

Ethnobotany, total phenolic and flavonoid content in the species *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson

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ABSTRACT

After the emergence of COVID-19, respiratory sequelae persisted, and one alternative worth reconsideration is *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson. The objective is to determine the ethnobotany and the total phenolic and flavonoid content of *C. citriodora*. The ethnobotanical information was gathered using a semi-structured questionnaire from herbalists in markets in the city of Trujillo, La Libertad, Peru. *Corymbia citriodora* was collected in Agallpampa, Otuzco, and identified in the *Herbarium Truxillense*. Three extracts were prepared and evaluated (infuse, decoct, and hydroethanolic). The total phenols were quantified using the Folin Ciocalteu method, and total flavonoids were quantified using the aluminum chloride method. *Corymbia citriodora* reported a higher medicinal use in bronchitis (39.1%), in the preparation of a frequent infusion, made from leaves, often in combination with *Piper aduncum* (35.7%). Total phenols and flavonoids were reported as 118.65 mg GAE and 33.95 mg QCE per gram of dried drug, with the hydroethanolic extract standing out. The high presence of phenolic and flavonoid bioactives is directly related to anti-inflammatory activity, suggesting that *C. citriodora* remains a viable option and demonstrates efficacy in a traditional context for improving respiratory conditions.

INTRODUCTION

Traditional medicine is an alternative to conventional therapy, due to the adverse effects they present, there is a tendency for patients to use medicinal plants, as they are readily available and, in many cases, more accessible [1].

Today, the practice of herbal medicine continues to provide health benefits, which is why there is a focus on documenting the ethnobotanical knowledge of each country [2,3]. Ethnobotanical knowledge can disappear if the plant species is threatened with extinction. This serves as an indicator of biodiversity, providing ample scope and opportunities for new research to discover new medicines [4–6]. These therapeutic effects could be explained by the presence of bioactive

metabolites, including phenols, flavonoids, carotenoids, and tocopherols, capable of interacting with the human body and reducing the burden of free radicals often associated with chronic diseases [7].

Corymbia citriodora is a species that thrives in low-precipitation areas, and there are plantations that have shown genetic improvements [8]. It is important for its ability to survive, its resistance to endemic diseases and pests, and its high wood production [9]. It is highly profitable and less risky for forest formation and conservation in subtropical areas; *C. citriodora* stands out from others for the quality of wood it provides to the paper industry [10].

It is a widely distributed species, and its wood is used as raw material for making furniture, poles, fences, houses, and fuel [11]. Similarly, the aerial parts of the tree provide important metabolites for traditional medicine [12] and essential oils that are significantly useful in the chemical and pharmaceutical industry [13].

Within the chemical composition of *C. citriodora*, important components have been reported, including essential

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oils (citronellal, citronellol, p-cymene, and spathulenol); phenolic acids (quinic acid, rosmarinic acid, ferulic acid, caffeic acid, gallic acid, ellagic acid, catechol, and 3-O-methylellagic); flavonoids (quercetin, kaempferol, isoquercitrin, isomyricitrin, myricetin, myricitrin, eucalyptin, citriodolic acids, and citriodol A), and glycosides (quercetin-3-O- β -D-galactoside, kaempferol-3-O- β -D-glucoside, quercetin-3-O- β -D-glucuronide, quercetin-3-O-rutinoside, and kaempferol-3-O- α -L-rhamnoside) [14,15].

This species is of great benefit in traditional medicine, as it has been described to be used in the treatment of pain, inflammation, fever, viral respiratory infections, and cancer [15,16]. Furthermore, studies have reported pharmacological and biological properties such as antibacterial, antioxidant, antiproliferative, acaricidal, antifungal, herbicidal, and insecticidal properties [17,18].

Given the information mentioned above, this research aims to describe the ethnobotany for traditional medicine knowledge and quantify the total phenolic and flavonoid compounds in the plant extracts of the species *C. citriodora* (Hook.) K.D. Hill & L.A.S. Johnson. With this, we aim to reevaluate and endorse the use of the species *C. citriodora* in traditional medicine. Likewise, we seek to explore how to harness the bioactive compounds present to ensure medicinal properties, thereby considering it the most accessible natural alternative for the treatment of respiratory conditions.

