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Update Review: Extraction, Purification, and Pharmacological Activities of Gotu Kola Terpenoids

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Abstract: *Centella asiatica*, which belongs to the family Apiceae, is widely used in cosmetics manufacturing and the drug industry. *Centella asiatica* contains many chemical compounds, and terpenes compounds such as asiaticoside, madecassoside, asiatic acid, and madecassic acid are a biomarker of the Gotu Kola plant. This review aims to compare the extraction method, purification, and pharmacological activities of gotu kola terpenoids from articles. The extraction methods consist of conventional (Maceration, Reflux, and Soxhlet) and non-conventional extraction (Microwave Assisted Extraction, Ultrasonic Extraction, and Subcritical Water Extraction). At the same time, the purification process uses HPLC, HPLC-UV, and RP-HPLC. In addition, pharmacological studies of *Centella asiatica* terpenoids have been confirmed as antimicrobial, wound healing, antioxidant, neuroprotective, anti-inflammatory, hepatoprotective, anticancer, antidiabetic, and hyperglycemic.

Keywords: extraction methods; purification; pharmacological activities; terpenoids; gotu kola (*Centella asiatica*).

1. Introduction

Centella asiatica (L.) Urb is a plant belonging to the Apiaceae family [1]. Gotu kola can be cultivated or can grow wild. Gotu kola grows in various tropical and subtropical areas, such as in Asian countries, India, Malaysia, China, Sri Lanka, North Australia, eastern Australia, Bangladesh, Iran, Indonesia, South America, Madagascar, and South and Central Africa. Based on its geographical origin, the gotu kola plant has common names in other languages, such as pennywort (English), gotu kola (America), byeong pul (Korea), asiatisches Wassernabelkraut (German), Trachiek kramh (Khmer), silabola (Malagasy), tabao en Amhara (Africa), mandookaparni (India), violette marron (French), ji xue cao (Chinese), ohtosammakonputki (Finland), ab-boshghabi (Iran), gotu kola (Sri Lanka), Pegaga (Malaysia), and pegagan (Indonesia) [2-6]. Morphologically, gotu kola is a creeping herb with a length of 15 cm that can grow in shady, humid, and swampy places, has five petioles, each petiole has a leaf, the leaves are shaped like a kidney, is green, and have hairs on the leaves, and have stalks long leaves. The flowers are white to purple, and the fruit is flat with single-seeded peppers. The geographical differences where the gotu kola plant grows cause gotu kola to have a variety

of morphology and leaf size, where the morphological differences are influenced by several factors such as environmental factors, genetic factors, and the interaction of the two [3-5]. Traditionally the gotu kola plant is used in Ayurvedic and Chinese medicine, namely as brain food to improve memory, improve brain function, and prevent cognitive deficits, while in Indonesia, the gotu kola plant can be used as a vegetable, supplement, and herbal medicine [79]. The gotu kola plant contains terpenoid compounds that can be used to make cosmetics and medicines. These terpenes consist of asiaticoside and madecassoside as triterpene saponins, asiatic acid and madecassic acid as sapogenins [5]. Our review aims to study the updated review of extraction methods, purification, and pharmacological activities of gotu kola terpenoids.

2. Materials and Methods

This article was written by collecting and reviewing scientific articles that compare conventional and non-conventional extraction methods, purification, and pharmacological activity of terpenoids from *Centella asiatica*. The article has been published in the last 15 years, including at least 20 articles in the last 2 years. Articles presented in Science Direct, Elsevier, Springer, PubMed, PubChem, and Google Scholar also have a DOI.

3. Results and Discussion

3.1. Comparison of Extraction Methods

There are many extraction methods used to extract chemical compounds present in plants. The extraction methods include conventional methods; several new methods are still being developed, and the existing methods have been modified. The selection of this extraction method is very important for extracting chemical compounds in plants [10]. Extraction is the process of separating the desired active compounds from raw materials or plants using suitable solvents. The efficiency of the extraction process is influenced by several factors, so these factors must be considered during the extraction process. The extraction factors are the nature of the extraction solvent, the extraction temperature, the extraction time, the ratio of the solvent to solids, and the particle size of the raw material [11].

Previous research revealed a comparison of conventional and non-conventional extraction methods for terpenoid compounds, including asiaticoside, madecassoside, asiatic acid, and madecassic acid, summarized in Table 1.

Table 1. Comparison of conventional and non-conventional extraction methods for terpenoid compounds.

