC/g DW), and hexane extract (8.20 ± 0.33 mmol TEAC/g DW) (**Figure 2(C)**). The scavenging capacities determined by both the DPPH and ABTS assays showed a consistent trend, aligning with previous findings for methanol extracts of *C. pulcherrima* flowers and leaves [33]. A recent study assessing antioxidant capacity in plants from the

Lamiaceae family using multiple methods, including DPPH and ABTS assays, found a positive correlation between the 2 approaches [35]. The results of the current ABTS assay suggest that antioxidants in *C. pulcherrima* flower extracts may retain their functionality where the pH is similarly 7.4 *in vivo* as in the *in vitro* ABTS assay.

Ferric reducing antioxidant power

The FRAP assay was used to ascertain the antioxidant capacity of water-soluble *C. pulcherrima* flower in the acidic condition (pH 3.6). The FRAP assay quantifies antioxidant capacity by assessing the development of a blue coloration indicative of the reduction of the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to its ferrous (Fe^{2+}-TPTZ) form.

The highest FRAP scavenging capacity was found in the methanol (0.89 ± 0.01 mg TEAC g^{-1} DW) extract, followed by the aqueous (0.62 ± 0.01 mg TEAC g^{-1} DW), ethyl acetate (0.44 ± 0.02 mg TEAC g^{-1} DW), and hexane (0.31 ± 0.06 mg TEAC g^{-1} DW) extracts (**Figure 2(D)**). Therefore, the pattern of antioxidant activity produced by the *C. pulcherrima* flower extracts in the FRAP assay was similar to the patterns they produced in the DPPH and ABTS antioxidant assays and was again supported by the results of TPC analysis. This similar pattern of results has also been seen in other studies that compared different parts of *C. pulcherrima* [32,33].

In three *in vitro* antioxidant assays, our *C. pulcherrima* flower extracts demonstrated a consistent pattern of results within different environments. The DPPH assay was performed in ethanol, the ABTS assay was conducted in a phosphate buffer at pH 7.4, and the FRAP assay was performed at pH 3.6. The methanolic extract exhibited the highest antioxidant capacity in every assay. The findings of these assays suggest that TPC was the principal determinant of bioactivity, representing a spectrum of secondary metabolites, each of which contributed to the effective scavenging of free radicals *in vitro* across diverse chemical environments.

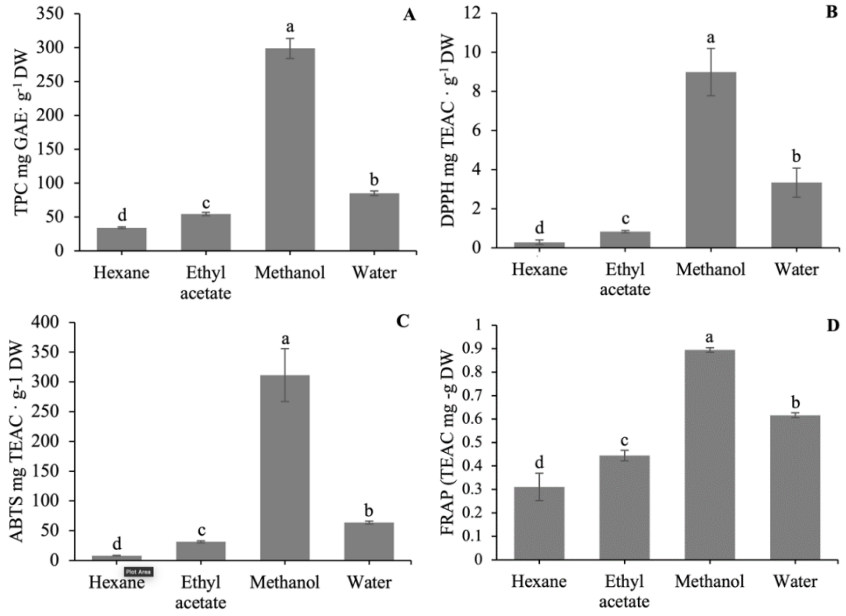


Figure 1 Total phenolic contents (TPC) (A) and *in vitro* antioxidant capacities of the yellow flower of *C. pulcherrima* extracted with different solvents were determined by (B) DPPH, (C) ABTS and (D) FRAP assays. Data are reported as means ± SD. Different letters indicate a significant difference ($p < 0.05$) indicated by ANOVA. Five replicate experiments were conducted.

Antibacterial activity

The screening using overlay spotted assay revealed antibacterial variations of the four extracts against pathogenic bacteria. All extracts were active against *Bacilli* showing a clear spot on *Bacilli* lawn. Moreover, it was observed that 3 extracts: Hexane, ethyl acetate and aqueous extract except methanol extract interestingly exhibited a wide range of antimicrobial activity for other Gram-positive bacteria: *Enterococcus faecalis* 4232 (Clinical isolates), *Enterococcus faecalis*

3532 (Clinical isolates), *Listeria monocytogenes* (Clinical isolates), *Staphylococcus agalactiae* (Clinical isolates), *Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC29213, Methicillin Resistant *Staphylococcus aureus* (Clinical isolate), *Staphylococcus epidermitis* 35984, *Streptococcus mutans* ATCC19615 and *Streptococcus pyogenes* ATCC49619. The extracts, including hexane, ethyl acetate and aqueous extract were active against *Acinetobacter baumannii* ATCC19606, and the hexane, methanolic and aqueous extracts were active against a multidrug-resistant isolate of *A. baumannii* where other Gram-negative bacteria were resistant to all extracts (**Table 1**).

To determine the lowest concentration using overlay spotted assay of all extracts inhibiting pathogenic bacteria, the concentrations of all extracts varied from 0.391 to 100 mg/mL depending on the types of bacteria and extract solvents used for examining the MICs. The ethyl acetate and hexane extracts emerged as particularly potent against Gram-positive bacteria, especially *Bacillus spp*, exhibiting the lowest MIC values from 0.391 to 1.563 mg/mL. *Staphylococcus aureus* strains, including MRSA, were the second most susceptible, with MICs ranging from 3.125 to 12.5 mg/mL for both hexane and ethyl acetate extracts. In contrast, the MICs of the aqueous extract ranged from 6.25 to 100 mg/mL for all susceptible Gram-positive bacteria. Specific to *A. baumannii* isolates, the lowest concentrations of the 3 extracts - hexane, ethyl acetate and aqueous - against *A. baumannii* ATCC19606 were in a range of 25 - 100 mg/mL and lower in multidrug-resistant isolate at a range of 3.125 - 25 mg/mL with different choices of hexane, methanol and aqueous extract as shown in **Table 2** possibly due to the morphological changes of the cell wall found in the resistance isolates as found in green tea extract [36].

The previous studies on antimicrobial properties have shown variations of antimicrobial activities against different bacteria due to variations of bacteria, antimicrobial resistance profiles, different parts of the *C. pulcherrima*, different solvents and different geographical locations. The study by Pulipati *et al.* [20] using orange flower macerated by hexane, chloroform, acetone, ethanol, methanol and water solvent showed antimicrobial activity against *B. subtilis*, *S. aureus*, *E. faecalis*, *P. aeruginosa* and *K. pneumoniae*, producing inhibition zones with diameters ranging from 5 to 15 mm. In addition, there were other studies on the antimicrobial activity of the orange flower of *C. pulcherrima* against a wider range of pathogens including *Candida albicans* [37] and *E. coli* [38]. Apart from the flower of *C. pulcherrima*, other parts such as leaves and bark were used to demonstrate antimicrobial properties against Gram-negative bacteria: *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S.* Typhi [39]. These findings suggest that the *C. pulcherrima* flower extract can be a promising herbal candidate used for infective control.

