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Phytochemical screening and biological activity of *Centella asiatica* (L.) Urban extracts by different methods

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ABSTRACT

Purpose: Study on the effects of extraction methods on the biological activities of *Centella asiatica*, including antibacterial and antioxidant properties. **Research Method:** The main components of *C.asiatica* include triterpenoid saponins, polyphenols, flavonoids, and other health-beneficial compounds found through phytochemical screening. Ethanol extraction is performed using three extraction methods: immersion extraction, ultrasonic extraction, and reflux. *Centella asiatica* extract was tested for polyphenol content, total flavonoids, total triterpenoid saponins, and the ability to scavenge the free radicals DPPH and ABTS. **Findings:** The reflux extraction method was more effective than the other extraction methods in extracting chemical components, yielding relatively higher polyphenol, total flavonoid, and total triterpenoid saponins contents. All three types of extracts have the ability to fight oxidation, protecting cells from harmful free radicals. The IC_{50} value of pennywort extract in DPPH and ABTS free radical scavenging tests ranged from 455.52 and 270.05 µg/mL (soaked) < 333.63 and 206.56 µg/mL (ultrasound) < 239.75 and 199.75 µg/mL (reflux). The minimum inhibitory concentration (MIC) for the two bacterial strains *Staphylococcus aureus* and *Escherichia coli* was less than 2.188 mg/mL in all three methods. **Research limitations:** No limitations were encountered. **Originality/Value:** The development of extraction processes and evaluation of high quality extracts from gotu kola requires a combination of traditional methods and modern technology such as the use of advanced chemical and biological analytical techniques. This may create opportunities for the development of new technologies in the field of herbal extraction.

INTRODUCTION

Gotu Kola, whose scientific name is *Centella asiatica* (L.) Urb, is a small, herbaceous, perennial plant native to wetlands in Asia (Prakash et al., 2017). It is a member of the Apiaceae family and is known for its diverse application properties (Shakir Jamil et al., 2007). *Centella asiatica* thrives in tropical and subtropical regions, especially in moist, shady environments such as wetlands, riverbanks, and marshes. Its natural habitat ranges across the world in Southeast Asia, India, Sri Lanka, China, Indonesia, and South Africa (Jantwal et al., 2021; Loc & Nhat, 2013; Torbati et al., 2021). The plant has small, round, fan-shaped leaves with a smooth and palmate texture. The leaves are usually green to light green in color. The stems are slender, creeping, and spiny, allowing the plant to easily spread above ground. The flowers are arranged in clusters near the leaf nodes. The fruit is small, oblong, and ribbed, containing seeds.

The rich biological activities of gotu kola are attributed to constituents such as triterpenoid saponins (Asiaticoside, madecassoside, asiatic acid, and madecassic acid), flavonoids (quercetin, kaempferol, and catechins) that contribute to the total phenolic content, etc (Monton et al., 2019; Sen et al., 2019; Tsaltaki et al., 2019). These substances contribute to the antioxidant and anti-inflammatory activities of the plant (Hoang & Rehman, 2023; Shohel Hossain, 2018). Triterpenoids are components present in most *C. asiatica* species in different regions (James & Dubery, 2009). The presence and concentration of these compounds can vary depending on factors such as plant growth conditions, harvest time, and extraction method used. Some of the extraction methods used are maceration, reflux, microwave-assisted, ultrasound-assisted, enzymatic, soxhlet extraction, and supercritical extraction (Mohapatra et al., 2021). These techniques all have more or less the same goal in extracting biological activity from plants. However, new extraction techniques that are considered more effective and environmentally friendly are currently being widely used, on both laboratory and industrial scales, in nutraceuticals, food additives, pharmaceuticals, and many other fields.

Centella asiatica is famous for its antioxidant activities, mainly due to its rich content of bioactive compounds, including polyphenols, flavonoids, and triterpenoids. These compounds are known for their potent antioxidant activities, by eliminating free radicals and strengthening the body's antioxidant defense system. According to Zainol et al. (2003), the highest antioxidant activity is found in *C. asiatica* leaves compared to other parts and is also the part containing the highest phenolic content contributing to the antioxidant activity of *C. asiatica* (Zainol et al., 2003). Phenolic compounds and flavonoids were also found and demonstrated to contribute to antioxidant activity (Pittella et al., 2009). Other research in 2021 also demonstrated the anti-aging skin activity of *C. asiatica* extract in pharmaceutical and cosmetic products (Buranasudja et al., 2021).