Extraction Method	Solvent Type	Handling Technique	Yield of Chemical Compounds				References
			Asiaticoside	Madecassoside	Asiatic Acid	Madecassic Acid	
Maceration	Methanol	1 g of sample was put into a 100 ml Erlenmeyer and then dissolved in 40 ml of each organic solvent for 7 days at room temperature.	1.78 (%w/w)	0.54 (%w/w)	1.83 (%w/w)	0.30 (%w/w)	[5]
	50% Methanol		1.61 (%w/w)	0.50 (%w/w)	1.43 (%w/w)	0.15 (%w/w)	
	Ethanol		0.99 (%w/w)	0.32 (%w/w)	1.36 (%w/w)	0,10 (%w/w)	
	50% Ethanol		1.16 (%w/w)	0.40 (%w/w)	1.35 (%w/w)	0.08 (%w/w)	
	Water		0.71 (%w/w)	0.26 (%w/w)	1.26 (%w/w)	0.07 (%w/w)	
	Acetonitrile		0.42 (%w/w)	0.19 (%w/w)	1.12 (%w/w)	0.06 (%w/w)	

Extraction Method	Solvent Type	Handling Technique	Yield of Chemical Compounds				References
			Asiaticoside	Madecassoside	Asiatic Acid	Madecassic Acid	
Dynamic Maceration	95% Etanol	20 g was put into a 250 ml Erlenmeyer and then extracted with 100 ml of 95% ethanol using a water bath at 60°C for 120 minutes.	0.174 (%w/w)	0.855 (%w/w)	0.025 (%w/w)	0.053 (%w/w)	[12]
Heat Reflux	Methanol	1 g of sample was put into a 100 ml volumetric flask and dissolved in 40 ml of organic solvent, each using a controlled water bath at 60°C for 1 hour.	1.43 (%w/w)	0.54 (%w/w)	1.69 (%w/w)	0.21 (%w/w)	[5]
	50% Methanol		1.49 (%w/w)	0.41 (%w/w)	1.55 (%w/w)	0.19 (%w/w)	
	Ethanol		0.97 (%w/w)	0.42 (%w/w)	1.34 (%w/w)	0.16 (%w/w)	
	50% Ethanol		1.13 (%w/w)	0.45 (%w/w)	1.28 (%w/w)	0.15 (%w/w)	
	Water		1.24 (%w/w)	0.28 (%w/w)	1.19 (%w/w)	0.11 (%w/w)	
	Acetonitrile		1.02 (%w/w)	0.26 (%w/w)	1.02 (%w/w)	0.09 (%w/w)	
Soxhlet	Methanol	For 8 hours, with 100 ml of methanol.	1.75	1.64	0.72	0.72	[13]
Microwave-Assisted Extraction	Methanol	1 g of the sample was dissolved in 40 ml of each organic solvent using a microwave at 800 W.	2.66 (%w/w)	0.76 (%w/w)	1.98 (%w/w)	0.32 (%w/w)	[5]
	50% Methanol		1.90 (%w/w)	0.64 (%w/w)	1.59 (%w/w)	0.25 (%w/w)	
	Ethanol		1.27 (%w/w)	0.30 (%w/w)	1.41 (%w/w)	0.17 (%w/w)	
	50% Ethanol		1.19 (%w/w)	0.52 (%w/w)	1.29 (%w/w)	0.21 (%w/w)	
	Water		0.90 (%w/w)	0.44 (%w/w)	1.02 (%w/w)	0.18 (%w/w)	
	Acetonitrile		0.75 (%w/w)	0.34 (%w/w)	0.44 (%w/w)	0.05 (%w/w)	
	Absolute Ethanol	Using a modified microwave oven with variable magnetron voltage input with microwave power ranging from 0-800 W at a frequency of 2.45 GHz	28.35 mg/g	102.78 mg/g	6.65 mg/g	5.70 mg/g	[14]
	80% Ethanol	Microwave with 100 W power and 80% ethanol solvent for 7.5 minutes.	4.56 (%w/w)	7..332 (%w/w)	0.209 (%w/w)	0.357 (%w/w)	[15]
Ultrasonic Extraction	Methanol	1 g of sample was dissolved in 40 ml of organic solvent, each using an ultrasonic bath at 60°C for 1 hour.	2.27 (%w/w)	0.59 (%w/w)	1.60 (%w/w)	0.19 (%w/w)	[5]
	50% Methanol		1.87 (%w/w)	0.53 (%w/w)	1.49 (%w/w)	0.11 (%w/w)	
	Ethanol		0.82 (%w/w)	0.41 (%w/w)	1.32 (%w/w)	0.16 (%w/w)	
	50% Ethanol		1.86 (%w/w)	0.32 (%w/w)	1.04 (%w/w)	0.20 (%w/w)	
	Water		0.54 (%w/w)	0.23 (%w/w)	0.78 (%w/w)	0.09 (%w/w)	

