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Phytochemical Screening, Antibacterial Activity, and Toxicity of *Calathea lutea* Leaf Extracts

Katherine Paternina-Sierra[1], Piedad Montero-Castillo[1], Diofanor Acevedo-Correa[2], Marlene Duran-Lengua[3], and Barbara Arroyo-Salgado[4]

[1]Food Engineering Program, Research Group in Agricultural and Agro-Industrial Innovation and Development, Faculty of Engineering,
[2]Tourism Administration Program, Research Group in Agricultural and Agro-Industrial Innovation and Development, Faculty of Economic Sciences, [3]FARMABAC Research Group, and [4]Toxicology and Environmental Research Group-BIOTOXAM, Faculty of Medicine, Universidad de Cartagena, Cartagena 130015, Colombia

ABSTRACT: In Colombia, there is a long tradition of using bijao (*Calathea lutea*) leaves to package or wrap various foods. However, scientific studies on *C. lutea* are limited, and research to evaluate its toxicity and/or antibacterial activity has not yet been conducted. The objective of this research, therefore, was to evaluate the content of phytochemical compounds, levels of toxicity, and antibacterial activity of the extracts, fractions, and essential oil derived from *C. lutea* leaves. The plant material was subjected to extraction by maceration, Soxhlet extraction, and steam distillation, and fractions of hexane, dichloromethane, ethyl acetate, and a residual ethanol-water fraction were obtained. Preliminary phytochemical screening was performed using standard procedures with staining reagents. Estimation of the toxicity was carried out using the *Caenorhabditis elegans* biological model. Antibacterial activity was determined by broth microdilution against *Staphylococcus aureus* and *Escherichia coli*. The results showed that the characteristic metabolites were flavonoids, triterpenes, and tannins. At the concentrations tested, the extracts, fractions, and essential oil showed minimal toxicity levels. In terms of antibacterial activity, *E. coli* showed no susceptibility; meanwhile, the dichloromethane fraction had high antibacterial activity against *S. aureus*, with a growth inhibition rate of 81.2%. The results suggested that the of dichloromethane fraction of *C. lutea* has antibacterial activity against *S. aureus*, suggesting its potential as a possible candidate as a natural antibacterial agent in the food industry. This alternative could offer a safer and more sustainable solution compared to the conventional synthetic preservatives.

Keywords: food preservation, phytochemicals, plant extract, *Staphylococcus aureus*

INTRODUCTION

Plants are a source of bioactive compounds (secondary metabolites) (Fuentes-Gandara et al., 2019; Oliveros-Díaz et al., 2021), for which a variety of actual and potential applications in the food and pharmaceutical industries have been reported (Siddique et al., 2020). Natural extracts obtained from the leaves, stems, roots, peels, and seeds of certain plants are rich in bioactive compounds and have been shown to have antimicrobial activity against food spoilage microorganisms and foodborne pathogens (Bertéli et al., 2021; Puvača et al., 2021), making them potential substitutes for synthetic preservatives (Farahmandfar et al., 2017; Martínez et al., 2019). One of the biggest challenges faced in the food industry is food spoilage caused by microbial contamination; therefore, antimicrobial compounds, such as sodium sulfite, potassium metabisulphite, sulfur dioxide, sorbates, and/or nitrates are often added to food products to preserve them (Cagnini et al., 2021). However, concerns exist around the safety of the daily consumption of these synthetic antimicrobials, as some may induce vitamin degradation, for example, the deterioration of thiamine by sulfides (Bensid et al., 2022; Bertéli et al., 2022). To minimize this problem, the food industry has recently focused on the search for natural substances and extracts with antimicrobial activity as an alternative to synthetic antimicrobials. This has been accompanied by an increase in the demand for natural-source antimicrobials (Munekata et al., 2020; Cagnini et al., 2021; Bertéli et al., 2022).

The *Calathea lutea* plant, traditionally known as bijao, belongs to the family Marantaceae. *C. lutea* is native to

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Correspondence to Katherine Paternina-Sierra, E-mail: kpaterninas@unicartagena.edu.co

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the tropical Americas and is distributed throughout the Colombian rainforest, across 20 of Colombia's 32 provinces (known as *departamentos* in Colombia) (Higuera Mora et al., 2020). This herbaceous plant, which has been used as food packaging or wrapping since ancient times, has played an important role in the consumption of native Colombian food products, such as tamales, hayacas, and bocadillo veleño, mainly on the Caribbean Coast and in the Santanderes (Peña Castellanos and Muñoz Suárez, 2015) and represent part of the country's culture and tradition. *C. lutea* is also a source of income for an important segment of farmers in Colombia (López Camacho, 2008; Gonzalez and Suspe, 2017).