Table 1 Antimicrobial activity using overlay spotted assay of different solvent extracts of the yellow flower of *C. pulcherrima* screened against various pathogenic bacteria.

Pathogenic isolates	Antimicrobial activity of extracts (100 mg/mL)				Positive control (amp/gen)	Negative control (1 % DMSO)
	Hexane	Ethyl acetate	Methanol	Aqueous		
Gram-positive bacteria						
Bacillus cereus ATCC11778	+	+	+	+	+*	–
Bacillus subtilis 7988 (Clinical isolate)	+	+	+	+	+*	–
Bacillus subtilis ATCC6051	+	+	+	+	+*	–
Enterococcus faecalis 4232 (Clinical isolates)	+	+	–	+	+*	–
Enterococcus faecalis 3532 (Clinical isolates)	+	+	–	+	+*	–
Listeria monocytogenes (Clinical isolates)	+	+	–	+	+*	–

Pathogenic isolates	Antimicrobial activity of extracts (100 mg/mL)				Positive control (amp/gen)	Negative control (1 % DMSO)
	Hexane	Ethyl acetate	Methanol	Aqueous		
Staphylococcus agalactiae (Clinical isolates)	+	+	–	+	+*	–
Staphylococcus aureus ATCC25923	+	+	–	+	+*	–
Staphylococcus aureus ATCC29213	+	+	–	+	+*	–
Methicillin Resistant *Staphylococcus aureus* (Clinical isolate)	+	+	–	+	–*	–
Staphylococcus epidermitis 35984	+	+	–	+	+*	–
Streptococcus mutans ATCC19615	+	+	–	+	+*	–
Streptococcus pyogenes ATCC49619	+	+	–	+	+*	–
Gram-negative bacteria						
Acinetobacter baumannii ATCC19606	+	+	–	+	+**	–
Multidrug-resistant *Acinetobacter baumannii* (MDR)	+	–	+	+	–**	–
Escherichia coli ATCC25922	–	–	–	–	+**	–
Escherichia coli O157:H7	–	–	–	–	+**	–
Klebsiella pneumoniae ATCC70063	–	–	–	–	+**	–
Pseudomonas aeruginosa ATCC27853	–	–	–	–	+**	–
Salmonella enterica serotype Typhi (Clinical isolates)	–	–	–	–	+**	–
Shigella enteritis (Clinical isolates)	–	–	–	–	+**	–
Vibrio cholerae (Clinical isolates)	–	–	–	–	+**	–

Ten µg/mL of ampicillin (amp) was used as a positive control for Gram-positive bacteria marked as * and 10 µg/mL of gentamycin (gen) was used as a positive control for Gram-negative bacteria marked as **. DMSO was used as a negative control. Symbol + and – stand for making a clear spot and not making a clear spot on bacterial lawn respectively. All tests were conducted in triplicate.

Table 2 Minimum inhibitory concentrations using overlay spotted assay of different solvent extracts of the yellow flower of *C. pulcherrima* against various pathogenic bacteria.

Pathogenic isolates	Minimum Inhibitory Concentration (MIC) (mg/mL)				Negative control (1 % DMSO)
	Hexane	Ethyl acetate	Methanol	Aqueous	
Gram-positive bacteria					
Bacillus cereus ATCC11778	0.391	0.391	100	6.25	R
Bacillus subtilis 7988 (Clinical isolate)	1.563	0.391	100	50	R
Bacillus subtilis ATCC6051	1.563	0.391	6.25	50	R
Enterococcus faecalis 4232 (Clinical isolates)	100	100	R	100	R
Enterococcus faecalis 3532 (Clinical isolates)	100	100	R	100	R

Pathogenic isolates	Minimum Inhibitory Concentration (MIC) (mg/mL)				Negative control (1 % DMSO)
	Hexane	Ethyl acetate	Methanol	Aqueous	
Gram-positive bacteria					
Listeria monocytogenes (Clinical isolates)	12.5	12.5	R	25	R
Staphylococcus agalactiae (Clinical isolates)	12.5	12.5	R	25	R
Staphylococcus aureus ATCC25923	12.5	12.5	R	50	R
Staphylococcus aureus ATCC29213	6.25	6.25	R	50	R
Methicillin Resistant *Staphylococcus aureus* (Clinical isolate)	6.25	6.25	R	50	R
Staphylococcus epidermitis 35984	100	25	R	50	R
Streptococcus mutans ATCC19615	6.25	12.5	R	25	R
Streptococcus pyogenes ATCC49619	100	12.5	R	25	R
Gram-negative bacteria					
Acinetobacter baumannii ATCC19606	100	100	R	25	R
Multidrug-resistant *Acinetobacter baumannii* (MDR)	3.125	R	25	12.5	R
Escherichia coli ATCC25922	R	R	R	R	R
Escherichia coli O157:H7	R	R	R	R	R
Klebsiella pneumoniae ATCC70063	R	R	R	R	R
Pseudomonas aeruginosa ATCC27853	R	R	R	R	R
Salmonella enterica serotype Typhi (Clinical isolates)	R	R	R	R	R
Shigella enteritis (Clinical isolates)	R	R	R	R	R
Vibrio cholerae (Clinical isolates)	R	R	R	R	R

DMSO was used as a negative control diluted by MHB broth. The letter R denotes resistance, a parameter not employed in the investigation of the minimum inhibitory concentration (MIC). All tests were conducted in triplicate.

Anticancer activity

We examined the anticancer efficacy of different extracts of *C. pulcherrima* using an MTT-based assay. The results revealed that all four extracts exhibited anticancer activity to some extent against the four cancer cell lines tested (**Figure 2**). The ethyl acetate and methanol fractions were the most and second most effective in suppressing cancer cells, respectively (**Figures 2(B)** - **2(C)**). On the other hand, the aqueous and hexane fractions showed moderate to mild effects on cancer cells (**Figures 2(A)** and **2(D)**).