C. asiatica exhibits remarkable antibacterial potential, contributing to the treatment of various infections and promoting wound healing. The antibacterial properties of *C. asiatica* are mainly attributed to its phytochemical components such as triterpenoids, flavonoids, polyphenols, and saponins. Here are some key points regarding its antibacterial activity. Ethanol extract of *C. asiatica* showed significant antibacterial activity against both Gram-negative and Gram-positive bacteria. Minimum inhibitory concentration (MIC) values were determined for different bacterial strains, indicating the effectiveness of the extract (Jagtap et al., 2009). The solvents (ethanol, chloroform, and hexane) used in gotu kola extract all have antibacterial activity against gram-positive and gram-negative strains at a concentration of 50 mg/mL (Rattanakom & Yasurin, 2015). Therefore, research on the extraction process and

evaluation of high-quality extract from *Centella asiatica* is necessary to ensure effectiveness and safety in use in medical and cosmetic practice.

MATERIALS AND METHODS

Raw material

C. asiatica was collected in Ben Tre (10° 14′ 25″ N, 106° 22′ 44″ E) and identified at the City Ginseng and Medicinal Center in Ho Chi Minh, Vietnam. The above ground parts of *C. asiatica* were collected and transported to the laboratory, washed, removed damaged parts and dried at 50ºC. The raw materials were ground and stored in glass zip bags as raw materials for extraction, qualitative and quantitative analysis of compounds in *C. asiatica*.

Chemicals

Analytical chemicals used in the study were purchased from Sigma-Aldrich, such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and Folin & Ciocalteu's phenol reagent. Some other chemicals were purchased from China, such as ethanol, ascorbic acid, sodium carbonate (Na_2CO_3), gallic acid, hydrochloric acid (HCl), aluminum chloride ($AlCl_3.6H_2O$),... etc.

Plant extraction

Centella asiatica powder samples were extracted using three methods: soaking, reflux, and ultrasound using ethanol solvent (Mohapatra et al., 2021). The maceration process was carried out in a 100 mL conical flask containing 1 g of sample dissolved in 40 mL of ethanol solvent for 72 hours at room temperature. 1 g of raw material powder dissolved in 40 mL of ethanol solvent was placed in the flask, reflux extraction was carried out at 60 °C for 1 h. Ultrasonic extraction was carried out for 1 hour at 60°C with 1 g of powder using 40 ml of ethanol solvent in an ultrasonic apparatus. At the end of the process, the mixture was filtered to remove residue with a vacuum filter and Whatman filter paper in a round plate with a diameter of 90 mm. The extract was then evaporated by vacuum. The concentrated extract, about 5 ml, was collected in a small vial and allowed to dry by evaporation in a 50°C oven, finally obtaining the extract from all three methods.

Analytical methods

Qualitative Phytochemical Screening

Phytochemical analysis is the process of studying chemical compounds in plants. Bioactive compounds include phenolics, tannins, flavonoids, alkaloids, saponins, and many others. The presence of phytochemical components is noted by the sign (+), and vice versa, the absence is indicated by the sign (-) by identifying the phenomenon of chemical reactions.

Quantitative Phytochemical Screening

Total Polyphenol Content: Gallic acid is used as the standard in the Folin-Ciocalteu colorimetric method. The Folin-Ciocalteu reagent reduces polyphenol compounds to form a blue reaction product, whose absorbance is measured at 765 nm using a UV–Vis spectrophotometer (Segaran & Chua, 2021). Total polyphenol content is expressed as milligrams of gallic acid equivalents per 1 g of extract (mgQE/g DW).

Total Flavonoid Content: Flavonoid content was determined based on the color method with aluminum chloride at 415 nm, following the research by Mahboubi et al. (2013). Quercetin was used as a standard (Mahboubi et al., 2013). Total flavonoid content is expressed as milligrams of quercetin equivalents per 1 g of extract (mgQE/g DW).

Saponin Triterpenoid Content: Construction of a standard curve to determine saponin triterpenoid content was performed as described by Segaran and Chua et al. (2021). Oleanolic acid is used in a sugar formulation (0.1 mg/mL). The extracts were dissolved in ethanol, and 0.2 ml of the extract was used for the reaction.Add 0.2 ml of 5% (w/v) vanillin-acetic acid solution and 1.2 ml of perchloric acid, shake well and incubate at 70°C for 15 min. Cool and add ethyl acetate to a total volume of 5 ml. Determine the absorbance at 550 nm using a UV-Vis instrument (Agilent Cary 60).

Antioxidant Activity

Free Radical Scavenging Assay by DPPH Method: The antioxidant capacity of pennywort samples was determined using the modified DPPH free radical scavenging method (Brand-Williams *et al*., 1995). The reaction mixture included 1.5 mL of DPPH (6.10^{-4}M, mixed in ethanol, OD_{517} nm = 1.1 ± 0.02) into each test tube containing 0.5 mL of extracts with different concentrations. The control sample used ascorbic acid. Absorbance was analyzed on a UV-Vis instrument (Agilent Cary 60) at a wavelength of 517 nm. The antioxidant capacity of a sample is expressed through the IC_{50} value - the concentration of antioxidants at which 50% of DPPH free radicals can be inhibited (1).

$$\text{DPPH}\ (\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \qquad (1)$$

Where Abs_C is the optical absorbance of the control sample, and Abs_T is the optical absorbance of the test sample.