Different extracts and fractions from species of Marantaceae have been demonstrated to act against various microorganisms, indicating that they are candidates for the development of naturally occurring antimicrobial agents (Fadahunsi et al., 2021). In addition, significant quantities of phenolic compounds have been detected in Marantaceae extracts (Aguirre et al., 2010; Tomás et al., 2010). However, the previous investigations into the antibacterial activity of *C. lutea* leaf extracts have employed only the agar disk diffusion method, without showing its potential biological activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Apagüeño Arévalo and Tamani Guerra, 2020). In the present investigation, antibacterial activity was evaluated via the broth microdilution method, one of the most suitable methods for studies with bacteria that contaminate food. The broth microdilution method is a highly reproducible method, with greater sensitivity, more accurate evaluation, and better-controlled conditions compared to the agar disk diffusion method (Jorgensen and Turnidge, 2015).

Research into the antibacterial activity of *C. lutea* leaf extracts or essential oil is currently limited, and in Colombia to date no previous studies have investigated its toxicity or antibacterial activity, which could reveal the value of this plant to contribute to the development of new natural antibacterial agents. Therefore, this research aimed to evaluate the phytochemical composition, potential toxicity, and antibacterial activity of the extracts and essential oil of *C. lutea* leaf.

MATERIALS AND METHODS

Selection and conditioning of plant material

C. lutea leaves, grown in the municipality of Santa Rosa de Lima, were purchased from commercial establishments located in Bazurto Market in the city of Cartagena, Bolivar. They were inspected for color, texture, odor, and the absence of fungal or insect colonies. The leaves were disinfected by immersion in sodium hypochlorite (100 ppm) for 2 min (Chaves et al., 2020). They were then cut into pieces of approximately 10 cm, weighed, and finally freeze-dried at −66.5°C (Biobase freeze-dryer model BK-FD10P). Subsequently, the dried plant material was ground using a Hamilton Beach grinder (80350/R). Maceration and Soxhlet extraction were used to obtain the total extracts. The essential oil was obtained by steam distillation. All reagents used were of analytical grade.

Ethanolic extract (EtOH) of *Calathea lutea* leaves by maceration

The milled plant material was subjected to extraction by maceration with ethanol (96% v/v; Merck) at a ratio of 1:10 and intermittent shaking at room temperature (25°C±3°C) for 3 days in the dark (Karim et al., 2020). Successive extractions (solid-liquid) and filtration through Whatman No. 1 filter paper (Sigma-Aldrich) were performed until the material was exhausted. The filtrate was then concentrated in a rotary evaporator (Heidolph HeiVAP Silver 3) under reduced pressure and controlled temperature (40°C±5°C) to obtain the ethanolic (EtOH) extract (Rivera et al., 2019; Lv et al., 2022). The EtOH extract was stored under refrigeration until subsequent fractionation and analysis were performed.

Extraction of total EtOH from *C. lutea* leaves by Soxhlet

The methodology for Soxhlet extraction followed that described by Hirondart et al. (2020), Alara et al. (2018), and Alara and Abdurahman (2019) with certain modifications. In brief, 30 g of ground *C. lutea* leaves was deposited into a cellulose thimble, which was covered with absorbent cotton and then placed in a Soxhlet apparatus (E&Q). The extraction was carried out using 600 mL of 96% ethanol, with a feed-to-solvent ratio of 1:20 g/mL. A heating mantle was used to subject the mixture to reflux during an extraction time of 2–3 h, until exhaustion of the plant material. After extraction, the extract was concentrated to dryness in a rotary evaporator operated at reduced pressure under a controlled temperature (40°C±5°C). Finally, the extract was stored under refrigeration until fractionation and further analysis were performed.

Fractionation of the total EtOH extracts from *C. lutea* leaves

The total EtOH extracts obtained by maceration and Soxhlet extraction were subjected to fractionation (liquid-liquid separation) in a 1-L separatory funnel using equal volumes of hexane, dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc), in increasing order of solvent polarity. According to the methodology described by Rivera et al. (2019) and Asuquo and Udobi (2016) with some adjustments, 20 g of the extract was dissolved in 200 mL of hexane in a separating funnel, which was then shaken vigorously and allowed to stand for approx-

imately 15 min, while the separation of both phases (organic and aqueous) was observed. The organic phase was then removed (process performed four times, 200 mL×4) and concentrated in a rotary evaporator at reduced pressure to obtain the hexane fraction. The process was repeated to obtain the CH_2Cl_2 and EtOAc fractions. The remainder comprised the residual ethanol-water (EtOH/H_2O) fraction, which was then freeze-dried (Fig. 1).