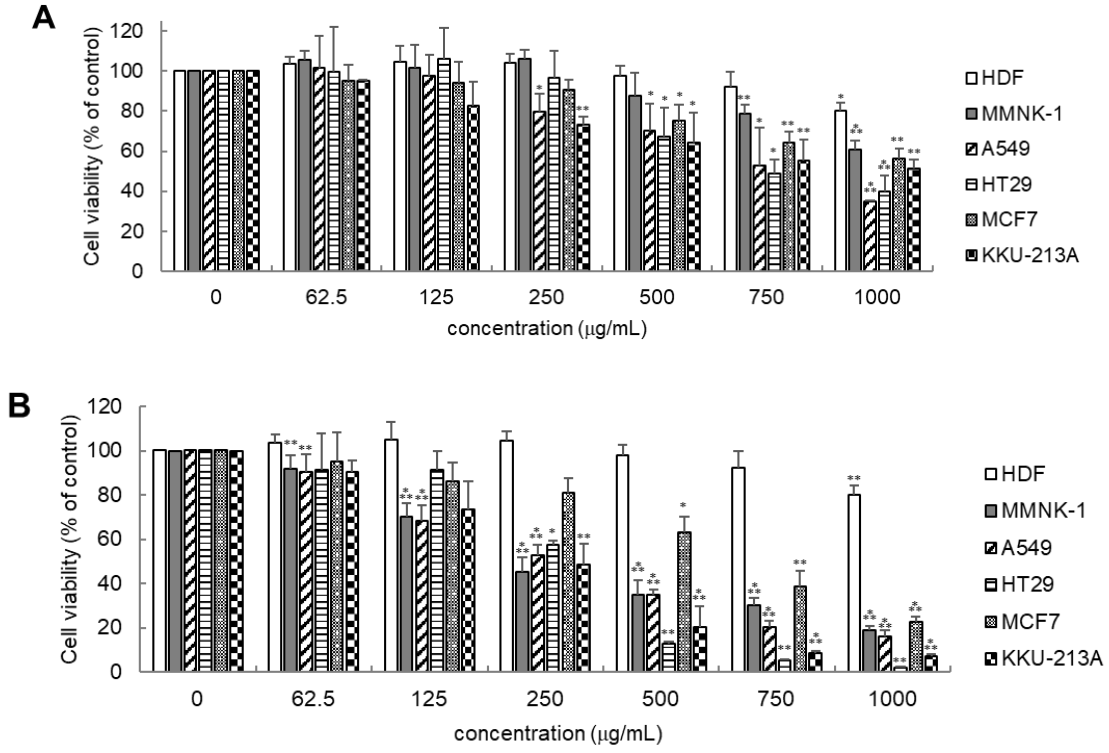
While the cancer cell lines were strongly affected by the ethyl acetate and methanol extracts even at low concentrations, normal fibroblast cells were only affected by the ethyl acetate extract at higher dosages (**Figure 2(C)**), while the methanol fraction showed very little effect on this cell line even at the highest dosage (**Figure 2(B)**). These findings were consistent with the recent work of Zengin and colleagues, who found that methanol and aqueous bark extracts of *C. bonduc* and *C. decapetala* showed stronger cytotoxic effects on cervical cancer cells (HeLa) than on normal VERO cells [25]. Previously, an ethyl acetate extract of *C. pulcherrima* was shown to selectively inhibit 2 breast cancer cell lines (MCF-7 and MDA-MB-453) compared with a normal breast cell line (MCF-12A) [40].

Surprisingly, our present methanol and ethyl acetate extracts suppressed the growth of MMNK-1 cells as well as the growth of cancer cells (**Figures 2(B) - 2(C)**).

The aqueous extract of *C. pulcherrima* exhibited very low cytotoxicity against HDF and MMNK-1, except at relatively high concentrations when it significantly suppressed the growth of all four cancer cell lines (**Figure 2(A)**). Zengin and colleagues reported that an aqueous extract of *C. bonduc* bark exhibited strong cytotoxicity toward HeLa cells [25].

The hexane extract demonstrated a mild influence on all 4 cancer cell lines and the 2 normal cell lines (**Figure 2(D)**). Nevertheless, a high dosage exhibited a significant effect on the 2 gastrointestinal tract cancer cell lines, namely the colon cancer cell line HT-29 and bile duct cancer cell line KKU-213A. These findings were consistent with the findings of Carvalho *et al*. [24] that an essential oil derived from *C. peltophoroides* had a strong cytotoxic effect on the HCT colon cancer cell line. Zengin and colleagues, however, reported that the human colon carcinoma cell line RKO showed the highest resistance to various extracts of *C. decapetala* and *C. bonduc* [25].

Crude extracts derived from different parts of *Caesalpinia* sp. and fractionated compounds from this plant have shown promising anti-cancer properties when tested on various types of human cancer cells [21-24,41,42]. A root extract of *C. pulcherrima* was reported to be a potential anticancer agent against breast, cervical, and prostate cancer cells [22]. In some studies, the mechanism underlying anticancer activity could be identified. In head and neck cancer, *C. pulcherrima* extract was shown to induce cancer cell death by activating the expression of the tumor suppressor genes p53 and p21 [43]. A *C. pulcherrima* extract suppressed tumor growth by suppressing telomerase enzymes in the oral carcinoma KB cell line and the osteosarcoma HOS cell line [23]. A study that used a yeast system showed that novel diterpene dibenzoates from *C. pulcherrima* induced DNA lesions by acting as a topoisomerase inhibitor, DNA intercalator, minor groove binder, DNA alkylator, and cleaver [44]. Based on the results of a gene ontology analysis, it was proposed that the activity of *Caesalpinia* extracts against prostate cancer might be supported by nuclear factor kappa B (NF-kappa B) signaling in cancer pathways [25]. Lastly, a breast cancer study found that *C. pulcherrima* arrested the cell cycle and triggered ROS-mitochondrial-mediated apoptosis and necroptosis by modulating estrogen and estrogen receptors [40].



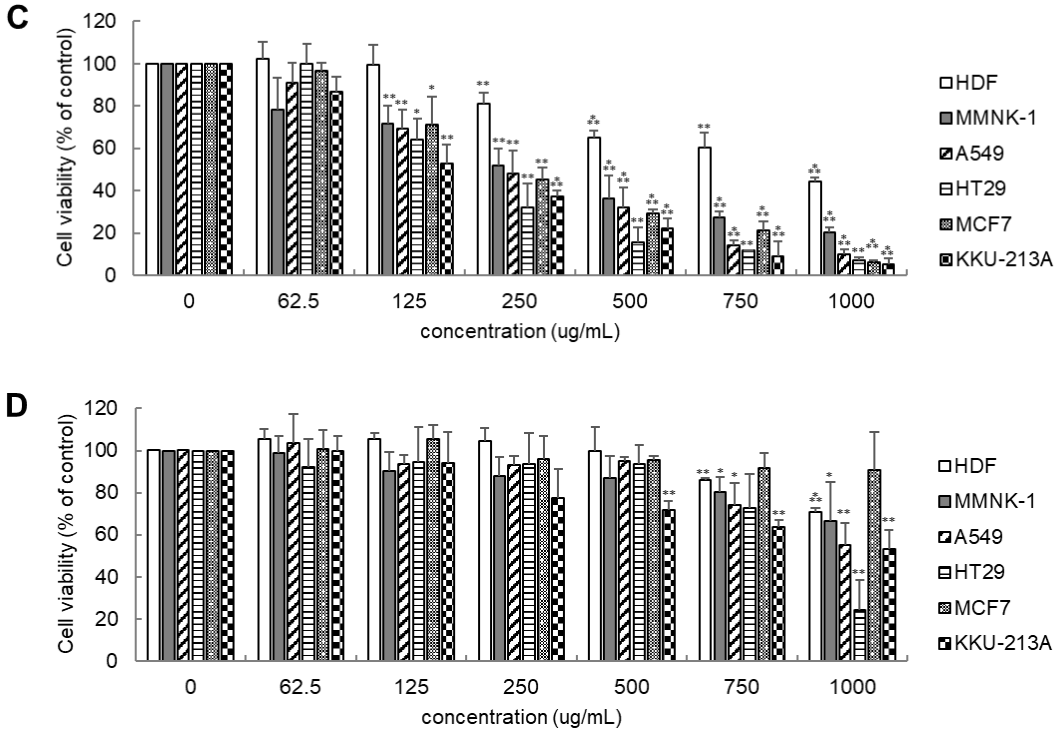


Figure 2 Determination of the anticancer activity of *Caesalpinia* extracts. A cytotoxicity assay was conducted on HDF, MMNK-1, A549, HT-29, MCF-7, and KKU-213A cells at 72 h after exposure to various concentrations of *Caesalpinia* extracts obtained through water extraction (A), methanol extraction (B), ethyl acetate extraction (C), and hexane extraction (D). Statistical significance was denoted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