Extraction Method	Solvent Type	Handling Technique	Yield of Chemical Compounds				References
			Asiaticoside	Madecassoside	Asiatic Acid	Madecassic Acid	
	Acetonitrile		0.30 (%w/w)	0.20 (%w/w)	0.57 (%w/w)	0.06 (%w/w)	
	80% ethanol	Using Ultrasonic at 48°C for 50 minutes and 80% ethanol solvent.	1.325 (%w/w)	2.262 (%w/w)	0.052 (%w/w)	0.082 (%w/w)	[15]
Subcritical Water Extraction	Deionized water	Extracted under optimum conditions at 250 C and 40 MPa.	10.0 mg/g	-	7.8 mg/g	-	[16]

The choice of solvent to be used is the main thing in the extraction method, especially the conventional extraction method because the solvent will determine the nature of the medium in which the solvent will interact with the processed material and the extracted compounds so that the properties of the solvent to be used must be considered and observed. Where the properties of the solvent, such as boiling point, melting point, polarity, specific gravity, viscosity, pH, and their effect on the active compound extracted and the level of purity [17]. In addition, the choice of solvent also depends on the type of plant used, the part of the plant to be extracted, and the nature of the active compound. Organic solvents generally consist of polar organic solvents and non-polar organic solvents. Polar organic solvents such as water, methanol, and ethanol were used to extract polar compounds, while non-polar organic solvents such as n-hexane and dichloromethane were used to extract non-polar compounds [10]. Conventional extraction methods use organic solvents such as ethanol (EtOH), methanol (MeOH), hexane, acetone, dichloromethane, ethyl acetate, acetonitrile, petroleum ether, and water. Several conventional extraction methods are often used to extract compounds from plants, namely maceration, percolation, reflux, and soxhlet extraction [17].

This conventional extraction method has advantages and disadvantages. The advantages are low cost and ease of operation, while the disadvantages of conventional methods are that it takes a long time to process and uses large amounts of organic solvents that can be harmful to health and the environment because the organic solvents used are toxic. In addition, the extraction duration is long; it is difficult to completely remove the remaining solvent, and the possibility of compound degradation due to the use of high temperatures. Due to the shortcomings of conventional extraction methods, a renewable technology approach that is more efficient is needed in extracting active compounds in natural or plant materials, one of which is by using non-conventional extraction methods or renewable extraction such as supercritical fluid extraction, pressurized-liquid extraction, ultrasound-assisted extraction, solid-phase micro-extraction, microwave-assisted extraction, and solid-phase extraction [18].

Mohapatra *et al*. (2021) conducted research to obtain the recovery of bioactive compounds using various extraction methods and using various organic solvents. The extraction methods used are conventional methods, such as maceration and hot reflux, while the non-conventional extraction methods are Microwave-Assisted Extraction and Ultrasonic Extraction. The organic solvents used were methanol, 50% methanol, ethanol, 50% ethanol, water, and acetonitrile. The results showed that Microwave-Assisted Extraction using methanol was the most effective extraction method because it showed the highest recovery of asiaticoside

compounds (2.66% w/w), madecassoside (0.76% w/w), asiatic acid (1.98% w/w), and madecassic acid (0.32% w/w) [5].

Yingngam *et al*. (2020) research conducted modeling and optimization of the extraction technique conditions with microwave-assisted extraction (MAE) using the response surface methodology (RSM) to obtain maximum yields of pentacyclic triterpene compounds from *C. asiatica*. The results showed that the MAE technique could accelerate the mass transfer rate and can increase the recovery yield of pentacyclic triterpene compounds with the use of less solvent. The results of the recovery of pentacyclic triterpene compounds consisted of asiaticoside compounds (28.35 mg/g), madecassoside (102.78 mg/g), asiatic acid (6.65 mg/g), and madecassic acid (5.70 mg/g) [14].