The extraction yields of the total extracts and their fractions were determined using Equation 1:

$$\text{Extract yield}\left(\% \frac{p}{p}\right) = \frac{\text{Mass of dry extract (mg)}}{\text{Dry leaf mass (mg)}} \times 100 \quad (1)$$

Obtaining the essential oil from *C. lutea* leaves by steam distillation

To obtain the essential oil, *C. lutea* leaves were added to CH_2Cl at a ratio of 1:10, and were then subjected to steam distillation by entrainment for approximately 3 h, following the methodology described by Peng et al. (2004), Azmir et al. (2013), and Islam et al. (2020) with some modifications. The yields were calculated using Equation 2:

$$\text{Oil yield}\left(\% \frac{v}{p}\right) = \frac{\text{Volume of } C.\ lutea \text{ oil (mL)}}{\text{Dry leaf mass (mg)}} \quad (2)$$

Preliminary phytochemical screening of extracts, fractions, and essential oil derived from *C. lutea* leaves

The total extracts, fractions, and essential oil were subjected to qualitative screening for various phytochemical constituents, following standard procedures. These included the determination of alkaloids by Dragendorff assay, the determination of tannins by $FeCl_3$ ferric chloride assay, the determination of coumarins and anthracene derivatives by KOH reagent assay and UV fluorescence at 365 nm, the determination of flavonoids by citroboric reagent assay, the determination of triterpenes and steroids by Liebermann-Burchard assay, the determination of saponins by foam test, and the determination of cardiotonic glycosides by Baljet assay (Beltrán Villanueva et al., 2013; Ochoa Pacheco et al., 2015). Each assay was performed in triplicate. The results were manifested as a change in color, the formation of a precipitate, or the release of a gas, which indicated the presence (+) or absence (−) of each secondary metabolite.

Evaluation of the toxicity of the extracts, fractions, and essential oil

The nematode biological model, *Caenorhabditis elegans* (*C. elegans*), was used for the toxicity assays, following the methodology described by Miao et al. (2020) with some modifications. The methodology was developed through the following phases:

Phase 1. Preparation of solutions of extracts, fractions, and essential oil of C. lutea: The methodology described by Rangsinth et al. (2019) was followed, with some modifications. In brief, working solutions of 5,000 μg/mL were prepared using dimethyl sulfoxide (DMSO) as the solvent. Subsequently, dilutions of 2,000, 1,000, 500, 100, 50, 10, 5, 1, and 0.5 μg/mL were made in K buffer (NaCl, KCl, and distilled water) and stored at −20°C until use.

Phase 2. Cultivation and maintenance of Caenorhabditis elegans: Wild-type *C. elegans* N2 Bristol strain nematodes were used in this study. The nematodes were incubated (FTC 120 Velp Scientifica incubator) at 20°C in Petri dishes containing nematode growth medium (NaCl, agar, peptone, cholesterol, KCl, $CaCl_2$, and $MgSO_4$), and *E. coli* OP50 was inoculated into the media as a food source. The nematodes' larval stages were synchronized by sus-

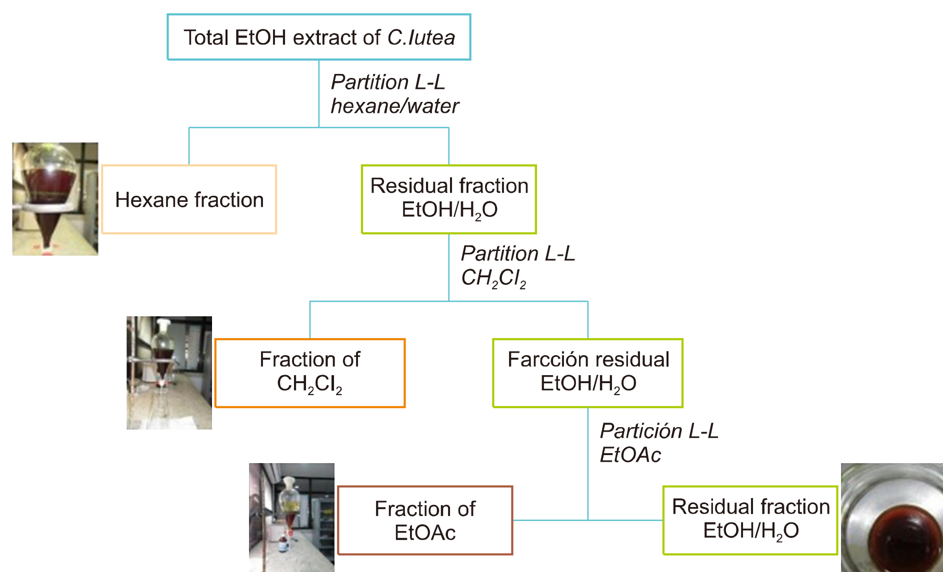

Fig. 1. Systematic representation of the fractionation of the total EtOH extract of *Calathea lutea*. EtOH, ethanol; L-L, liquid-liquid; CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; EtOH/H_2O, ethanol-water.

pension in an alkaline solution composed of 5.25% hypochlorite and NaOH (Dengg and van Meel, 2004; Liao, 2018).