Free Radical Scavenging Assay by ABTS Method: Prepare the ABTS stock solution by mixing 7.4 mM ABTS solution into 10 mL of 2.6 mM $K_2S_2O_8$ solution and incubate in the dark for 24 hours (OD_{734} nm = 1.1 ± 0.02) (Pham et al., 2017). The extracts were dissolved in ethanol and mixed into a series of different concentrations. 0.5 mL of the extract was mixed with 1.5 mL of clear $ABTS^+$ solution and incubated for 30 minutes in the dark at room temperature. Absorbance was determined at 734 nm. Ascorbic acid was used as a positive control. Each experiment was repeated 3 times, and the IC_{50} value was calculated similarly to the DPPH method mentioned above (2).

$$\text{ABTS}(\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \qquad (2)$$

Where Abs_C is the optical absorbance of the control sample, and Abs_T is the optical absorbance of the test sample.

Antimicrobial Activity

The antibacterial activity of the extract was evaluated based on the agar disk diffusion method, with Gram-positive bacterial strains *Staphylococcus aureus* ATCC 6538 and Gram-negative *Escherichia coli* ATCC 8739 (Palaksha et al., 2010). Then, the bacterial density was determined in the range of 10^6-10^7 CFU/ml by measuring optical density at 660 nm wavelength. The antibacterial ability of the extract was tested by pipetting 50 µl of sample solutions of different concentrations in the sample diluent into wells on agar plates spread with test bacteria. Use chloramphenicol (1 mg/mL) as a positive control. Use solvent to dissolve the sample as a negative control. After 24 hours, results are recorded by image and diameter of the sterile zone. The experiment was performed 3 times. The minimum inhibitory concentration of the test sample was investigated using the dilution method on a 96-well microplate (Cockerill, 2010). Each well contains 150 µl of bacterial medium and 50 µl of

sample diluted in the medium. Samples were diluted according to different concentration series. Incubate at 37ºC for 24 hours. After 24 hours, 20 µL of 0.01% resazurin reagent was added to each well. Observe the color change and record the MIC value.

RESULTS

Physicochemical properties and effectiveness of *C. asiatica* extract

Issues related to the preservation of raw materials during the extraction process were identified. High humidity can reduce extraction efficiency due to incomplete dissolution of compounds. Additionally, *C. asiatica* extract has a moisture content of less than 5%, making it suitable for preservation and storage, meeting the moisture standards of dry extract (Table 1). The total ash and HCl insoluble content of pennywort ingredients were evaluated at 13.62 ± 0.094% and 1.994 ± 0.215%, respectively.

C. asiatica extract is obtained using different methods such as soaking, reflux, and ultrasound, which directly impact the performance and separation of biological activity in the plant. Table 2 illustrates that there is not a significant difference in performance among the three methods. Specifically, ultrasound and reflux extraction show higher efficiency (> 22%) compared to immersion extraction at 20.77%. Furthermore, the choice of extraction solvent significantly influences antioxidant activity, as different compounds with varying polarities and solubilities are present in the plant and are soluble in specific extraction solvents. The yield and composition of compounds from *C. asiatica* are regulated by factors such as the processing of raw materials post-harvest, extraction method, and plant parts used. For example, drying leaves with hot air and extracting with 50% ethanol yielded 37.3%, while freeze-drying the whole plant and extracting with water resulted in a yield of 23.3% (Shin et al., 2021).

Table 1. Humidity of *C. asiatica* extract.

Samples	Humidity (%)
C. asiatica powders	7.47 ± 0.123
Immersion extraction	0.63 ± 0.04
Reflux extraction	0.78 ± 0.03
Ultrasonic extraction	0.77 ± 0.04

Table 2. Efficiency of *C. asiatica* extraction process.

Samples	Yield (%)
Immersion extraction	20.462
Reflux extraction	22.909
Ultrasonic extraction	24.121

Table 3. Preliminary phytochemical composition of *C. asiatica* extract.