GC analysis

Various bioactive compounds were identified in the 4 solvent extractions of *C. pulcherrima* flower (**Tables 3 - 6**). In the hexane extract, the primary chemical component was the alcohol 1-methyl-3-methylidene-8-propan-2-yltricyclo-decane. Other hydrocarbon and natural products included 4,11,11-trimethyl-8-methylidenebicyclo-undec-4-ene; 5-(5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylpent-1-en-3-ol; 3,7,11,11-tetramethylbicyclo-undeca-2,6-diene; 1,3-dimethyl-8-propan-2-yltricyclo-dec-3-ene; 1,1,7-trimethyl-4-methylidene-1a,2,3,4a,5,6,7a,7b-octahydrocyclopropa[h]azulen-7-ol, and 2,2-dimethyl-3-3,7,12,16,20-pentamethylhenicosa-3,7,11,15,19-pentaenyl]oxirane (**Table 3**). In the ethyl acetate extract, benzoic acid was the main component (**Table 4**). Natural products including 4,11,11-trimethyl-8-methylidenebicyclo-undec-4-ene; 5,5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol, and 1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene were also found. The sesquiterpenes: 1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene and 4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene were also detected. In the methanol extract, 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one was detected, and carboxylic substances including benzoic acid and acetic acid were found. A cluster of furans was present including 5-(hydroxymethyl)furan-2-carbaldehyde; furan-2-ylmethanol; 2,4-dihydroxy-2,5-dimethylfuran-3-one; furan-2-carbaldehyde, and 4-hydroxy-2,5-dimethylfuran-3-one. In addition, an ester cluster was detected that included hexadecanoic acid, methyl ester; methyl-octadeca-9,12-dienoate, methyl-octadeca-9,12,15-trienoate and methyl octadecenoate (**Table 5**). The aqueous extract contained furans, benzene, ester and natural products, including 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one;1-hydroxypropan-2-one; 1-hydroxy, 2-furanmethanol, 2,3-dihydro-benzofuran; hydroxy dimethyl furanone, 2,3-dihydro-1-benzofuran; benzoic acid; 5-(hydroxymethyl)furan-2-carbaldehyde; hexadecanoic acid; 4-ethenyl-2-

methoxyphenol; cyclopent-4-ene-1,3-dione; cyclopentane-1,2-dione; propane-1,2,3-triol and 3,5-dihydroxy-2-methylpyran-4-one (**Table 6**).

The chemical constituents identified in *Caesalpinia pulcherrima* flowers in this study are consistent with those reported in previous research. Ogunbinu *et al*. [45] identified monoterpenoids, sesquiterpenoids, and aliphatic compounds in *C. pulcherrima*. Similarly, our analysis also detected sesquiterpenoids and aliphatic compounds, further supporting these findings across different compound profiles. In our hexane extract, the detected sesquiterpenoids were oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl), cadinene, and muurolene. In our ethyl acetate extract, cadinene, muurolene and 1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene were among the sesquiterpenoids found. In the hexane extract, aliphatic hydrocarbons such as bicycloundec-4-ene, 4,11,11-trimethyl-8-methylene and 3,7,11,11-tetramethylbicycloundeca-2,6-diene were detected.

The major classes of chemicals identified in dried flower extracts of *C. pulcherrima* have been alkaloids, flavonoids and tannins in hexane extract, saponins and tannins in methanol extract and glycosides, steroids and tannins in aqueous extract [20]. Specific phytochemicals found in our hexane extract were alcohol (8-Isopropyl-1-methyl-3-methylenetricyclodecane-rel); hydrocarbon and natural products (Bicycloundec-4-ene, 4,11,11-trimethyl-8-methylene; 1-Naphthalenepropanol-ethenyldecahydro-5,5,8a-tetramethyl-2-methylene; 3,7,11,11-Tetramethylbicycloundeca-2,6-diene; Copaene; 1,1,7-trimethyl-4-methylenedecahydro-1h-cyclopropa[E]azulen-7-Ol; Oxirane and 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)). GC-MS analysis of a methanolic extract revealed carboxylic acids and many types of furans. In an aqueous extract of the flower of *C. pulcherrima*, different phytochemicals were found.

Azevedo *et al*. (2018) analyzed the chemical composition of the yellow flower of *C. pulcherrima* identifying 4aα, 7α, 7aβ-Nepetalactone (0.83 %), α-Copaene (4.25 %), E-Caryophyllene (2.10 %), γ-Muurolene (2.41 %), α-Muurolene (1.91 %), γ-Cadinene (1.15 %), E-Nerolidol (2.08 %), Caryophyllene Oxide (49.13 %), Humulene Epoxide II (2.46 %), 1-epi-Cubenol (1.22 %), epi-α-Muurolol (4.96 %), α-Cadinol (4.47 %) and Isoamil hexonoate (4.53 %) some of which were also detected in some of our extracts. Our hexane extract contained copaene (2.25 %), cadinene (0.79 %) muurolene (1.16 %), and cardinol (0.36 %) which were also found in the ethyl acetate extract at different concentrations. However, caryophyllene was found only in the methanolic extract and at less than 1 % (0.86 %), and caryophyllene oxide was absent from all our extracts. However, the phytochemical composition detected in the leaf of the same species differed from the flower extracts by presenting 2(1H)-Naphthalenone,octahydro-4a-methyl-7-(1-methylenthyl)-,(4aa,7a,8aa) (21.17 %); Flavone (15.68 %); 2-Propenal,3-(2-methoxyphenyl) (11.52 %); 4’,5,7,-Trihydroxy isoflavone (10.90 %) and 10-Octadecenoic acid, methyl ester (9.94 %) [46].

The variations in both the quantity and concentration of volatile compounds in the present extracts of *C. pulcherrima* yellow flower confirm the robust antioxidant and antimicrobial activities of the species. Additionally, these observed variations are implicated in the inhibition of specific cancer cell lines. While GC-MS facilitated the identification of a wide range of compounds, the substantial molecular weight of the key phytochemical components found may necessitate the application of LC-MS to more completely analyze the extracts. Consequently, further investigations into employing LC-MS are essential for a comprehensive understanding of the potential correlations between phytochemical compounds and their respective properties.

Table 3 The major chemical components of the hexane extract of the yellow flower of *C. pulcherrim*a were identified by GC-MS analysis.