Thong-on *et al*. (2021) conducted research and investigated the optimum conditions with non-conventional extraction methods such as ultrasound-assisted extraction and microwave-assisted extraction using the response surface (RSM) methodology for the recovery of triterpenoid glycosides. The results showed that using the MAE method recovered asiaticoside compounds (4.56% w/w), madecassoside (7.332% w/w), asiatic acid (0.209% w/w), and madecassic acid (0.357% w/w). At the same time, using the UAE method, recovery of asiaticoside compounds (1.325% w/w), madecassoside (2.262% w/w), asiatic acid (0.052% w/w), and madecassic acid (0.082% w/w) [15].

Kim *et al*. (2009) conducted a study using the Subcritical Water Extraction method to increase bioactive components such as asiaticoside and asiatic acid extracted from *C. asiatica* using subcritical water as the solvent. Kim reported that pressure or temperature could increase the bioactive components, from the results of the study showed that extraction using subcritical water at a temperature of 250^{o}C and a pressure of 40 MPa yields asiaticoside compounds of 10.0 mg/g and asiatic acid of 7.8 mg/g which higher than the conventional extraction method with methanol or ethanol solvent at room temperature [16].

Monton *et al*. (2019) researched by developing an extraction technique using dynamic maceration with a surface response methodology to increase the recovery of bioactive compounds, especially asiaticoside, madecassoside, asiatic acid, madecassic acid. The results showed that the content of the four compounds increased when extracted at 60^{o}C for 120 minutes, with the results being asiaticoside (0.174% w/w), madecassoside (0.855% w/w), asiatic acid (0.025% w/w), and madecassic acid (0.053% w/w) [12].

Rafamantanana *et al*. (2009) conducted a study using the HPLC-UV method and evaluated the efficient extraction to increase the content of asiaticoside, madecassoside, asiatic acid, and madecassic acid compounds from *C. asiatica*. The results showed that extraction using Soxhlet for 8 hours and the HPLC-UV method could increase the content of asiaticoside (1.75), madecassoside (1.64), asiatic acid (0.72), and madecassic acid (0.72) [13].

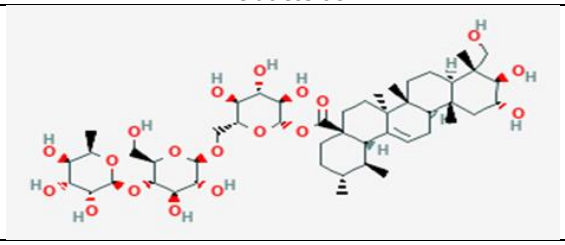
Several studies have been conducted to extract terpenoid compounds, including asiaticoside, madecassoside, asiatic acid, and madecassic acid, using conventional and non-conventional extraction methods. From the comparison of these extraction methods, non-conventional extraction methods yielded the higher recovery of asiaticoside, madecassoside, asiatic acid, and madecassic acid than conventional extraction methods. Thus, non-conventional extraction methods can be recommended to extract *C. asiatica* for higher production or recovery of compounds.

3.2. Terpenoid compounds from Gotu Kola (Centella asiatica).

Results Based on various studies of the gotu kola plant (*Centella asiatica*), the pegagang plant contains chemical compounds such as terpenoids, flavonoids, alkaloids, tannins, steroids, and phenolic compounds. Terpenoid compounds are the main components or biomarker compounds of the gotu kola plant. Terpenoid compounds mostly consist of asiaticoside, madecassoside, asiatic acid, and madecassic acid, which have pharmacological activity. These terpenoids act as anti-inflammatory, and antioxidant agents and increase the rate of skin cell migration. Several factors, such as location, place of growth, and environmental conditions of gotu kola plant, will affect the content of terpenoids in gotu kola plant. Gotu kola extract, which contains high terpenoids, is widely used in the manufacture of health products and supplements [19-21].

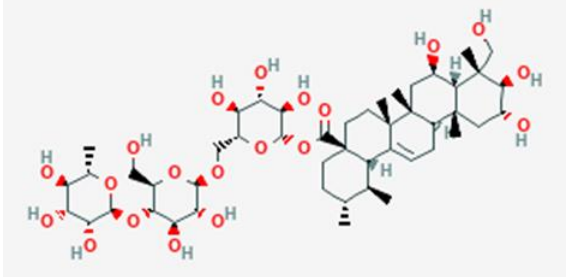
Asiaticoside (pubchem CID: 52912190) has the molecular formula $C_{48}H_{78}O_{19}$ and a molecular weight of 959.1. Asiaticoside is widely distributed in the brain, skin, and stomach after oral administration. Madecassoside (pubchem CID: 131801373) has the molecular formula $C_{48}H_{78}O_{20}$ and a molecular weight of 975.1 g/mol. Madecassoside can be widely distributed in the brain, liver, lung, kidney, heart, spleen, and stomach after oral administration. Asiatic acid (pubchem CID: 119034) has the molecular formula $C_{30}H_{48}O_5$ and a molecular weight of 488.7 g/mol. Asiatic acid is widely distributed in the brain, liver, heart, kidney, bladder, and large intestine and can be absorbed in the jejunum after oral administration. Madecassic acid (pubchem CID: 73412) has the molecular formula $C_{30}H_{48}O_6$ and a molecular weight of 504.7 g/mol. Madecassic acid is widely distributed in the brain, liver, kidney, heart, bladder, colon, and plasma after oral administration [20].