Parameters	Response	Results	Phenomenon
Alkaloid	Reaction with Mayer's reagent	+	Reddish-brown precipitate
Saponin	Foaming phenomenon	+	Durable foam
Flavonoid	$Pb(CH_3COO)_2$ (10%)	+	Yellow precipitate
Terpenoid	Chloroform and concentrated H_2SO_4	+	Brick-red color
Tannins	$FeCl_3$ 0.5% solution	-	Blue-black precipitate

Phytochemicals

Compounds that contribute to the biological activity of *C. asiatica* extract include alkaloids, flavonoids, terpenoids, and saponins, while tannins are not found in pennywort extract (Table 3). The medicinal potential is demonstrated by the presence of phytochemical components in *C. asiatica* extract. *C. asiatica* is rich in terpenoids, which contribute to antioxidants, wound healing, and the treatment of inflamed tissues (CU et al., 2020). Additionally, the presence of flavonoids and polyphenols in pennywort has important functions in antioxidant, anti-inflammatory, and antibacterial properties against bacteria such as *E. coli*, *Shigella flexneri*, and *S. aureus* (Utami et al., 2011). Tiwari et al. (2011) also pointed out that alkaloids are nitrogen-containing heterocyclic compounds that have analgesic and antibacterial effects. Differences between published studies may be due to growth environmental conditions or extraction methods used (Chaudhary et al., 2020). Specifically, secondary metabolites such as steroids, flavonoids, saponins, coumarins, etc., were also found in *C. asiatica* extracts from parts of India using solvents like methanol and petroleum ether (Shobana, 2014) and water, acetone, chloroform, and methanol (Saranya et al., 2017). Kavisa Ghosh et al. (2014) conducted the chemical analysis of ethanol extract of *Centella asiatica* leaves, which showed the presence of alkaloids, saponins, glycosides, triterpenoids, sterols and absence of tannins (Ghosh & Indra, 2014).

Total Polyphenol and Flavonoid Contents

Polyphenol and flavonoid compounds are commonly found in all *C. asiatica* extracts. The effect of the extraction method on total polyphenol content (TPC) and total flavonoid content (TFC) is shown in Figure 1. The reflux method showed high amounts of TPC and TFC with values of 168.97 ± 1.16 mg gallic acid (GAE)/g DW and 144.69 ± 1.61 mg (QE)/g DW, respectively. Similarly, TPC content with values of 147.96 ± 1.89 and 123.46 ± 0.83 mg gallic acid equivalent (GAE)/g DW and TFC are 140.29 ± 1.76 and 111.83 ± 2.24 mg (QE)/g DW for ultrasound and maceration methods. Using the reflux method to extract compounds from pennywort ensures the maximum yield of its beneficial components, such as flavonoids, triterpenoids, and polyphenols. The reflux system facilitates a consistent flow of the solvent, promoting a steady and efficient interaction between the solvent and the plant matrix. This results in increased dissolution and transfer of polyphenol and flavonoids from the plant material to the solvent. Ultrasound-assisted extraction (UAE) may extract flavonoids at a faster rate, but its lower temperature might not fully release all polyphenol and flavonoid glycosides or reach deep into the plant matrix. While extreme heat can cause flavonoids to degrade, the controlled temperatures in reflux extraction are typically gentle enough to prevent significant degradation, especially when ethanol is present as a stabilizing solvent.

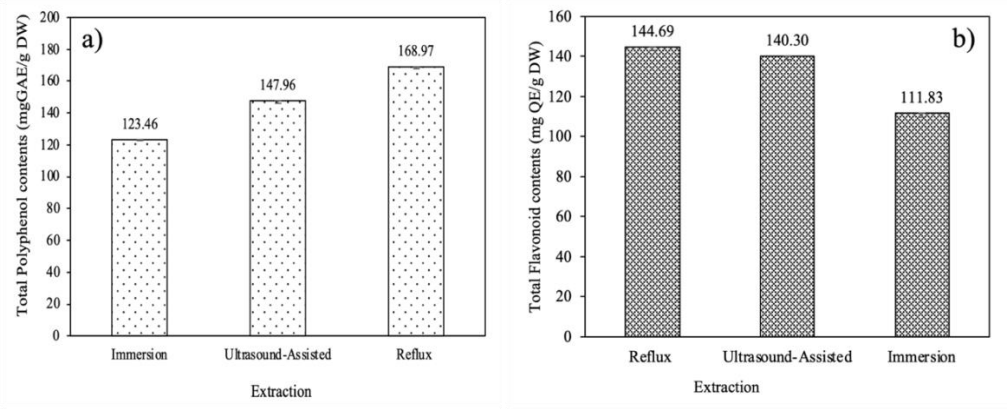

Fig. 1. Total polyphenol (a), and flavonoid (b) content in *C. asiatica* extract.