MATERIALS AND METHODS

Botanical material

The species used was *C. citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, which was acquired from herbalists in the markets located in the city of Trujillo, region La Libertad, Peru. They demonstrated knowledge of the use of medicinal plants in the region. The medicinal plant was collected in Agallpampa, Otuzco, at geographical coordinates (7°57'08" S, 78°31'00").

Taxonomic identification

A complete specimen was pressed and then prepared according to the standard protocols of the *Herbarium Truxillense* and taxonomically identified with registration code N° 60829 by the Biologist-Botanist Eric Rodriguez.

Reagents and solvents

Ethanol 96° GL (CKF®), distilled water (Dropaksa®), Folin Ciocalteu reagent (Sigma Aldrich®), sodium carbonate, aluminum chloride, sodium nitrite, and sodium hydroxide (Merck®) were used. Gallic acid (Merck®) and quercetin (Sigma Aldrich®) were used as standards.

Ethnobotanical study

Field and exploration studies on the *C. citriodora* species were conducted between April and July. Three major markets in the city of Trujillo, Peru, were selected due to their high distribution of medicinal plants. Sampling was conducted randomly among the herbalists as proposed by Weckerle *et al.* [19]. The information was collected through semi-structured interviews using a questionnaire from herbalists and verbal

informed consent was obtained before the interview. The level of fidelity was not considered because it is the only medicinal plant. The study followed the Code of Ethics of the International Society of Ethnobiology [20], and the protocol was approved by the Ethics Committee of the National University of Trujillo with registration code N° PR003P–2022/CEIFYB.

Preparation and extraction of plant extracts

The acquired *C. citriodora* species had its leaves selected as the plant material, ensuring they were intact, without inert material or decomposition. The plant material was shade-dried and then dried in a Memmert oven at 40°C for 24 hours, followed by mechanical milling with a Corona mill to obtain particles no smaller than 2 mm. Three extraction systems were prepared, including two aqueous extracts obtained by decoction and infusion, and a hydroethanolic extract of 70% obtained by reflux. The concentration of the extracts was 10% w/v [21].

Quantification of total phenols

This was carried out using the Folin Ciocalteu method. In 10 ml volumetric flasks, 200 μ l of the plant extract was taken, 200 μ l of 10% v/v Folin Ciocalteu reagent was added, and the mixture was gently mixed. Then, 400 μ l of 4% Na₂CO₃ was added, and the flask was filled with distilled water. After waiting for 90 minutes, the absorbance was measured at 750 nm using a UV-visible spectrophotometer, Peak Instruments. Each measurement was performed in triplicate. Gallic acid was used as the standard for the calibration curve at concentrations of 10–100 μ g/ml. The total phenol concentration was expressed as gallic acid equivalents per gram of dry material (mg GAE/g DM) [22,23].

Quantification of total flavonoids

This was conducted using the colorimetric method with aluminum chloride. In 10 ml volumetric flasks, 200 μ l of the extract was taken, and 150 μ l of 5% w/v NaNO₂ was added and gently mixed. Then, 150 μ l of 10% w/v AlCl₃ was added. After 5 minutes, 1000 μ l of 1 M NaOH was added, and the solution was filled with distilled water. After waiting for 30 minutes, the absorbance was measured at 510 nm using a UV-visible spectrophotometer, Peak Instruments. Each measurement was performed in triplicate. Quercetin was used as the standard for the calibration curve at concentrations of 1–100 μ g/ml. The total flavonoid concentration was expressed as quercetin equivalents per gram of dry material (mg QCE/g DM) [24,25].

Statistical analysis

All data were obtained in triplicate and expressed as mean and standard deviation ($\bar{X} \pm SD$). Statistical differences ($p < 0.05$) were calculated using the analysis of variance (ANOVA) test with Microsoft Office Excel 2019.