No.	RT (min)	Percentage	Identification	Formula
1	27.0968	14.74	1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	$C_{15}H_{24}$
2	24.5888	9.27	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$
3	55.0769	5.09	5-(5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylpent-1-en-3-ol	$C_{20}H_{34}O$
4	27.6908	2.64	3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene	$C_{15}H_{24}$
5	22.8247	2.25	1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene	$C_{15}H_{24}$
6	30.7758	1.22	1,1,7-trimethyl-4-methylidene-1a,2,3,4a,5,6,7a,7b-octahydrocyclopropa[h]azulen-7-ol	$C_{15}H_{24}O$
7	70.0251	1.01	2,2-dimethyl-3-[(3E,7E,11E,15E)-3,7,12,16,20-pentamethylhenicosa-3,7,11,15,19-pentaenyl]oxirane	$C_{30}H_{50}O$
8	28.4311	0.8	4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.01,5]decan-4-ol	$C_{15}H_{26}O$
9	28.7861	0.79	1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$
10	30.9642	0.75	4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.04,6]dodecane	$C_{15}H_{24}O$
11	26.9487	0.71	7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	$C_{15}H_{24}$
12	27.8817	0.45	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
13	33.3833	0.42	1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	$C_{15}H_{26}O$
14	26.2441	0.39	2,6-dimethylocta-2,4,6-triene	$C_{15}H_{24}$
15	33.6741	0.36	1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	$C_{15}H_{26}O$
16	37.3955	0.34	[(2E)-3,7-dimethylocta-2,6-dienyl] hexanoate	$C_{16}H_{28}O_2$
17	30.4062	0.32	3,7,11-trimethyldodeca-1,6,10-trien-3-ol	$C_{15}H_{26}O$
18	24.9848	0.31	1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	$C_{15}H_{24}$
19	28.04	0.29	1-methyl-4-[(2Z)-6-methylhepta-2,5-dien-2-yl]cyclohexene	$C_{15}H_{24}$
20	33.2174	0.24	1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	$C_{15}H_{26}O$
21	47.7106	0.24	1,1,4a-trimethyl-7-propan-2-yl-2,3,4,4b,5,6,8a,10a-octahydrophenanthrene	$C_{20}H_{32}$
22	34.9336	0.21	3-ethenyl-3-methyl-6-propan-2-yl-2-prop-1-en-2-ylcyclohexan-1-ol	$C_{15}H_{26}O$
23	50.2495	0.17	heptyl (Z)-tetradec-9-enoate	$C_{21}H_{40}O_2$
24	25.9427	0.16	1,5,9,9-tetramethylcycloundeca-1,4,7-triene	$C_{15}H_{24}$

No.	RT (min)	Percentage	Identification	Formula
25	23.1761	0.15	1-methyl-5-methylidene-8-propan-2-yltricyclo[5.3.0.02,6]decane	$C_{15}H_{24}$
26	40.339	0.15	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$
27	23.4347	0.13	1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	$C_{15}H_{24}$
28	21.7616	0.1	10-methyl-4-methylidene-7-propan-2-yltricyclo[4.4.0.01,5]decane	$C_{15}H_{24}$
29	33.0746	0.1	1,1,4,7-tetramethyl-2,3,5,6,7a,7b-hexahydro-1aH-cyclopropa[h]azulen-7-ol	$C_{15}H_{24}O$
30	31.8651	0.1	2,3,8-dimethyl-1,2,3,4,5,6,7,8-octahydroazulen-5-yl]propan-2-yl acetate	$C_{17}H_{28}O_2$
31	27.4803	0.08	4-methyl-7-methylidene-1-propan-2-yl-2,3,4,4a,5,6-hexahydro-1H-naphthalene	$C_{15}H_{24}$
32	5.6707	0.07	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$
33	22.3377	0.06	4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.01,5]decan-4-ol	$C_{15}H_{24}$
34	29.3053	0.06	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
35	30.5611	0.06	1,2-dimethyl-8-propan-2-yltetracyclo[4.4.0.02,4.03,7]decane	$C_{15}H_{26}O$
36	17.6219	0.05	7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	$C_{11}H_{22}O_2$
37	25.6002	0.05	3-methylbutyl hexanoate	$C_{15}H_{24}$
38	43.4165	0.04	7-ethenyl-1,1,4a,7-tetramethyl-3,4,4b,5,6,9,10,10a-octahydro-2H-phenanthrene	$C_{20}H_{32}$

Table 4 The major chemical components of the ethyl acetate extract of the yellow flower of *C. pulcherrima* were identified by GC-MS analysis.

No.	RT (min)	Percentage	Identification	Formula
1	15.0442	6.12	benzoic acid	$C_7H_6O_2$
2	24.5845	3.8	4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	$C_{15}H_{24}$
3	55.0554	2.74	5,5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol	$C_{20}H_{34}O$
4	28.7949	2.2	1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$
5	26.9324	1.74	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
6	77.6784	1.68	10,13-dimethyl-17-5-propan-2-ylhept-5-en-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta-phenanthren-3-ol	$C_{29}H_{48}O$
7	44.3899	1.42	hexadecanoic acid	$C_{16}H_{32}O_2$

No.	RT (min)	Percentage	Identification	Formula
8	22.8309	1.02	1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene	$C_{15}H_{24}$
9	28.3971	0.82	1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$
10	27.646	0.75	7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene	$C_{15}H_{24}$
11	27.8868	0.61	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
12	33.6816	0.55	1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	$C_{15}H_{26}O$
13	30.786	0.34	1,1,7-trimethyl-4-methylidene-1a,2,3,4a,5,6,7a,7b-octahydrocyclopropa[h]azulen-7-ol	$C_{15}H_{24}O$
14	25.3695	0.31	2,6-dimethylocta-2,4,6-triene	$C_{15}H_{24}$
15	27.0733	0.3	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
16	29.1072	0.28	1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,7-hexahydronaphthalene	$C_{15}H_{24}$
17	29.311	0.25	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
18	24.9877	0.25	1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	$C_{15}H_{24}$
19	33.389	0.25	1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol	$C_{15}H_{26}O$
20	28.1527	0.21	1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	$C_{15}H_{24}$
21	25.8494	0.19	4,7-dimethyl-1-propan-2-yl-1,2,3,4,4a,5-hexahydronaphthalene	$C_{15}H_{24}$
22	26.2508	0.19	2,6-dimethylocta-2,4,6-triene	$C_{15}H_{24}$
23	27.4836	0.18	4-methyl-7-methylidene-1-propan-2-yl-2,3,4,4a,5,6-hexahydro-1H-naphthalene	$C_{15}H_{24}$
24	22.6471	0.12	1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene	$C_{15}H_{24}$
25	25.6996	0.1	4,7-dimethyl-1-propan-2-yl-1,2,3,4,4a,5-hexahydronaphthalene	$C_{15}H_{24}$

Table 5 The major chemical components of the methanol extract of the yellow flower of *C. pulcherrima* were identified by GC-MS analysis.