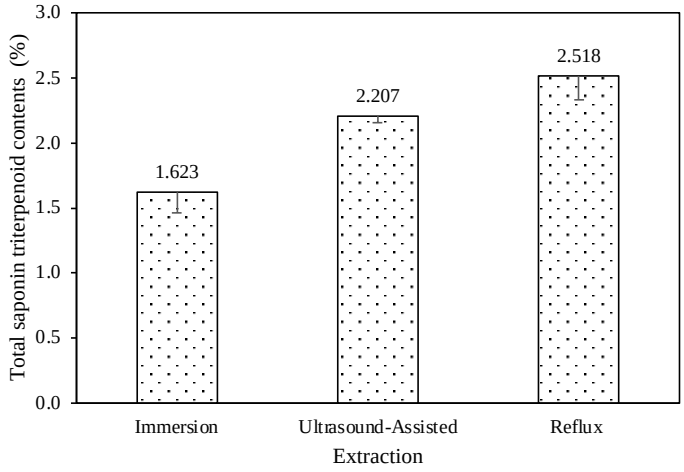

Fig. 2. Triterpenoid saponin content from *C. asiatica* extract by three different methods.

Triterpenoid saponins content

C. asiatica extract has a high triterpenoid saponin content, making it highly sought after in cosmetics, dietary supplements, and pharmaceutical products (AR, 2012; Huang et al., 2011). In this study, the triterpenoid saponin content obtained from immersion, ultrasound, and reflux extraction methods was 1.623%, 2.207%, and 2.518%, respectively (Fig. 2). The triterpenoid saponin content in *C. asiatica* extract may vary depending on factors such as the extraction method, the part of the plant used, and geographical differences. The elevated temperature during reflux extraction enhances the solubility of triterpenoid saponins in the solvent and disrupts cell walls, making the bioactive compounds more accessible. Ethanol, a polar solvent used in reflux extraction, is suitable for extracting triterpenoid saponins. While methods like ultrasound-assisted extraction (UAE) or enzyme-assisted extraction may offer advantages in speed or specificity, reflux extraction typically excels in maximizing compound yield due to prolonged exposure to optimal extraction conditions. This is particularly beneficial for triterpenoid saponins, which may require sustained high temperatures and sufficient solvent interaction for full extraction.

Antioxidant Activity Analysis

Extraction with ethanol solvent, which is related to the polarity of the solvent, can extract different fractions of polar/nonpolar components from plants. This finding was in good agreement with the total polyphenol and flavonoid content. The free radical scavenging activity of the compounds can be measured by the deuteration effect after trapping unpaired electrons by DPPH (Table 4). Experimental results show that *C. asiatica* extract has DPPH free radical scavenging activity with an IC_{50} value of 455.52 µg/mL (immersion) < 333.63 µg/mL (ultrasound) < 239.75 µg/mL (reflux), the inhibition rate is higher the higher the sample concentration. Similarly, the ABTS method is commonly used to measure the antioxidant capacity of compounds. In this assay, the ABTS radical cation ($ABTS^{\bullet+}$) is generated, which is blue-green in color. When an antioxidant is introduced, it donates electrons or hydrogen atoms to neutralize the ABTS radical cation, leading to decolorization. The degree of decolorization is measured spectrophotometrically at 734 nm, which corresponds to the absorbance of the ABTS radical. A greater reduction in absorbance indicates a stronger antioxidant capacity, as the compound stabilizes the free radicals. The obtained results also clearly showed that the tested extract had the ability to scavenge free radicals. As in the case of the described assay, the reflux extract showed the highest

antioxidant capacity, at the highest analytical concentration (1000 µg/mL) > 90% of ABTS radicals could be removed. Table 4 shows the analysis results from the ABTS method, *C. asiatica* extract has an IC_{50} value of 270.05 µg/mL (immersion) < 206.56 µg/mL (ultrasound) < 199.75 µg/mL (reflux).

Antimicrobial Activity

Antibacterial testing using the agar disk diffusion method of *C. asiatica* extract using ethanol solvent is presented in Table 5 and Figure 3. A test dose of 1 mg/well with a sterile ring diameter of 6 mm corresponds to no resistance. *C. asiatica* extract, obtained by all three methods (reflux, immersion, and ultrasound), showed inhibitory ability against the Gram-negative bacterium *E. coli*. In contrast, Gram-positive bacteria did not exhibit any zone of inhibition against *S. aureus*. The positive control Chloramphenicol had inhibition zones of 24.0 ± 0.2 mm (*E. coli*) and 24.5 ± 0.2 mm (*S. aureus*). However, *C. asiatica* extracts from the three methods showed MIC against both *E. coli* and *S. aureus* strains (Table 6 and Fig. 4). Specifically, the minimum inhibition value for the reflux method was 1.313 mg/mL, for immersion were 1.750 mg/mL and 2.188 mg/mL, and for ultrasound it was 1.131 mg/mL, corresponding to the two bacterial strains *E. coli* and *S. aureus*. The difference in results between the disk diffusion test and the 96-well test with resazurin reagent may be due to several factors. In the disk diffusion method, the ability of the antibacterial agent to diffuse through the agar medium may be limited, resulting in the antibacterial agent not having direct and consistent contact with *S. aureus* bacteria, which may reduce its effectiveness. The disk diffusion method uses a smaller extract concentration than the 96-well assay. In the 96-well test, the antibacterial agent is mixed directly with the bacteria in solution, ensuring direct and uniform contact.