RESULTS AND DISCUSSION

The information obtained from herbalists has a significantly positive impact on the use and sustainability of traditional knowledge. Table 1 presents the demographic and ethnobotanical information collected from herbalists in the

Table 1. Information on ethnobotanical knowledge of *C. citriodora* and demographic information of herbalists interviewed in markets.

Ethnobotanical information						
Specie	Common name	Medicinal use	Application method	Used part	Condition	Combinations with species
<i>C. citriodora</i> (Hook.) K.D. Hill & L.A.S. Johnson.	Eucalyptus	Common cold, bronchitis, flu, cough, muscle pain, asthma, COVID-19	Infusion, steam bath, inhalation, smudging, friction	Whole plant, leaves, stems	Fresh, dry, both	<i>P. aduncum</i> (matico), <i>R. officinalis</i> (romero), <i>P. multiflora</i> (escorzonera), <i>M. alborea</i> (malva blanca), <i>C. auriculatum</i> (hierba santa), <i>S. molle</i> (molle), <i>S. officinalis</i> (salvia)
Demographic information						
Gender			Age			
Male (25%)			25–34 (25%)			
Female (75%)			35–49 (58.3%)			
			50≥ (16.7%)			

markets of Trujillo, where a higher percentage of indications for relieving bronchitis (39.1%) and the common cold (21.7%) were found, categorized by the type of ailment (Fig. 1A). In addition, infusion (54.5%) was the predominant method of administration (Fig. 1B).

The demographic information of the interviewees indicates that 75% of the respondents were women, and 25% were men. The predominance of women aligns with Mohamadi *et al.* [26], attributed to the culture of the study area and the greater concern for maternal health [27]. On the other hand, the prevalence in the age group of 35–49 years contrasts with the similarity found in the study by Abba and Dogara [27]. This is due to increased activity and time dedicated to the collection of medicinal plants; furthermore, during this stage, it demonstrates the transmission of traditional medicinal knowledge in the region [28].

Research demonstrates the relationship between popular usage and its biological effects, reinforcing what is documented in traditional medicine. Magalhães *et al.* [29] report its use as a remedy for fever, flu, cough, sneezing, sinusitis, bronchitis, and asthma. Similarly, Paniagua-Zambrana *et al.* [30] reported its use in respiratory conditions such as colds, flu, and pneumonia, both as an infusion and in steam baths. On the other hand, Abbass [31] claims that it reduces muscle and stomach pain, cough, and shortness of breath in COVID-19 patients, thereby reducing risk factors for morbidity. In addition, Sharifi-Rad *et al.* [32] describe its use in the treatment of respiratory conditions, including wheezing, difficulty breathing, cough, chest tightness, and shortness of breath. Among the isolated molecules, tereticornate A and euciptin have been identified with the potential to reduce the activity of NF-κB/AP-1 and attenuate the secretion of IL-1β [33].

Goodine and Oelgemöller [34] mention it as an antifungal and antibacterial agent. Similarly, Sarma *et al.* [35] report its potent antimicrobial, anti-inflammatory, and cytotoxic properties, while Tolba *et al.* [36] describe it as an antioxidant and antimicrobial. Salehi *et al.* [37] reiterate, highlighting its antimicrobial, antiseptic, antioxidant, and anti-inflammatory properties. The bioactive components serve specific beneficial functions for the human body. Ethnobotanical studies report

the use of leaves (48%), with infusions (60%) being the most common preparation method, often directed towards the treatment of respiratory diseases (16%) [38]. Dried leaves and buds are used for inhalation to relieve asthma [39]. Samois and Mahomoodally [40] report the use of leaf decoctions for baths. According to Paniagua-Zambrana *et al.* [30], it is used in the form of infusions and steam baths.

Corymbia citriodora shows that the most frequently used part is the leaves (78.6%), and the predominant plant condition can be both fresh or dry, with 91.7% (Fig. 1C and D). Leaves are commonly used as the predominant plant part in formulations [41]. Afolayan *et al.* [42] also reported that leaves (34.82%) were the most frequently used, followed by root barks and stems (15.17%). Other studies report that leaves (31.16%) and stems (19.6%) are the most frequently used organs [43]. Bibi *et al.* [44] reported that the most frequent preparation method was decoction (31%), but infusion (16%) was also commonly used. Magalhães *et al.* [29] often report consumption in the form of infusions and/or decoctions made from the leaves. The selection of the plant part used often depends on the location of its bioactive metabolites.