Table 2. Physicochemical properties of Asiaticoside [22].

Asiaticoside	
Synonyms	**Asiaticoside**
Structure	
Molecular Formula	C48H78O19
Molecular Weight	959.1 g/mol
PubChem CID	52912190

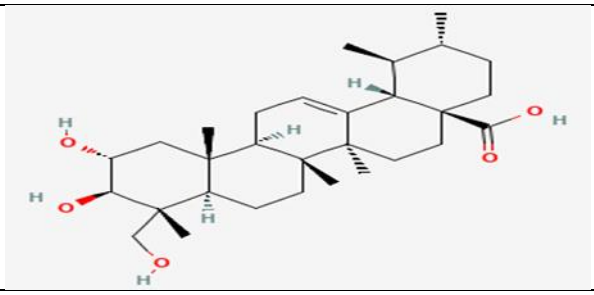
Source: National Center for Biotechnology Information

Table 3. Physicochemical properties of Madecassoside [22].

Madecassoside	
Synonyms	**Madecassoside**
Structure	
Molecular Formula	C48H78O20
Molecular Weight	975.1 g/mol
PubChem CID	131801373

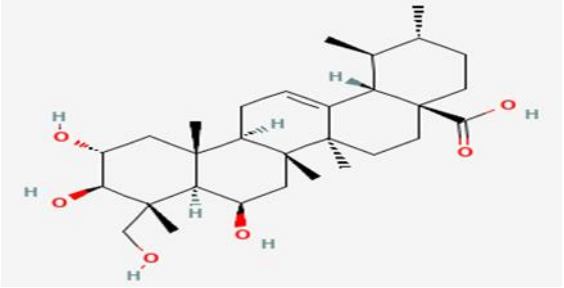
Source: National Center for Biotechnology Information

Table 4. Physicochemical properties of Asiatic acid [22].

Asiatic acid	
Synonyms	**Asiatic acid**
Structure	
Molecular Formula	C30H48O5
Molecular Weight	488.7 g/mol
PubChem CID	119034

Source: National Center for Biotechnology Information

Table 5. Physicochemical properties of Madecassic acid [22].

Madecassic acid	
Synonyms	**Madecassic acid, Brahmic acid, Brahmanic acid**
Structure	
Molecular Formula	C30H48O6
Molecular Weight	504.7 g/mol
PubChem CID	73412

Source: National Center for Biotechnology Information

3.3. Purification of Gotu Kola (Centella asiatica).

In plant extracts, there are various kinds of bioactive compounds with different polarities of compounds, so further separation of these bioactive compounds must be carried out for the purification process of bioactive compounds. Before that, it is necessary to do the phytochemical characteristics first. Phytochemical characteristics are a qualitative first step carried out to determine the presence of secondary metabolites in plant extracts, which are then tested for pharmacological activity to identify and determine which bioactive compounds provide the desired pharmacological activity. Furthermore, bioactive compounds were isolated and purified using several separation techniques, such as HPLC, to obtain pure compounds. Likewise, terpenoid compounds such as asiaticoside, madecassoside, asiatic acid, and madecassic acid can be separated using HPLC [23,24].

Table 6. Purification of Gotu Kola (*Centella asiatica*) using HPLC.