Table 4. IC_{50} value of ethanol extract with *C. asiatica* extraction methods.

Methods	IC_{50} DPPH (µg/mL)	IC_{50} ABTS (µg/mL)
Immersion extraction	455.52	270.05
Reflux extraction	239.75	199.75
Ultrasonic extraction	333.63	206.56
Ascorbic aicd	4.47	18.88

Table 5. Antibacterial properties of three types of *C. asiatica* extracts.

Samples	Resistance index (sterile ring diameter – mm)	
	Escherichia coli ATCC 8739	*Staphylococcus aureus* ATCC 6538
Immersion extraction	6.3 ± 0.2	6.0 ± 0.0
Reflux extraction	6.5 ± 0.3	6.0 ± 0.0
Ultrasonic extraction	6.4 ± 0.2	6.0 ± 0.0
Chloramphenicol (20µg)	24.0 ± 0.2	24.5 ± 0.2

Table 6. Minimum inhibitory concentration (MIC) of bacterial strains by three *C. asiatica* extracts.

Samples	MIC (mg/ml)	
	Escherichia coli ATCC 8739	*Staphylococcus aureus* ATCC 6538
Immersion extraction	1.750	2.188
Reflux extraction	1.313	1.313
Ultrasonic extraction	1.313	1.313
Chloramphenicol (ppm)	0.2	0.2

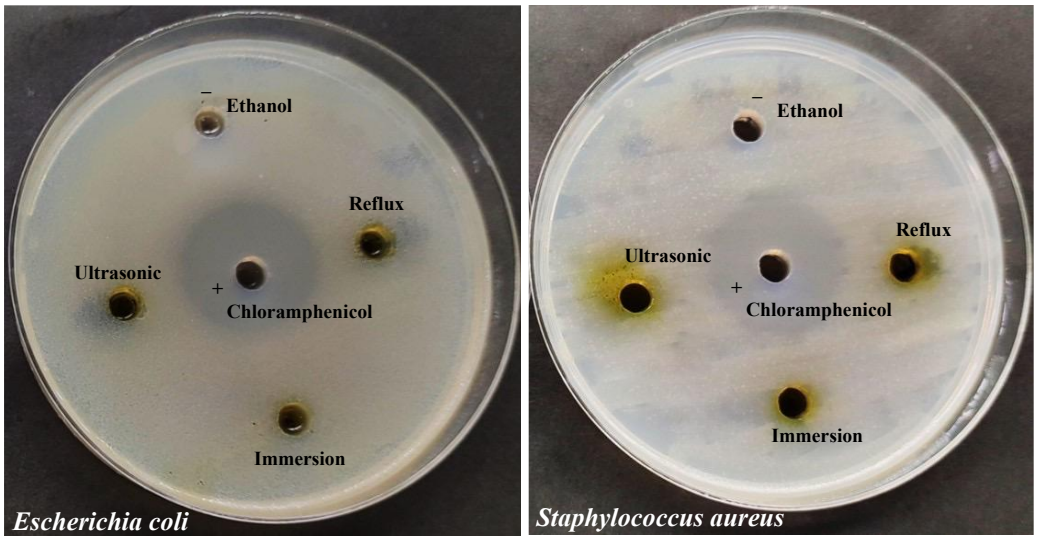

Fig. 3. Circles of inhibition of bacterial strains of three different methods: a) *E. coli* ATCC 8739 and b) *S. aureus* ATCC 6538