They also report that *C. citriodora* is used in combination with other plants to synergize therapeutic properties, with 35.7% in combination with *Piper aduncum* and 7.1% in combination with *Rosmarinus officinalis*, *Perezia multiflora*, or *Malva alborea* (Fig. 1E). The combination of *C. citriodora* with *P. aduncum* (matico) is mentioned in the studies and is reaffirmed by Mejía and Estela [45]. A list of medicinal plants used in the treatment of respiratory tract diseases and against SARS-CoV-2 was compiled, where *P. aduncum* was found to be effective in treating bronchitis, chills, colds, cough, tuberculosis, pharyngitis, and pneumonia, as confirmed by Delgado-Paredes *et al.* [46]. The knowledge base about its medicinal uses is attributed to family members (54%), followed by practical experience (20%), which aligns with the idea that ethnobotanical knowledge is passed down vertically within a family and learned through practice [47,48].

On the other hand, *P. aduncum* is used for the treatment of other respiratory conditions such as pneumonia, tuberculosis,

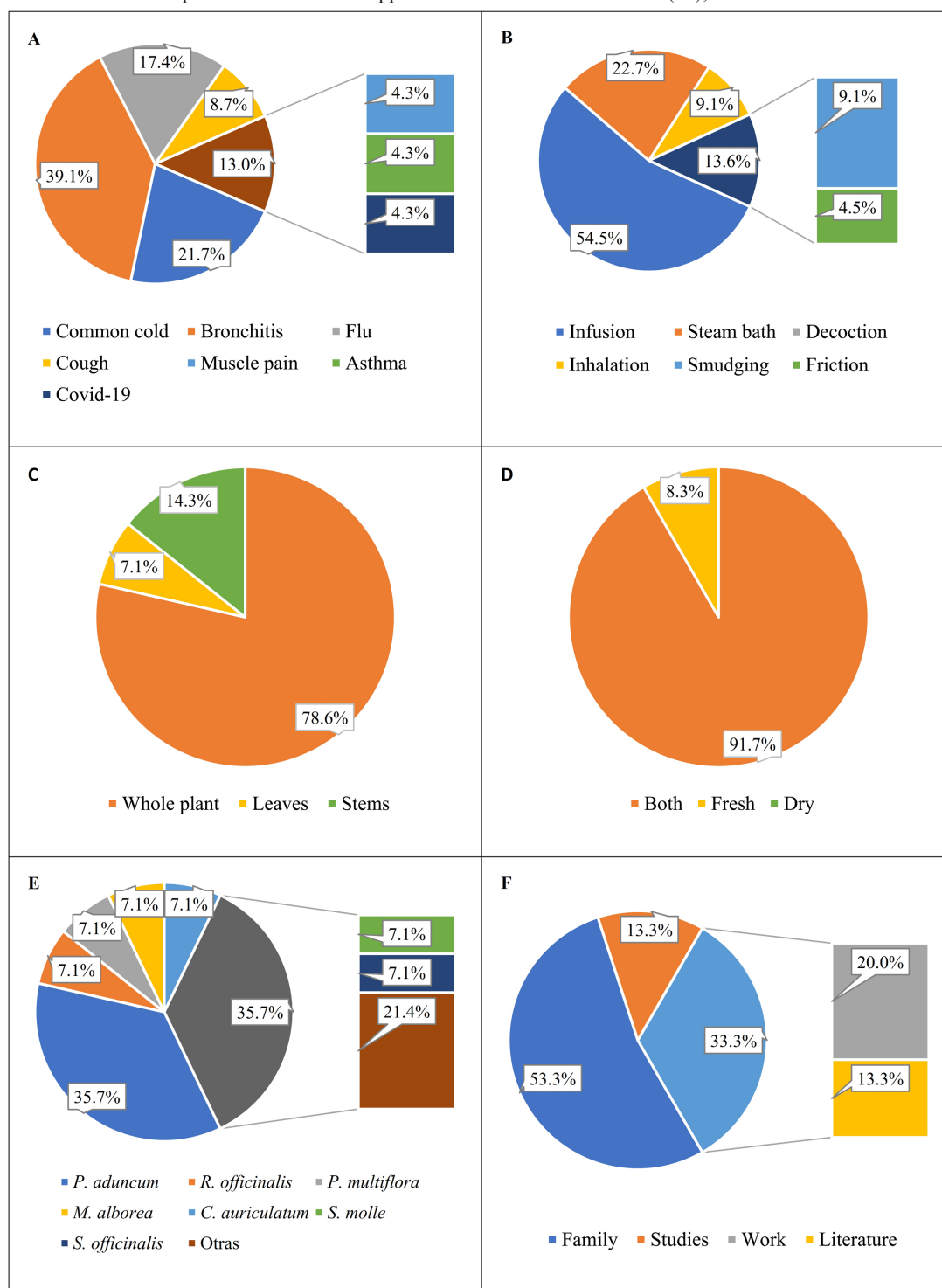


Figure 1. Ethnobotanical information of *C. citriodora* collected from herbalists; (A) medicinal use, (B) method of application, (C) used part, (D) condition, (E) combinations with species, and (F) basic of their knowledge.

fever, and the flu in traditional medicine [49,50]. It is also reported that species such as *P. multiflora*, *R. officinalis* L., and *Salvia officinalis* L. [49,51] can address respiratory conditions, and thus, they may synergize with *C. citriodora*.

Regarding the total phenol content (Fig. 2A), it was found to be 118.65, 94.44, and 68.31 mg GAE/g DM in hydroethanolic, decoction, and infusion extracts, respectively.

The hydroethanolic extract exhibited the highest concentration. The studies indicate that the concentration of *C. citriodora* is high compared to the 57.02 mg GAE/g DM reported by Bibi *et al.* [44]. Other authors reported concentrations of 106 g/kg dry matter [52], 58.1 µg GAE/mg of extract [53], and 28.23 mg GAE/g DM [54]. In addition, Toumi *et al.* [55] reported phenol concentrations ranging from 375 to 400 mg GAE/100 g DM in