Sample	**Method**	**Solvent System**	**Reference**
Centella asiatica	HPLC-UV	The mobile phase was 30% acetonitrile and deionized water, using a gradient elution technique.	[25]
	HPLC	The mobile phase was 0.3% phosphoric acid and acetonitrile, using a gradient elution technique.	[26]
	HPLC	The mobile phase was 0.2% phosphoric acid and acetonitrile, using the isocratic elution technique.	[27]
	HPLC	The mobile phase (A) acetonitrile, mobile phase (B) 1% formic acid in the water, by gradient elution technique.	[28]
	HPLC	The mobile phase (A) acetonitrile, mobile phase (B) 0.01% phosphoric acid aqueous solution, using gradient elution technique.	[12]

RP-HPLC	The mobile phase was 0.1% orthophosphoric acid and acetonitrile, using the isocratic elution technique.	[29]
HPLC	The mobile phase was acetonitrile in water, using a gradient elution technique.	[9]
HPLC-UV	The mobile phase was 0.2% phosphoric acid and acetonitrile, using a gradient elution technique.	[30]
HPLC	The mobile phase (A) 0.2% phosphoric acid in water, mobile phase (B) 0.2% phosphoric acid in acetonitrile, by gradient elution technique.	[31]
HPLC	The mobile phase was acetonitrile and phosphate buffer (50 : 50%), using the isocratic elution technique.	[32]
HPLC	The mobile phase is acetonitrile and water (70 : 30%), using the isocratic elution technique.	[33]
HPLC	The mobile phase (A) 3 ml of orthophosphoric acid in 1000 ml of water, and mobile phase (B) acetonitrile, using a gradient elution technique.	[34]
HPLC	The mobile phase was acetonitrile and water pH 3 (50 : 50%)	[35]
HPLC	The mobile phase was potassium dihydrogen phosphate and acetonitrile, using a gradient elution technique.	[36]
HPLC	The mobile phase (A) methanol and water (70:30), mobile phase (B) acetonitrile and water, using gradient elution technique	[37]

3.4. Pharmacology activities.

3.4.1. Antimicrobal activity.

Mudaliana, 2021 researched the antimicrobial activity of five pathogenic bacteria consisting of *E. coli, Staphylococcus aureus, Salmonella typhi, Bacillus subtilis,* and *Mycobacterium tuberculosis* from the ethanol extract of *Centella asiatica*. Testing for antimicrobial activity used the Kirby-Bauer test method, Mueller Hinton agar medium (MHA) and Lowenstein Jensen (LJ) medium. From the results of the research that has been done, it shows that the ethanol extract of *C. asiatica* can only inhibit the growth of the four pathogenic bacteria, namely *E. coli, Staphylococcus aureus, Salmonella typhi,* and *Mycobacterium tuberculosis,* but cannot inhibit the growth of *Bacillus subtilis* [38].

Wong and Ramli, 2021 researched the antimicrobial activity of four extracts (hexane, ethanol, methanol, and water) from *C. asiatica* against foodborne pathogens and food spoilage microorganisms. Antimicrobial test using bacteria and fungi. The bacteria used *E. coli, Bacillus cereus, S. aureus, S. Typhimurium*. Fungi using *Candida albicans* and *Aspergillus niger*. The test method uses agar disc diffusion, then the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicide concentration (MFC) are determined. The results of the research that has been done show that of the four *C. asiatica* extracts that give the highest yields, the ethanol extract of *C. asiatica* has the potential to be an alternative synthetic preservative against foodborne pathogens [39].

Owusu *et al*. (2021) researched the antimicrobial activity of three extracts (70% ethanol, absolute ethanol, and water) from *C. asiatica*, in testing the antimicrobial activity using pathogenic bacteria consisting of *Salmonella typhi, E. coli,* and *Staphylococcus aureus.* The test method uses the good diffusion method. Antimicrobial activity testing was carried out by determining the value of the minimum inhibitory concentration (MIC) of the three *C. asiatica* extracts. From the results of the research that has been done, it shows that of the three *C. asiatica* extracts that give the highest minimum inhibitory concentration (MIC) value is *C. asiatica* water extract with a yield of 6.25 mg/ml. In contrast, the lowest minimum inhibitory concentration (MIC) value is 70% ethanol extract with a yield of 1.56 mg/ml [40].

Sieberi *et al*., 2020 conducted research on the antimicrobial activity of the dichloromethane-methanol extract of *C. asiatica* against *E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella sonnei*, and *Bacillus subtilis* bacteria. In testing using the agar disk diffusion method, the microdilution method, and the time-kill kinetics method. The results of the research that has been done, shows that the antimicrobial activity of the dichloromethane-methanol extract of *C. asiatica* is dose-dependent. Doses with a concentration of 500 mg/ml showed higher inhibitory activity of the dichloromethane-methanol extract of *C. asiatica* against the five bacterial species tested compared to concentrations of 250 mg/ml and 125 mg/ml [41].