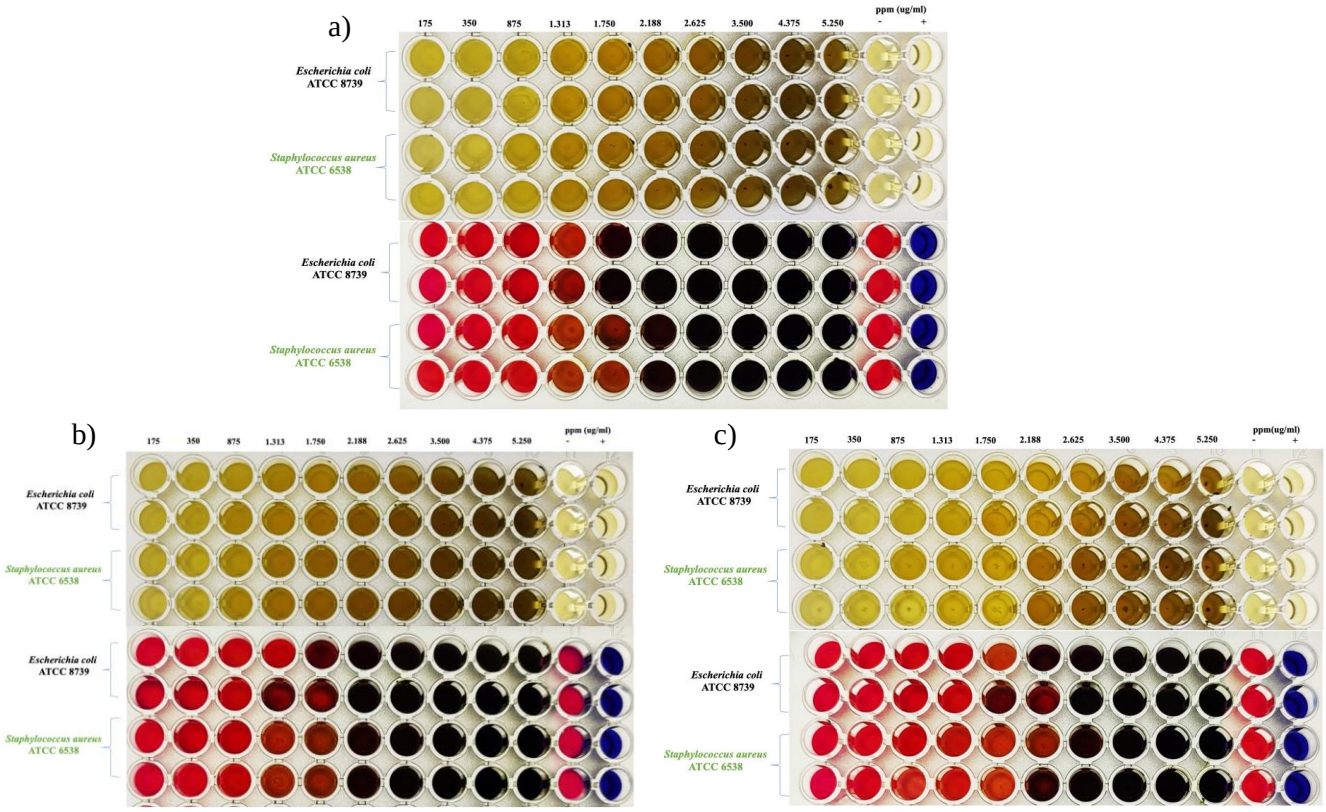

Fig. 4. Minimum inhibitory concentrations of three types of *C. asiatica* extracts on 96-well plates: a) Immersion, b) Ultrasonication, c) Reflux.

DISCUSSION

C. asiatica raw materials were evaluated for physicochemical parameters such as moisture, total ash, and ash insoluble in acid. The total ash content can reflect the purity level of the raw material. Raw materials with high ash content may contain many unwanted impurities or minerals. The determination of insoluble ash in HCl acid helps evaluate the content of insoluble minerals such as silica and other oxides, which are often related to the quality and purity of the raw material.

Each extraction method has its own advantages and disadvantages. The ultrasound method facilitates the separation of heat-sensitive compounds, reduces extraction time, and utilizes ultrasound waves to break down cell walls for quick release of compounds in the solvent. Reflux extraction is effective in recovering active ingredients and is highly efficient, but it requires careful attention to thermal degradation. Thermal degradation is minimized when using the soaking method, but this method is time-consuming and inefficient as solvent diffusion into the plant is static and slower compared to the other two methods.

Measuring polyphenol and flavonoid content is important to predict the antioxidant capacity of each extract. Similarly, the total phenolic content of *C. asiatica* in Thailand sample ranges from 98.5 mg GAE/g to 183.24 mg GAE/g (Chanwitheesuk et al., 2005). Polyphenol and flavonoid compounds are found in all *C. asiatica* extracts. Polyphenols and flavonoids, a diverse group of secondary metabolites known for their potent antioxidant properties. These compounds can neutralize free radicals, preventing oxidative stress and lipid peroxidation. Measuring polyphenol and flavonoid content is key to assessing the antioxidant potential of plant extracts, as higher levels generally correlate with better free radical scavenging ability. Sellathoroe et al. (2019) conducted an evaluation and comparison of biologically active ingredients in *C. asiatica* between different extraction methods, in which ultrasound was more effective than both immersion and soxhlet extraction with 0.47% saponin, 0.34% terpenoids, 0.11% flavonoids, and 0.03% alkaloids (Sellathoroe et al., 2019).

Table 7. Biological activities of *C. asiatica* analyzed by extraction methods.