***Phase 3. Lethality assay*:** For the *in vivo* toxicity assays, different concentrations of the extracts, fractions, and essential oil were used to measure nematode lethality, following the methodology described by Miao et al. (2020), with some modifications. In brief, 15±2 *C. elegans* nematodes in larval stage L4 (developmental stage of the reproductive life cycle of *C. elegans*, ideal for a wide range of research) (Duran-Izquierdo et al., 2022; Long et al., 2023) were exposed for 24 h to the previously prepared solutions of the extracts and fractions, and to buffer K as a control group, in 96-well plates. Subsequently, the number of dead nematodes was counted by visual recording using a microstereoscope (Motic SMZ-161). Nematodes that did not show movement within 30 s were considered dead. Three technical replicates were established for each treatment, and two biological replicates were established on different days. The lethality percentage was calculated using Equation 3:

$$\% \text{ lethality} = \frac{NM}{NM+NV} \times 100 \qquad (3)$$

where NM represents dead nematodes and NV represents live nematodes.

C. elegans is a model organism that is widely used in toxicology studies due to its short life cycle, ease of handling, small size, and high sensitivity to toxins (Nagar et al., 2020; Yu et al., 2022; Long et al., 2023). Its genome, which has 60%–80% similarity to that of humans, suggests that the results of toxicology studies in *C. elegans* closely reflect the effects in mammalian models for most compounds (Feng et al., 2017; Moyson et al., 2018; Yu et al., 2022), thus this model is useful for the evaluation of toxicity pathways relevant to humans (Hunt, 2017; Long et al., 2023).

Antibacterial activity of extracts, fractions, and essential oil of *C. lutea* leaves

The broth microdilution method was used to evaluate the antibacterial activity of the extracts, fractions, and essential oil derived from *C. lutea* leaves, as established by the Clinical & Laboratory Standards Institute (CLSI) protocol M07-A9 (Qaiyumi, 2007; CLSI, 2012), with some modifications. The solvent-free extracts, fractions, and essential oil were weighed and dissolved in DMSO in Eppendorf tubes to obtain an initial concentration of 41,000 μg/mL (stock solution) (Pájaro-González et al., 2022), and the assays were performed in 96-well plates.

Working solutions were prepared from the extracts, fractions, and essential oil in cation-adjusted Mueller Hinton broth (CMHC, adjusted with magnesium) at a concentration of 1,024 μg/mL, and 100 μL of the solutions was poured into the plate in triplicate. Four or five isolated colonies were taken from the *S. aureus* and *E. coli* plates, and a suspension was prepared in CMHC. The suspension was adjusted to a turbidity equivalent to the 0.5 McFarland standard (optical density at 620 nm, 0.08–0.10). This resulted in a suspension containing approximately $1-2\times10^8$ CFU/mL. A 1:150 dilution of the suspension was made in CMHC to obtain a suspension containing 1×10^6 CFU/mL. From this suspension, 100 μL was taken and deposited in the wells that contained the solutions of the extracts, fractions, essential oil, and the respective controls. Therefore, they were evaluated at a final concentration of 512 μg/mL with an inoculum of 5×10^5 CFU/mL. An antibiotic solution corresponding to the susceptibility of the microorganism was used as a positive control of antibacterial activity, a suspension of each microorganism was used as a growth control, and an inoculation of each microorganism in CMHC broth in the presence of the respective solvent was used as a negative (inhibition) control. The percentage inhibition was calculated using Equation 4 (Quave et al., 2008):

$$\text{Inhibition percentage (\%PI)} = 1 - \frac{[OD\ (Tt0) - OD\ (C1)]}{[OD\ (C3) - OD\ (C6)]} \times 100 \qquad (4)$$

where *OD* (*T*t0) represents the optical density of the inoculated treatment, *OD* (*C*1) represents the optical density of the treatment without inoculum, *OD* (*C*3) represents the optical density of the inoculated DMSO control, and *OD* (*C*6) represents the optical density of the DMSO control without the inoculum.

Statistical analysis

The toxicity results were expressed as the mean±standard deviation. The antibacterial activity results were expressed as the mean±standard error of the mean. IBM SPSS Statistics 25 statistical software (IBM Corp.) was used for the statistical analyses. One-way analysis of variance (ANOVA) was employed to analyze the data, followed by Tukey's comparison test. *P*-values less than 0.05 ($P<0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

Performance of extracts, fractions, and essential oil of *C. lutea* leaves

A higher yield was evidenced in the total EtOH extract obtained by Soxhlet extraction compared to that obtained by cold maceration (Table 1). This result was possibly related to the fact that a moderate increase in tem-

Table 1. Yield of *Calathea lutea* leaf extracts and their fractions according to extraction method