No.	RT (min)	Percentage	Identification	Formula
1	20.4098	8.35	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$
2	23.5273	6.16	benzoic acid	$C_7H_6O_2$
3	7.7811	6.01	Acetic acid	$C_2H_4O_2$
4	24.9155	5.3	5-(hydroxymethyl)furan-2-carbaldehyde	$C_6H_6O_3$
5	19.9863	3.14	methyl hexadecanoate	$C_{17}H_{34}O_2$
6	32.8503	3.01	hexadecanoic acid	$C_{16}H_{32}O_2$

No.	RT (min)	Percentage	Identification	Formula
7	10.3927	2.87	furan-2-ylmethanol	$C_5H_6O_2$
8	22.845	2.81	2,3-dihydro-1-benzofuran	C_8H_8O
9	8.8457	2.25	2,4-dihydroxy-2,5-dimethylfuran-3-one	$C_6H_8O_4$
10	8.0576	2.15	furan-2-carbaldehyde	$C_5H_4O_2$
11	5.9635	2.03	1-hydroxypropan-2-one	$C_3H_6O_2$
12	19.0859	2	4-ethenyl-2-methoxyphenol	$C_9H_{10}O_2$
13	25.3154	1.83	methyl-octadeca-9,12-dienoate	$C_{19}H_{34}O_2$
14	26.5742	1.54	methyl-octadeca-9,12,15-trienoate	$C_{19}H_{32}O_2$
15	16.0983	1.52	4-hydroxy-2,5-dimethylfuran-3-one	$C_6H_8O_3$
16	35.7905	1.46	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	$C_{30}H_{50}$
17	21.2804	1.3	propane-1,2,3-triol	$C_3H_8O_3$
18	20.8417	1.08	3,5-dihydroxy-2-methylpyran-4-one	$C_6H_6O_4$
19	24.092	1.01	methyl octadecanoate	$C_{19}H_{38}O_2$
20	9.581	0.92	cyclopent-4-ene-1,3-dione	$C_5H_4O_2$
21	9.8516	0.86	4,11,11-trimethyl-8-methylidenebicyclo-undec-4-ene	$C_{15}H_{24}$
22	27.6565	0.51	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$
23	9.4634	0.48	5-methylfuran-2-carbaldehyde	$C_6H_6O_2$
24	24.5625	0.37	methyl-octadec-9-enoate	$C_{19}H_{36}O_2$
25	10.9515	0.31	4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	$C_{15}H_{24}$
26	10.0574	0.29	methyl benzoate	$C_8H_8O_2$
27	11.6985	0.25	2H-furan-5-one	$C_4H_4O_2$

Table 6 The major chemical components of the aqueous extract of the yellow flower of *C. pulcherrima* were identified by GC-MS analysis.

No.	RT (min)	Percentage	Identification	Formula
1	7.6518	10.78	ammonium ethanoate	$C_2H_7NO_2$
2	20.1805	8.38	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$
3	5.9166	4.29	1-hydroxypropan-2-one	$C_3H_6O_2$
4	10.2987	3.54	furan-2-ylmethanol	$C_5H_6O_2$
5	22.6573	3.05	2,3-dihydro-1-benzofuran	C_8H_8O
6	15.9514	2.52	5-hydroxy-3,4-dimethyl-3H-furan-2-one	$C_6H_8O_3$
7	8.7753	2.08	2,4-dihydroxy-2,5-dimethylfuran-3-one	$C_6H_8O_4$
8	23.398	2.06	benzoic acid	$C_7H_6O_2$
9	24.6744	1.9	5-(hydroxymethyl)furan-2-carbaldehyde	$C_6H_6O_3$
10	32.6563	1.79	hexadecanoic acid	$C_{16}H_{32}O_2$
11	18.9041	1.79	4-ethenyl-2-methoxyphenol	$C_9H_{10}O_2$
12	9.487	1.59	cyclopent-4-ene-1,3-dione	$C_5H_4O_2$
13	11.7674	1.58	cyclopentane-1,2-dione	$C_5H_6O_2$

No.	RT (min)	Percentage	Identification	Formula
14	21.0687	1.21	propane-1,2,3-triol	$C_3H_8O_3$
15	20.657	1.11	3,5-dihydroxy-2-methylpyran-4-one	$C_6H_6O_4$
16	34.1739	1.09	benzene-1,4-diol;cyclohexa-2,5-diene-1,4-dione	$C_{12}H_{10}O_4$
17	15.3985	1.03	Phenol	C_6H_6O
18	15.1926	0.87	3H-pyran-2,6-dione	$C_5H_4O_3$
19	10.487	0.65	2-methylbutanoic acid	$C_5H_{10}O_2$
20	16.5043	0.52	5-acetyloxolan-2-one	$C_6H_8O_3$
21	10.0458	0.5	oxolan-2-one	$C_4H_6O_2$
22	11.581	0.39	2H-furan-5-one	$C_4H_4O_2$
23	9.3811	0.3	5-methylfuran-2-carbaldehyde	$C_6H_6O_2$
24	12.6457	0.28	2-hydroxy-3-methylcyclopent-2-en-1-one	$C_6H_8O_2$
25	14.0338	0.14	2,6-ditert-butyl-4-methylphenol	$C_{15}H_{24}O$

Conclusions

In this study, we evaluated the phytochemical properties of the yellow flower of *Caesalpinia pulcherrima* through sequential extraction with four solvents. Our findings demonstrated that methanol was the most effective solvent for extracting antioxidants. In contrast, hexane, ethyl acetate, and water extracts exhibited the strongest antimicrobial activity against all tested gram-positive bacteria and one of the gram-negative bacteria: *Acinetobacter baumannii*. Moreover, our investigation revealed that extracts, particularly those from ethyl acetate and methanol, exhibited cytotoxicity against the bile duct cancer cell line KKU-213A, a novel finding in *C. pulcherrima* research. The volatile metabolites identified were consistent with those reported in previous studies. This comprehensive re-examination of the phytochemical properties of the yellow flower's crude extract suggests promising directions for future research. Subsequent studies focused on synthesizing or purifying key chemical constituents from *C. pulcherrima* may hold significant potential for drug development.

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References

[1] Z Popović, R Matić, S Bojović, M Stefanović and V Vidaković. Ethnobotany and herbal medicine in modern complementary and alternative medicine: An overview of publications in the field of I&C medicine 2001 - 2013. *Journal of Ethnopharmacology* 2016; **181**, 182-192.

[2] H Yuan, Q Ma, L Ye and G Piao. The traditional medicine and modern medicine from natural products. *Molecules* 2016; **21(5)**, 559.

[3] DS Fabricant and NR Farnsworth. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* 2001; **109(S1)**, 69-75.

[4] RR Mendonça-Filho. *Bioactive phytocompounds: New approaches in the phytosciences*. *In*: I Ahmad, F Aqil and M Owais (Eds.). Modern phytomedicine: Turning medicinal plants into drugs. John Wiley & Sons, New Jersey, 2006.

[5] TCSP Pires, L Barros, C Santos-Buelga and ICFR Ferreira. Edible flowers: Emerging components in the diet. *Trends in Food Science & Technology* 2019; **93**, 244-258.

[6] M Teixeira, W Tao, A Fernandes, A Faria, IMPLVO Ferreira, J He, VD Freitas, N Mateus and H Oliveira. Anthocyanin-rich edible flowers, current understanding of a potential new trend in dietary patterns. *Trends in Food Science & Technology* 2023; **138**, 708-725.

[7] M Skrajda-Brdak, G Dąbrowski and I Konopka. Edible flowers, a source of valuable phytonutrients and their pro-healthy effects - A review. *Trends in Food Science & Technology* 2020; **103**, 179-199.

[8] P Kumari, Ujala and B Bhargava. Phytochemicals from edible flowers: Opening a new arena for healthy lifestyle. *Journal of Functional Foods* 2021; **78**, 104375.

[9] TK Lim. *Caesalpinia pulcherrima*. *In*: Edible medicinal and non-medicinal plants. Springer Dordrecht, Netherlands, 2013, p. 766-772.

[10] ARS Ribeiro, PBF Diniz, CS Estevam, MS Pinheiro, RLC Albuquerque-Júnior and SM Thomazzi. Gastroprotective activity of the ethanol extract from the inner bark of *Caesalpinia* pyramidalis in rats. *Journal of Ethnopharmacology* 2013; **147(2)**, 383-388.