Method	Conditions	Solvent	Compounds	References
Immersion	Dried plants, extraction time 110.5 min, and extraction temperature 70.20°C	Ethanol (37%)	Polyphenols 4.71 mg GAE/g DW	(Gunathilake et al., 2019)
Soxhlet	Dried plants, raw materil-solvent ratio 1:10 and extraction temperature 45°C.	Ethanol (50%)	β-carotene, Viatmin C, flavonoid và polyphenol total	(Rahman et al., 2013)
Microwave-based extraction	Dried plants, 40% microwave power, extraction time 6 min	Ethanol	Total phenolics and flavonoid, and total triterpenoids	(Sen et al., 2019)
Ultrasound-assisted extraction (UAE)	Dried plants, 1:10 raw materil-solvent ratio, 30 min	Water	Total phenolics 8.32 ± 0.105 mg GAE/g DW, DPPH antioxidant activity 86%	(Wan Zainal et al., 2019)
Solvent-Free Microwave-Assisted Extraction (SFME)	Dried plants, microwave power 450 W, extraction time 43.33 min		Total phenolics 2.39869 mg GAE/g	(Rahmawati et al., 2021)

The triterpenoid saponin content extracted from *C. asiatica* may vary depending on the solvent used due to the nature of triterpenoid saponins as amphipathic molecules. Some previous studies have shown that water is not effective as a solvent for extracting triterpenoid saponins from plants because water has poor solubility for many organic compounds, requiring longer time or higher temperature conditions to be effective (Zhao et al., 2010). Ethanol is generally considered a more effective solvent for triterpenoid saponin extraction due to its ability to solubilize a wide range of compounds, including nonpolar and semipolar components (Kim et al., 2009). The recovery efficiency of triterpenoid saponins from *C. asiatica* extract in Nakhon Pathom Province, Thailand reached 4.8% (dimethyl ether), 9.3% (ethanol), and 18.8% (dimethyl ether and ethanol mixture) (Pingyod et al., 2021).

Studies have demonstrated that *C. asiatica* extract exhibits significant DPPH radical scavenging activity, which in different ecotypes also has different IC_{50} values. This activity is due to the presence of various bioactive compounds, including triterpenoids (asiaticoside, madecassoside), flavonoids, and phenolic acids. The antioxidant activity of *C. asiatica* was determined to be higher when extracted with ethanol solvent than with water extraction, likely indicating high free radical scavenging activity due to efficient extraction of phenolics and flavonoids (Hamid et al., 2002).

Previously published studies showed the inhibitory effect on *S. aureus* strains with MIC values of 32 – 256 mg/mL by water extract and MIC values of 8 mg/mL by ethanol extract (Taemchuay et al., 2009). On the other hand, the water extract has the ability to inhibit *Helicobacter pylori* using the agar disk diffusion method, and the MIC value is 0.125 to 8 mg/mL (Zheng et al., 2016). In the study by Ferdous et al. (2017), positive effects were shown using ethanol as the extraction solvent. On the other hand, the extraction method soaked in *C. asiatica* with a methanol solvent could not inhibit *E. coli* strains (Gautam et al., 2007). These extracts can be prepared using different solvents, each of which affects the composition of the extract and the antibacterial efficacy. Ethanol is a solvent that has shown potential in the extraction of bioactive compounds from *C. asiatica* due to its effectiveness in solubilizing a variety of phytochemicals. In the present study, the antibacterial activity of *C. asiatica* may also be due to the similar effect of triterpenes, which are the active ingredients of *C. asiatica*. One of the important groups of compounds found in *C. asiatica* is flavonoids, which contribute to its wide range of biological activities, including antibacterial properties. Flavonoids can disrupt bacterial cell walls and membranes, leading to increased permeability and ultimately cell death.

CONCLUSION

In this study, three extraction methods: immersion, reflux, and ultrasound were used to recover extracts from *C. asiatica*. Identified natural compounds such as alkaloids, saponins, flavonoids, and terpenoids exist in *C. asiatica* extract. Ethanol extract from *C. asiatica* has been researched and proven to be effective in antioxidant and antibacterial properties. Ethanol extraction from *C. asiatica* by reflux method contains higher levels of polyphenols and flavonoids than other methods, which are known for their powerful antioxidant properties. These compounds have the ability to scavenge free radicals, help prevent cell damage due to oxidation, and have the best ability to inhibit the activity of free radicals with an IC_{50} value of 239.75 µg/mL (DPPH) and 199.75 µg/mL (ABTS) using reflux extraction. Triterpenoid saponins from *C. asiatica* also have antibacterial properties and can inhibit Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria such as *E. coli*.

Conflict of interest

The Authors declare that there is no conflict of interest.

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