Extracts	Performance (%)	
	Cold maceration	Soxhlet
Total EtOH extract[1)]	13.81±1.38^{B}	31.01±2.05^{A}
Hexane fraction	3.48±0.11^{bB}	7.19±0.38^{cA}
CH_2Cl_2 fraction	6.19±0.32^{aB}	14.69±0.78^{aA}
EtOAc Fraction	0.48±0.13^{c}	ND
Residual EtOH/H_2O fraction	3.61±0.16^{bB}	8.93±0.57^{bA}

[1)]Total ethanolic extract constituted 100% of the primary extraction.
Results represent the mean±standard deviation of three independent replicates.
Values with different lowercase letters within the same column indicate significant differences (P<0.05) between solvents. Values with different capital letters within the same row indicate significant differences (P<0.05) between extraction methods.
EtOH, ethanol; CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; EtOH/H_2O, ethanol-water; ND, not tested due to very low values, which could not be measured accurately.

perature causes an increase in the solubility of compounds, enhances the diffusion rate, causes disruptions in interactions between matrix components, and creates a decrease in the viscosity and surface tension of compounds and solvents, which lead to an increase in the yield of extracts (Zia et al., 2022). In addition, it was observed that the yields of the fractions varied significantly among the different solvents used in the eluotropic series, with CH_2Cl_2 fractions having the highest yields and EtOAc fractions having the lowest yields. The latter may be related to the fact that some secondary metabolites of the *C. lutea* leaf may have lower solubility in ethyl acetate, resulting in a lower extraction yield.

The higher yields obtained in the CH_2Cl_2 fractions may have been because some secondary metabolites, such as certain terpenoids and steroids (detected by phytochemical screening), are lipophilic and can be extracted more efficiently with dichloromethane; therefore, it is possible that this solvent extracted a greater amount of these metabolites, resulting in higher yields. It should also be considered that the chemical composition of plants vary widely, and therefore certain secondary metabolites may be present in different proportions. In general, the leaves of the *C. lutea* plant used in this study contained compounds that were easily extractable with CH_2Cl_2. In addition, since each solvent has its own chemical properties and may interact differently with the compounds present in plants, it is possible that CH_2Cl_2 may have established certain interactions with the particular compounds found in *C. lutea* leaves and facilitated their extraction. In the essential oil, the yields were approximately 0.24% (calculated based on the dry weight of freeze-dried leaves), which was similar to the yield reported by Chau et al. (2015) for *Amomum longiligulare* leaf oils (0.25%) and higher than the yield obtained by hydrodistillation reported by Ojekale et al. (2013) for *Thaumatococcus danielli* leaf oil (0.09%).

Phytochemical screening of extracts, fractions, and essential oil derived from *C. lutea* leaves

The results of preliminary phytochemical analysis showed that it was not possible to perform the test for the EtOH/H_2O residual fractions (obtained by cold maceration and Soxhlet extraction) due to their high affinity (polar) for the stationary phase. The results of the successful tests revealed the presence of flavonoids, tannins, triterpenes, and steroids; while metabolites such as alkaloids, coumarin/anthracene derivatives, saponins, and cardiotonic glycosides were absent (Table 2). These results correlate with those of Williams and Harborne (1977) and Abdullah et al. (2008), who reported the presence of flavonoids in the leaves of plants in the *Calathea*

Table 2. Results of preliminary phytochemical screening of extracts, fractions, and essential oil of *Calathea lutea* leaves

Extracts	Extraction method	Alc	Cum	Flav	Tri/est	Sap	Gli	Tan
Total EtOH extract	Cold maceration	−	−	+	+	−	−	+
Hexane fraction	Cold maceration	−	−	+	+	−	−	−
CH_2Cl_2 fraction	Cold maceration	−	−	+	+	−	−	+
EtOAc fraction	Cold maceration	−	−	+	+	−	−	−
Total EtOH extract	Soxhlet	−	−	+	+	−	−	−
Hexane fraction	Soxhlet	−	−	−	+	−	−	−
CH_2Cl_2 fraction	Soxhlet	−	−	+	+	−	−	+
Essential oil	SD	−	−	−	+	−	−	−

EtOH, ethanol; CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; SD, steam distillation; Alc, alkaloids; Cum, coumarins; Flav, flavonoids; Tri/est, triterpenes/steroids; Sap, saponins; Gli, cardiotonic glycosides; Tan, tannins; +, present; −, absent.

genera. Similarly, Tomás et al. (2010) detected tannins and flavonoids in their phytochemical study of *C. lutea*. Aguirre et al. (2010) conducted phytochemical analyses of the extracts of *C. lutea* leaves and detected the presence of saponins, coumarins, alkaloids, and phenolic compounds (flavonoids and tannins with catechin structures). Similarly, in a recent study by Chandran (2020), the presence of polyphenols, steroids, terpenoids, saponin, and triterpenes was detected in the ethanolic and aqueous extracts of *C. lutea* leaves. Differences in phytochemical constitution can be attributed to ecological and geographical conditions, as well as the age of the plant, the time of harvest, and the fact that individual plants have different ways of adapting to the natural environment, generating a change in metabolism, which induces variation in the composition of compounds or secondary metabolites according to its condition, distribution, and habitat (Bagamboula et al., 2004; Islam et al., 2020).