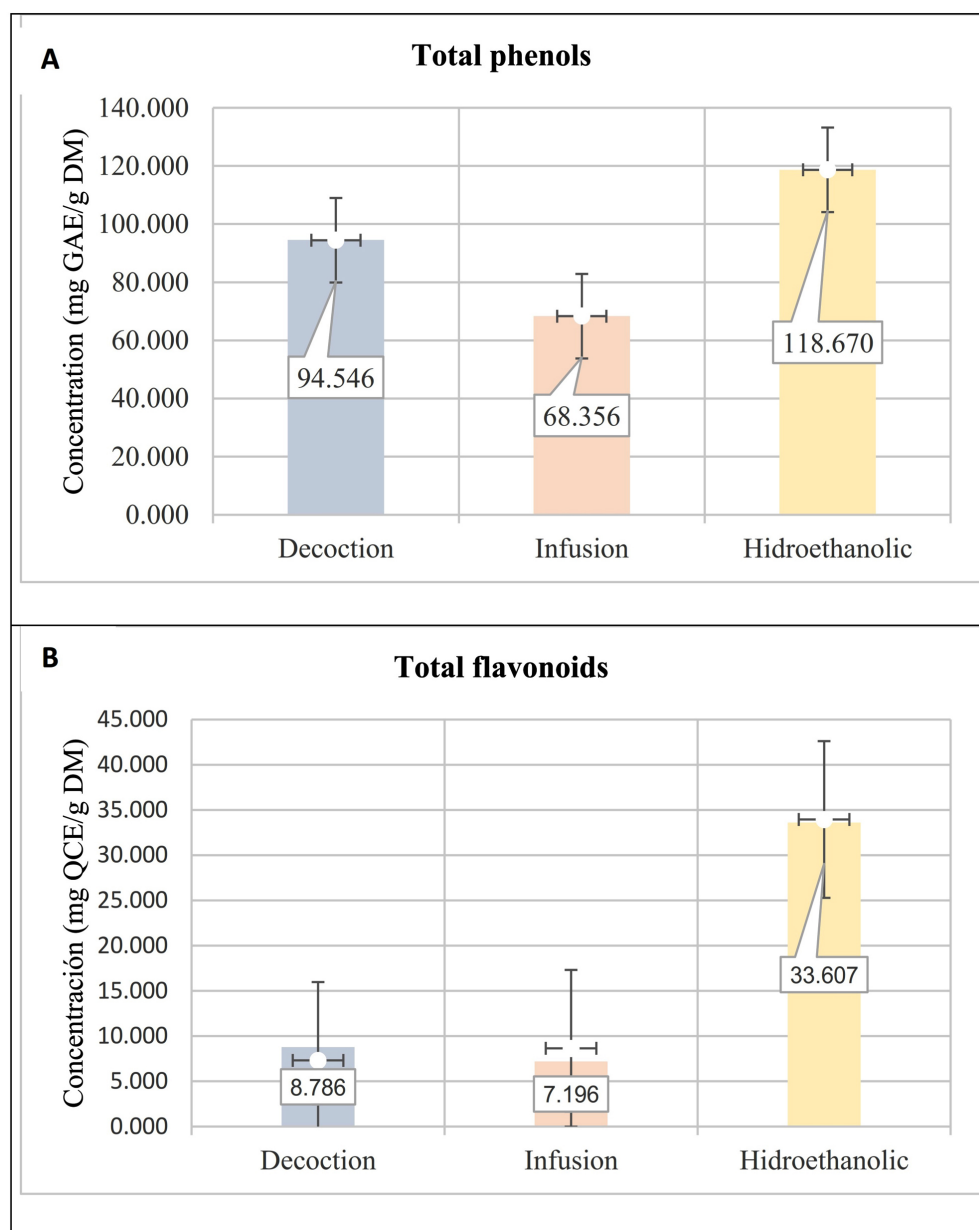


Figure 2. (A) Total phenols and (B) total flavonoids content in *C. citriodora*. GAE: gallic acid equivalent, QCE: quercetin equivalent, DM: dry material. Statistic: ANOVA ($p < 0.05$).

a 60% ethanol extract, which was influenced by an extraction method involving electric field pulsations.

Regarding total flavonoids (Fig. 2B), it was reported to be 33.95, 8.66, and 7.31 mg QCE/g DM in hydroethanolic, infusion, and decoction extracts, respectively. It can be observed that the hydroethanolic extract had a higher concentration compared to the study by Gonçalves *et al.* [54], which reported 4.46 mg EQ/g dry weight. Some authors also refer to concentrations of 4.89 mg/g extract [56] and 50–60 mg EC/100 g DM in a 60% ethanol extract [55], with yield intensified by the extraction method.

The main compounds, such as phenols and flavonoids, are influenced and react to certain climatic conditions and soil properties [22]. Variability in polyphenol concentration

is attributed to environmental factors such as light intensity, day length, temperature, and nutrient levels [57]. Furthermore, the composition of metabolites in the same species can vary according to geographical location and the different methods used for obtaining phenolic extracts [58].

CONCLUSION

Ethnobotany reported that the species *C. citriodora* is effective for treating bronchitis, the common cold, and flu, primarily consumed in the form of an infusion made from fresh or dried leaves, sometimes in association with matico (*P. aduncum*) and escorzonera (*P. multiflora*). High concentrations of total phenols and total flavonoids were reported at 118.65 mg GAE/g DM and 33.95 mg QCE/g DM in the hydroethanolic

extract. The predominant presence of polyphenols implies enhanced anti-inflammatory and antioxidant activity due to their inherent relationship and can positively ensure therapeutic efficacy in traditional use.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study followed the Code of Ethics of the International Society of Ethnobiology, and the protocol was approved by the Ethics Committee of the National University of Trujillo with registration code N° PR003P–2022/CEIFYB.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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