[11] JF Morton. Caribbean and Latin American folk medicine and its influence in the United States. *Quarterly Journal of Crude Drug Research* 1980; **18(2)**, 57-75.

[12] H Takawale, V Mute, D Awari, VI Hukkeri, P Mehta and P Vawhal. Screening of antiulcer activity of *Caesalpinia pulcherrima* L. bark. against aspirin induced ulcer in rats. *World Journal of Medical Sciences* 2011; **6(4)**, 168-172.

[13] SMU Gilani, S Ahmed, SG Baig and MM Hasan. Ethnopharmacognosy, phytochemistry and pharmacology of genus Caesalpinia: A review. *Journal of Pharmacognosy and Phytochemistry* 2019; **8(4)**, 2222-2229.

[14] SS Phuse and ZH Khan. Influence of extraction methods using different solvents on *Caesalpinia pulcherrima* leavaes. *International Journal of Pharma and Bio Sciences* 2017; **8(2)**, 829-837.

[15] C Rajaram, K Reddy and K Sekhar. Evaluation of anti-arthritic activity of *Caesalpinia pulcherrima* in freund's complete adjuvant induced arthritic rat model. *Journal of Young Pharmacists* 2015; **7(2)**, 128-132.

[16] A Raj, A Sharma, N Singh, P Kumar, A Singh, V Bhatia and SK Sharma. Studies on anti-fertility activity of peacock flower (*Caaesalpinia pulcherrima*) on uterine morphology of rat (*Rattus rattus*). *In*: Proceedings of the Reviewed Proceeding National Seminar on Internet: Applications in Research, Haryana, India. 2011, p. 7-15.

[17] S Kumar, J Singh, A Baghotia, V Mehta, V Thakur, M Choudhary, S Verma and D Kumar. Antifertility potential of the ethanolic extract of *Caesalpinia pulcherrima* Linn. leaves. *Asian Pacific Journal of Reproduction* 2013; **2(2)**, 90-92.

[18] GS Chakraborthy and RS Badujar. Antioxidant activity of the successive extracts of *Caesalpinia pulcherrima* leaves. *International Journal of Pharmaceutical and Clinical Research* 2009; **1(2)**, 75-76.

[19] F Hussain, SMR Dewan, MM Hassan, S Akter and M Meshkat. *In vitro* antimicrobial and antioxidant activities evaluation of methanolic extract of *Caesalpinia pulcherrima* flowers. *International Research Journal of Pharmacy* 2013; **4**, 30-32.

[20] S Pulipati, G Pallavi, B Sujan, KA Babu and PS Babu. Evaluation of antibacterial activity of fresh and dry flower extracts of *Caesalpinia pulcherrima* L. *International Journal of Biological Pharmaceutical Research* 2012; **3(3)**, 360-365.

[21] XM Chen, W Lu, ZH Zhang, JY Zhang, TML Tuong, LL Liu, YH Kim, CH Li and JM Gao. Cassane diterpenoids from the aerial parts of *Caesalpinia pulcherrima* and their antibacterial and anti-glioblastoma activity. *Phytochemistry* 2022; **196**, 113082.

[22] O Erharuyi, A Adhikari, A Falodun, A Jabeen, R Imad, M Ammad, MI Choudhary and N Gören. Cytotoxic, anti-inflammatory, and leishmanicidal activities of diterpenes isolated from the roots of *Caesalpinia pulcherrima*. *Planta Medica* 2017; **83(1-2)**, 104-110.

[23] N Promsawan, P Kittakoop, S Boonphong and P Nongkunsarn. Antitubercular cassane furanoditerpenoids from the roots of *Caesalpinia pulcherrima*. *Planta Medica* 2003; **69(8)**, 776-777.

[24] BAD Carvalho, OS Domingos, M Massoni, MHD Santos, M Ionta, JHG Lago, CR Figueiredo, AL Matsuo and MG Soares. Essential oil from *Caesalpinia peltophoroides* flowers-chemical composition and *in vitro* cytotoxic evaluation. *Natural Product Communications* 2013; **8(5)**, 679-682.

[25] G Zengin, MF Mahomoodally, MCN Picot-Allain, KI Sinan, G Ak, OK Etienne, E Sieniawska, M Maciejewska-Turska, Ł Świątek, B Rajtar and M Polz-Dacewicz. Chemical composition, biological properties and bioinformatics analysis of two Caesalpina species: A new light in the road from nature to pharmacy shelf. *Journal of Pharmaceutical and Biomedical Analysis* 2021; **198**, 114018.

[26] K Larsen, SS Larsen and JE Vidal. *Flora of Thailand: Leguminosae - caesalpinioideae*. Forest Herbarium, Royal Forest Department, Bangkok, Thailand, 1984.

[27] EA Ainsworth and KM Gillespie. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols* 2007; **2(4)**, 875-877.

[28] TJ Herald, P Gadgil and M Tilley. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture* 2012; **92(11)**, 2326-2331.

[29] A Nowak, A Klimowicz, W Duchnik, Ł Kucharski, K Florkowska, A Muzykiewicz, D Wira, J Zielonkabrzezicka, A Siedłowska and K Nadarzewska. Application of green-extraction technique to evaluate of antioxidative capacity of wild population of fireweed. *Herba Polonica* 2019; **65(4)**, 18-30.

[30] K Thaipong, U Boonprakob, K Crosby, L Cisneros-Zevallos and DH Byrne. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 2006; **19(6-7)**, 669-675.

[31] W Sirisarn, A Mordmuang, K Kerdkumthong and S Saeheng. Exploring *Bougainvillea glabra* flowers: A promising source of natural antimicrobial and anticancer agents. *Journal of Applied Biology & Biotechnology* 2024; **12(6)**, 174-184.

[32] GLTD Torre, EC Arollado, AA Atienza and RAM Manalo. Evaluation of antioxidant capacity and identification of bioactive compounds of crude methanol extracts of *Caesalpinia pulcherrima* (L.) swartz. *Indian Journal of Pharmaceutical Sciences* 2017; **79(1)**, 113-123.

[33] MN Vivek, HCS Swamy, M Manasa, S Pallavi, Y Kambar, MM Asha, M Chaithra, TRP Kekuda, N Mallikarjun and R Onkarappa. Antimicrobial and antioxidant activity of leaf and flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum*. *Journal of Applied Pharmaceutical Science* 2013; **3(8)**, 64-71.

[34] GS Chakraborthy. Antioxidant activity of the successive extracts of *Caesalpinia pulcherrima* flowers. *Pharmacologyonline* 2009; **1**, 362-365.

[35] N Chaves, A Santiago and JC Alías. Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants* 2020; **9(1)**, 76.

[36] A Farooqui, A Khan, I Borghetto, SU Kazmi, S Rubino and B Paglietti. Synergistic antimicrobial activity of Camellia sinensis and Juglans regia against multidrug-resistant bacteria. *PloS One* 2015; **10(2)**, e0118431.

[37] LA Refahy, TA Farghaly, MS Abdel-Aziz and TS Mohamed. Antimicrobial, antioxidant and cytotoxic potential of Caesalpinia pulcherrima flower. *Medicine, Environmental Science, Biology* 2015; **9(2)**, 150-158.

[38] HCS Swamy, MM Asha, M Chaithra, MN Vivek, Y Kambar and TRP Kekuda. Antibacterial activity of flower extract *of Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* against urinary tract pathogens. *International Research Journal of Biological Sciences* 2014; **3(4)**, 80-83.