Toxicity of extracts, fractions, and essential oil derived from *C. lutea* leaves

To evaluate the safety of the extracts, fractions, and essential oil derived from the *C. lutea* leaves, the *in vivo* toxicity was determined via *C. elegans* lethality assay using different concentrations of the extracts, fractions, and essential oil. As expected, no nematode deaths were observed in the control group (K medium) at all concentrations evaluated. Zero lethality was also observed at the lowest concentrations of the extracts, fractions, and essential oil (0.5, 1.0, and 5.0 μg/mL). Only the total EtOH extract at concentrations of 10.0 and 50.0 μg/mL conferred lethality in the exposed nematodes, albeit at a small percentage. This results was in contrast to the fractions and essential oil at the same concentrations, which did not show lethality against *C. elegans* (Table 3); however, there was a slight decrease in the survival rate at higher concentrations. Nonetheless, none of the concentrations of the fractions or essential oil evaluated (0.5, 1.0, 5.0, 10, 50, 100, 500, 1,000, and 2,000 μg/mL) conferred greater than 21.5% mortality; therefore, the fractions and essential oil were considered safe to use in the subsequent tests. Aquino et al. (2020) considered it safe to continue their studies because none of their *Lentinus strigosus* extracts conferred more than 50% mortality on *C. elegans*; in fact, they reported percentages of less than 35%. Our lethality findings were consistent with research conducted by Apagüeño Arévalo and Tamani Guerra (2020) in Peru, wherein the toxicity of aqueous extracts of *C. lutea* leaves at different concentrations (25, 200, and 2,000 mg/kg) were evaluated in mice. In this previous study, Apagüeño Arévalo and Tamani Guerra (2020) reported the absence of significant clinical alterations in the autonomic, sensory, neuromuscular, cardiovascular, respiratory, ocular, gastrointestinal, and cutaneous systems due to toxicity.

Antibacterial activity of extracts, fractions, and essential oil derived from *C. lutea* leaves

The antibacterial activity of the extracts, fractions, and essential oil *C. lutea* leaves against reference strains of *S. aureus* American Type Culture Collection (ATCC) 29213 and *E. coli* ATCC 25922 was determined. These bacteria were selected for their prevalence in food poisoning outbreaks (Fetsch and Johler, 2018; Odo et al., 2020; Le et al., 2021; Ágredo-Campos et al., 2023) and because they are considered to be major agents of food spoilage and foodborne diseases in Colombia (Contreras et al., 2020; Toscano et al., 2020; Barragán et al., 2021; De La Hoz,

Table 3. Percentage lethality of *Caenorhabditis elegans* when exposed to extracts, fractions, and essential oil from *Calathea lutea* leaves for 24 h

Type of extract	Extraction method	10 mg/mL	50 mg/mL	100 mg/mL	500 mg/mL	1,000 mg/mL	2,000 mg/mL
		% Lethality rate of *C. elegans*					
Total EtOH extract	Cold maceration	4.49±0.30^{aCD}	4.42±0.12^{bD}	5.57±0.31^{bcC}	12.01±0.43^{aB}	12.01±0.43^{dB}	18.01±0.64^{bcdA}
	Soxhlet	4.47±0.58^{aD}	4.70±0.26^{aD}	5.17±0.15^{cD}	11.54±0.38^{aC}	15.29±0.87^{abB}	19.37±0.55^{abA}
Hexane fraction	Cold maceration	0	0	5.67±0.18^{bcC}	11.54±0.38^{aB}	12.81±0.91^{bcdB}	16.57±1.13^{cdA}
	Soxhlet	0	0	6.83±0.27^{aD}	11.13±0.62^{aC}	12.78±0.48^{cdB}	18.38±0.64^{abcA}
CH_2Cl_2 fraction	Cold maceration	0	0	5.30±0.56^{cD}	8.12±0.37^{bC}	15.08±1.37^{abcB}	19.58±0.72^{abA}
	Soxhlet	0	0	5.04±0.90^{cB}	4.99±1.11^{dB}	5.70±1.07^{eB}	12.63±0.94^{eA}
EtOAc fraction	Cold maceration	0	0	6.69±0.45^{abC}	7.51±0.32^{bcC}	15.45±1.19^{aB}	20.48±0.83^{aA}
Residual $EtOH/H_2O$ fraction	Cold maceration	0	0	0	5.53±1.16^{dC}	7.56±0.84^{eB}	11.79±0.69^{eA}
	Soxhlet	0	0	0	5.90±0.35^{cdC}	10.35±0.31^{dB}	15.82±0.83^{dA}
Essential oil	SD	0	0	5.66±0.19^{bcB}	6.27±0.39^{cdB}	6.27±0.39^{eB}	13.06±0.48^{eA}

Results represent the mean±standard deviation of three independent replicates.
Values with different lowercase letters within the same column indicate significant differences ($P<0.05$) between extraction methods and solvents. Values with different capital letters within the same row indicate significant differences ($P<0.05$) between extract/fraction concentration.
EtOH, ethanol; CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; $EtOH/H_2O$, ethanol-water; SD, steam distillation.