3.4.2. Wound healing activity.

Tanga *et al*. (2022) conducted research *in vivo* wound healing in a mouse model using methanol extract of *C. asiatica* in carboxymethyl cellulose. Observed wound healing, such as wound contraction, tissue proliferation, and cell deposition, as well as relative gene expression levels in the mouse model. The study used CMC as a control and *C. asiatica* methanol extract with concentrations of 0.25, 0.5, and 1%. From the results of the research that has been done, it shows that *C. asiatica* methanol extract concentration of 0.5% in CMC gives higher results compared to controls and other concentrations in healing wound contractions, relative levels of gene expression, and levels of tissue deposition and polymorphonuclear cell infiltration [42].

Damkerngsuntorn *et al*. (2020) researched the healing of acne scars on thirty people who underwent treatment with the Er:YAG 2940 nm laser. Then proceed with treatment using *C. asiatica* ECa 233 extract gel 0.05% w/w and placebo gel. *C. asiatica* extract gel ECa 233 and placebo gel were used four times a day for 7 days, then used twice a day for 3 months, then acne scar healing was observed for erythema, melanin, and texture index by assessing photos and giving a rating scale on appearance acne scars. From the results of the research that has been done, it shows that the use of *C. asiatica* ECa 233 0.05% w/w extract gel can improve skin erythema and the appearance of scars after the Er:YAG 2940 nm laser resurfacing for acne scars [43].

Ahmed *et al*. (2019) conduct research on wound healing in rabbits in vivo using a formulation from the *C. asiatica* fraction with Polyvinyl alcohol or Polyethylene Glycol (PVA/PEG) hydrogels using the freeze-thaw method. The results of research that has been done show that the hydrogel formulation can accelerate wound healing 15% faster than creams sold in the market and does not cause signs of irritation to the skin of rabbits [44].

3.4.3. Antioxidant activity.

Buranasudja *et al*. (2021) conduct research on the antioxidant activity of the 50% ethanol extract of the *Centella asiatica* (L.) callus culture. The application of *C. asiatica* callus culture can produce more promising bioactive compounds compared to isolating bioactive compounds from *C. asiatica* plants. The RT qPCR results showed that *C. asiatica* callus extract could increase the regulation of antioxidant enzymes against oxidative stress [45].

Junsi and Siripongvutikorn (2022) conducted research on the antioxidant activity of *C. asiatica* juice on the effect of blanching time. *C. asiatica* juice is made by boiling fresh *C. asiatica* leaves in hot water for 0, 1, and 2 minutes. Then determine the total flavonoid content, and total phenolic content, and test the antioxidant activity of the juice filtrate. The research results show that *C. asiatica* juice obtained from blanching for 2 minutes gives the highest values of

total phenolic, total flavonoids, and antioxidant activity compared to 0 and 1-minute blanching [46].

Jatayu *et al*. (2018) conducted research on the antioxidant activity of the ethanol extract of *C. asiatica* on Superoxide Dismutase (SOD) levels in the liver of Cyprinus carpio. Cyprinus carpio was given *C. asiatica* ethanol extract at doses of 50, 100, 150, and 300 mg kg-1 every 3 days for 2 weeks. The research results show that the ethanol extract of *C. asiatica* can increase SOD levels in the liver of *Cyprinus carpio* fish so that it can become an antioxidant defense in the body [47].

Kesornbuakao *et al*. (2020) conducted research on the antioxidant activity of *C. asiatica* crude extract mixed with Bovine Serum Albumin. The mixing ratio between extract and Bovine Serum Albumin is 1:2, 1:3, and 1:4. Antioxidant activity testing was carried out using the DPPH method on the gastrointestinal system. The research results show that the ratio of crude extract and Bovine Serum Albumin 1:2 and pH 2.0 give the highest antioxidant activity results because a low pH can cause inhibition of active compounds in certain areas of the gastrointestinal system [48].

Thien *et al*. (2021) research the antioxidant activity of *C. asiatica* extract on chlorophyll and polyphenol content at 30-120 days of growth. Antioxidant activity testing was carried out by determining the total antioxidant value, reducing power activity, and DPPH free radical scavenging activity. The results of the research that has been done shows that the highest chlorophyll content of 5.104 ± 0.041 mg is found on the 45th growth day, the polyphenol content and the highest reducing power activity are respectively 14.156 ± 0.026 mg and 22.891 ± 0.040 mg which are on the 75th growth day, while the highest total antioxidant activity and DPPH free radical scavenging activity were respectively 6.059 ± 0.022 mg on the 60th growth day [49].