[39] OT Osuntokun, J Ju and T Oa. Bioprospective screening of antibacterial and phytochemical activity of *Caesalpinia pulcherrima* (pride of Barbados) on selected clinical isolate. *Bioequivalence & Bioavailability International Journal* 2017; **1(3)**, 118.

[40] NS Sakle, D Lokwani and SN Mokale. *Caesalpinia pulcherrima* arrests cell cycle and triggers reactive oxygen species-induced mitochondrial-mediated apoptosis and necroptosis via modulating estrogen and estrogen receptors. *Pharmacognosy Magazine* 2019; **15(S2)**, S288-S297.

[41] B Das, Y Srinivas, C Sudhakar, I Mahender, K Laxminarayana, PR Reddy, TV Raju, NM Jakka and JV Rao. New diterpenoids from *Caesalpinia* species and their cytotoxic activity. *Bioorganic & Medicinal Chemistry Letters* 2010; **20(9)**, 2847-2850.

[42] S Mallapur, PR Biradar, SA Kavatagimath, P Patil, V Patil and N Kudatarkar. Pharmacological evaluation of *Caesalpinia pulcherrima* leaf extract for anticancer activity against Ehrlich Ascites Carcinoma bearing mice. *Indian Journal of Pharmaceutical Education and Research* 2021; **55(1)**, 164-173.

[43] EC Kim, YS Hwang, HJ Lee, SK Lee, MH Park, BH Jeon, CD Jeon, SK Lee, HH Yu and YO You. *Caesalpinia sappan* induces cell death by increasing the expression of p53 and p21WAF1/CIP1 in head and neck cancer cells. *The American Journal of Chinese Medicine* 2005; **33(3)**, 405-414.

[44] AD Patil, AJ Freyer, RL Webb, G Zuber, R Reichwein, MF Bean, L Faucette and RK Johnson. Pulcherrimins A - D, novel diterpene dibenzoates from *Caesalpinia pulcherrima* with selective activity against DNA repair-deficient yeast mutants. *Tetrahedron* 1997; **53(5)**, 1583-1592.

[45] AO Ogunbinu, S Okeniyi, G Flamini, PL Cioni and IA Ogunwande. Monoterpenoid constituents of the volatile oils of *Cynometra megalophylla* harms., *Caesalpinia pulcherrima* L. swartz and *Pachylobus edulis* G. Don., growing in Nigeria. *Journal of Essential Oil Research* 2010; **22(6)**, 536-539.

[46] K Jaikumar, MSN Mohamed, WJ Wyson, M Deventhiran, D Anand and P Saravanan. Phytochemical analysis of *Caesalpinia pulcherrima* (L.) *Sw*. leaf extract using GC-MS analysis. *International Journal of Pharmaceutical Sciences and Drug Research* 2017; **9(2)**, 90-93.

Supplementary material

%Cell viability of various cell types after treatment with *Caesalpinia pulcherrima* extracts.

Hexane fraction

Concentration (µg/mL)	HDF	MMNK-1	A549	HT-29	MCF-7	KKU-213A
	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD
0	100	100	100	100	100	100
62.5	105 ± 5.0	99 ± 7.9	104 ± 13.7	92 ± 13.1	101 ± 8.7	100 ± 7.6
125	106 ± 2.9	90 ± 9.0	94 ± 4.2	95 ± 16.5	106 ± 6.5	94 ± 14.4
250	105 ± 6.1	88 ± 8.9	93 ± 4.6	94 ± 14.9	96 ± 10.7	77 ± 14.2
500	100 ± 11.6	87 ± 10.3	95 ± 2.0	94 ± 8.7	96 ± 1.9	72 ± 4.3**
750	86 ± 0.7**	80 ± 7.3*	74 ± 10.5*	73 ± 16.2	92 ± 7.0	64 ± 3.6**
1000	71 ± 1.6***	67 ± 18.4*	55 ± 10.9**	24 ± 14.0**	91 ± 18.1	53 ± 9.1**

Ethyl acetate fraction

Concentration (µg /mL)	HDF	MMNK-1	A549	HT-29	MCF-7	KKU-213A
	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD
0	100	100	100	100	100	100
62.5	102 ± 8.0	78 ± 15.0	91 ± 9.3	100 ± 9.4	96 ± 3.7	87 ± 6.9
125	100 ± 9.2	72 ± 8.4**	69 ± 9.3**	64 ± 10.0*	71 ± 13.5*	53 ± 8.9**
250	81 ± 4.8**	52 ± 8.0***	48 ± 10.8**	32 ± 11.7**	45 ± 5.5***	37 ± 2.8***
500	65 ± 3.3***	36 ± 10.8***	32 ± 9.5***	16 ± 6.7**	29 ± 1.5***	22 ± 4.5***
750	60 ± 7.1**	27 ± 2.6***	14 ± 2.1***	12 ± 0.0**	21 ± 4.2***	9 ± 6.9***
1000	44 ± 1.9***	20 ± 2.4***	10 ± 2.1***	7 ± 1.3**	6 ± 1.2***	5 ± 2.7***

Methanol fraction

Concentration (µg /mL)	HDF	MMNK-1	A549	HT-29	MCF-7	KKU-213A
	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD
0	100	100	100	100	100	100
62.5	104 ± 6.4	92 ± 5.9	90 ± 7.9	91 ± 16.3	95 ± 13.2	90 ± 5.4
125	99 ± 5.0	70 ± 5.7**	68 ± 7.2**	91 ± 8.4	86 ± 8.4	74 ± 12.4
250	82 ± 2.3**	45 ± 6.6***	53 ± 4.6***	57 ± 2.0*	81 ± 6.4*	48 ± 9.5**
500	80 ± 3.7**	35 ± 6.4***	35 ± 2.3***	13 ± 1.0**	63 ± 7.0**	20 ± 9.7***
750	65 ± 3.4***	30 ± 3.0***	20 ± 2.6***	5 ± 0.7***	39 ± 7.2***	8 ± 1.2***
1000	55 ± 13.3**	19 ± 2.1***	16 ± 2.5***	2 ± 0.2***	22 ± 2.8***	7 ± 0.5***

Aqueous fraction

Concentration (µg/mL)	HDF	MMNK-1	A549	HT-29	MCF-7	KKU-213A
	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD	%cell ± SD
0	100	100	100	100	100	100
62.5	103 ± 3.7	105 ± 4.7	102 ± 15.7	100 ± 22.4	95 ± 8.2	95 ± 0.7
125	105 ± 8.0	102 ± 11.3	98 ± 10.7	106 ± 15.5	94 ± 10.4	83 ± 12.2
250	104 ± 4.5	106 ± 4.3	80 ± 8.6*	96 ± 13.8	91 ± 5.2	73 ± 3.7**
500	98 ± 4.9	88 ± 11.1	70 ± 13.4*	67 ± 14.4	75 ± 7.9*	64 ± 14.5*
750	92 ± 7.7	79 ± 4.7**	53 ± 18.9*	49 ± 6.9*	64 ± 5.4**	55 ± 10.7**
1000	80 ± 4.2**	61 ± 4.8***	35 ± 0.5***	40 ± 7.7**	56 ± 4.9**	51 ± 4.7***

Note: Statistical significance: $^{*}(p < 0.05)$, $^{**}(p < 0.01)$, $^{***}(p < 0.001)$.