2021). A variation in the susceptibility of the bacterial strains to these extracts, fractions, and essential oil was observed, with a better antibacterial performance observed against *S. aureus* compared to *E. coli*. The *E. coli* strain tested was not susceptible to the extracts, fractions, or essential oil obtained in this study. The differences in susceptibility between gram-negative and gram-positive bacteria can be attributed to the low susceptibility of the outer membrane of gram-negative bacteria, which hinders the penetration of antimicrobial compounds to reach their site of action (Farahmandfar et al., 2019; Breijyeh et al., 2020). Gram-positive bacteria lack this important layer, which may be the main reason for the higher resistance of gram-negative bacteria to a wide range of antibiotics and antimicrobial agents (Breijyeh et al., 2020; Tavares et al., 2020). The antibacterial activity was found to be highest in the CH_2Cl_2 fraction of *C. lutea* leaves, which conferred 81.20%±3.90% inhibition against *S. aureus* ATCC 29213. The second highest antibacterial activity was conferred by the EtOAc fraction (58.18%±4.28%). Both of these fractions were obtained from the total EtOH extract via cold maceration (Table 4). Meanwhile, *S. aureus* was not sensitive to the other fractions, extracts, and essential oil at the concentrations tested, presenting an inhibition rate of less than 50%. This could indicate that the extracts, fractions, and essential oil could be active against *S. aureus* at concentrations higher than 512 μg/mL; however, further studies are required to confirm this.

The activity of the CH_2Cl_2 fraction may have been related to the higher content of active compounds in this fraction, especially the phenolic and terpenoid compounds identified during phytochemical screening, which are known for their antibacterial properties (Álvarez-Martínez et al., 2021; Cagnini et al., 2021; Otmani et al., 2021; Touzani et al., 2021). These two chemical classes have been associated with very similar mechanisms of action, mainly based on the disruption of the bacterial plasma membrane, which increases the permeability of bacterial cells and promotes leakage of the intracellular contents (Álvarez-Martínez et al., 2021; Ecevit et al., 2022). Liu et al. (2022) indicated that compounds in the dichloromethane fraction of *Eurycoma longifolia* may play an important role in antibacterial activity against *S. aureus*, being related to cell destruction and/or increased cell permeability. The authors reported that this fraction increased the leakage of electrolytes, nucleic acid, and protein from *S. aureus*.

The antibacterial activity of *C. lutea* was superior to that reported by Apagüeño Arévalo and Tamani Guerra (2020), who tested the biological activity of the aqueous extracts of *C. lutea* against *S. aureus*, *E. coli*, and *P. aeruginosa* at concentrations of 1,300, 1,500, and 1,700 mg/mL of the extracts, using the agar disk diffusion method, without presenting biological activity against these bacteria. Considering the antibacterial activity of the CH_2Cl_2 fraction of *C. lutea* against *S. aureus* ATCC 29213 at a concentration of 512 μg/mL (81.20%), the CH_2Cl_2 fraction of *C. lutea* could be a possible candidate for a natural antibacterial agent with activity similar to that of the conventional synthetic preservatives used in food, such as sodium sulfite and potassium metabisulfite. These synthetic preservatives have shown the following growth inhibition data in previous studies: sodium sulfite at concentrations of 4,000 and 2,000 μg/mL inhibited the growth of *S. aureus* and *E. coli*, respectively, by >90%; potassium metabisulfite at concentrations of 1,000 and 500 μg/mL inhibited the growth of *S. aureus* and *E. coli*, respectively, by >90% (Bertéli et al., 2021; Cagnini et al., 2021; Bertéli et al., 2022). It is important to mention

Table 4. Antibacterial activity (percentage inhibition of bacteria) of the total extract and fractions of *Calathea lutea* leaves at a concentration of 512 μg/mL against *Staphylococcus aureus* and *Escherichia coli* strains

Type of extract	Extraction method	*S. aureus*	*E. coli*
		ATCC 29213	ATCC 25922
Total EtOH extract	Cold maceration	10.85 ± 0.72^{ef}	–
	Soxhlet	42.31 ± 1.74^{c}	–
Hexane fraction	Cold maceration	32.69 ± 3.59^{cd}	–
	Soxhlet	33.27 ± 2.71^{cd}	–
CH_2Cl_2 fraction	Cold maceration	81.20 ± 3.90^{a}	–
	Soxhlet	37.68 ± 4.38^{cd}	–
EtOAc fraction	Cold maceration	58.18 ± 4.28^{b}	–
Residual EtOH/H_2O fraction	Cold maceration	NA	NA
	Soxhlet	5.55 ± 0.89^{f}	–
Essential oil	SD	24.80 ± 0.37^{de}	–

Results represent the mean±standard deviation of sample of three independent replicates.
Values with different lowercase letters within the same column indicate significant differences ($P<0.05$) between extraction methods and solvents.
EtOH, ethanol; CH_2Cl_2, dichloromethane; EtOAc, ethyl acetate; EtOH/H_2O, ethanol-water; SD, steam distillation; NA, failed to analyze; –, not available.

that synthetic preservatives are officially regulated in the food industry. In the CODEX STAN 192-1995 (2017) regulations, the maximum concentration of any synthetic preservative is stipulated according to the food category (30 – 1,000 μg/mL), and the use of sodium or potassium metabisulfite at concentrations higher than 1,000 μg/mL in food applications is prohibited. It is also worth mentioning that the above-mentioned preservatives present inhibition of microorganisms at concentrations higher than those allowed, which could be explained by considering that *in vitro* culture conditions are ideal for microbial growth. However, no maximum concentration is stipulated for natural extracts for food application; therefore, the CH_2Cl_2 fraction of *C. lutea* should be analyzed at concentrations higher than those tested in this study, especially as it did not demonstrate lethality in the biomodel. The absence of lethality in our toxicity tests, evidenced by an LD50 greater than 2,000 μg/mL, adds to the demonstrated antibacterial activity and supports the hypothesis that the CH_2Cl_2 fraction of *C. lutea* could be a promising candidate for use as a natural food preservative.

This study had certain limitations. The main limitation was that, in this first phase of research (basic research), solvents with a high affinity for the compounds of interest in *C. lutea* leaves were used, without their subsequent application in food matrices. Although these solvents allowed us to obtain high-quality extracts, it is essential to recognize that a certain amount of solvent may have escaped into the environment during the extraction process and that the resulting products may have contained a small quantity of residual solvent, which may result in environmental and food safety impacts (Prasad et al., 2022). In this sense, it will be essential to consider food safety in the further stages of development. Therefore, we propose the use of solvents (e.g., carbon dioxide) and extraction methods (e.g., supercritical fluid, microwave-assisted, and ultrasound) that are considered safe for food use to ensure the safety of the extract and its potential application in edible products (Picot-Allain et al., 2021; Sridhar et al., 2021; Jha and Sit, 2022). Nonetheless, the results obtained in this first phase are promising and provide a good basis for further studies.

In this work, the phytochemical compounds, potential toxicity, and antibacterial activity of the extracts, fractions, and essential oil of a traditional Colombian leaf, *C. lutea*, were investigated. The influence of the solvent (ethanol, hexane, dichloromethane, and ethyl acetate) and the extraction method (maceration, Soxhlet, and steam distillation) on the yield was evaluated, revealing that the Soxhlet extraction method and dichloromethane as the solvent conferred the highest yield among the fractions. In the phytochemical screening, the characteristic metabolites of this plant were found to be flavonoids, triterpenes, and tannins.

Regarding toxicity, all extracts, fractions, and essential oils were considered safe at the concentrations evaluated. Regarding antibacterial activity, although with low inhibition percentages, *S. aureus* was found to be moderately sensitive to the extracts, fractions, and essential oil, with the dichloromethane fraction obtained by cold maceration showing the highest antibacterial activity. It is possible that the extracts, fractions, and essential oil of *C. lutea* leaf may be active against *S. aureus* at concentrations higher than those tested in this research. Meanwhile, the extracts, fractions, and essential oil showed no antibacterial activity against *E. coli* ATCC 25299.

In future work, we plan to evaluate the antimicrobial activity of the dichloromethane fraction against microorganisms (both the ones tested in this study and others) at higher concentrations. We also plan to develop studies that use specific food matrices to assess the behavior of the extracts *in situ*, to evaluate the sensory effect on the product to be preserved, and to determine the effect of processing and additives on the antimicrobial activity. Overall, our results revealed that extracts, fractions, and essential oils obtained from *C. lutea* can be used as readily available sources with antibacterial properties against *S. aureus* for possible use in various food products. However, to guarantee the safety of the extract and its application in edible products, we propose the use of solvents and extraction methods that are already considered safe for food use.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: KPS. Analysis and interpretation: KPS, MDL, BAS. Data collection: KPS. Writing the article: KPS. Critical revision of the article: KPS, PMC, DAC. Final approval of the article: all authors. Statistical analysis: KPS. Obtained funding: KPS. Overall responsibility: KPS.

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