3.4.4. Neuroprotective activity.

Kumar and Kavita (2020) researched the neuroprotective and antidepressant activities of terpenoid compounds and asiaticoside isolated from *C. asiatica*. In this study, *C. asiatica* was extracted using the Soxhlet method using n-hexane and ethyl acetate, followed by phytochemical screening with GC-MS, then tested for its neuroprotective and neuroprotective and antidepressant activities. The results of the research that has been done show that the LOD of triterpenes in the extract was found to be 0.007 µg mL-1 with significant antidepressant properties so that it can be used to prevent depression, while asiatic acid can be used as an antidepressant by lowering corticosterone levels and increasing levels of the monoamine neurotransmitter corticosterone [50].

Yadav *et al*. (2019) conduct research on the neuroprotective activity of increasing the labyrinth, inflammation, acetylcholine esterase (AChE), *in vivo* antioxidant activity testing, and finally, histopathological analysis of the organs of the brain, heart, liver, and kidneys in Swiss albino rats. Testing the neuroprotective activity using ethanol extracts of *C. asiatica* and Evolvulus alsinoides with different doses. The research results show that neuroprotective and nootropic activity can reduce AChE levels in the brain homogenate of Swiss albino rats [51].

Doulah *et al*. (2020) conduct research on the effect of pre-treatment on long-term potentiation in Alzheimer's disease mice using *Centella asiatica* extract given for 6 weeks. Alzheimer's disease is a neurodegenerative disorder characterized by loss of cognitive function, impaired memory and language, and behavioral disturbances. From the results of the research that has been done, it shows that administration of *C. asiatica* extract to Alzheimer's

disease rats given for 6 weeks before the induction of Meynert's basalis nucleus lesions and in Alzheimer's can reduce memory, so it can be said that *C. asiatica* extract plays a protective role for neurons mice with Meynert nucleus basalis lesions [52].

3.4.5. Anti-inflammatory activity.

Baby *et al*. (2020) conduct research on the anti-inflammatory activity of the stability of human red blood cells in vitro by determining hypotonicity-induced inhibition of red blood cell membrane lysis. The results showed that *C. asiatica* extract could provide anti-inflammatory activity, with the value of the proportion of hemolysis reduced from 47.18% to 1.24% and the proportion of stabilization increased from 52.81% to 98.76% [53].

Hafiz *et al*. (2020) researched the anti-inflammatory activity of the ethanol extract of *C. asiatica* on acetylcholinesterase (AChE) activity, inflammation, and oxidative stress, which were carried out in vivo and in vitro. The results showed that the ethanol extract of *C. asiatica* could significantly suppress pro-inflammatory mediators and oxidative stress by reducing concentration-dependent acetylcholinesterase (AChE) activity [54].

Giribabu *et al*. (2020) research on the of *C. asiatica* extract on inflammation, oxidative stress, and apoptosis in the cerebrum in diabetic rats. The study was conducted by giving diabetic rats *C. asiatica* extract at different doses, namely 50, 100, and 200 mg/kg/b.w. for 28 days. The results of the study showed that the administration of *C. asiatica* extract to diabetic rats reduced insulin and increased FBG levels in diabetic rats. The ethanol extract of *C. asiatica* can also reduce apoptosis which is characterized by a decrease in inflammation and a decrease in pro-apoptosis [55].

Hernayanti *et al*. (2021) research the anti-inflammatory activity of *C. asiatica* extract on cadmium-induced rats. Cadmium is a toxic heavy metal that can cause liver inflammation. This research was carried out by giving *C. asiatica* extract with different doses, namely 100, 200, 300, and 400 mg/kgbb in rats induced by cadmium for 14 days, then determining the levels of blood Cd, GST, GSH, TNF-α, and COX- 2. The results showed that *C. asiatica* extracts at a dose of 200 mg/kgbb was the most effective dose for reducing Cd, TNF-α, and COX-2 levels in cadmium-induced rats [56].

3.4.6. Hepatoprotective activity.

Sivakumar *et al*. (2018) conduct research on the hepatoprotective activity of the ethanol extract of *C. asiatica* against paracetamol-induced hepatotoxicity in rats. Mice were given *C. asiatica* ethanol extract at a dose of 100 and 200 mg/kg for 14 days. The results showed that the ethanol extract of *C. asiatica* given to rats at a dose of 200 mg was better at reducing lipid peroxidation in liver tissue and could restore the activity of the enzymes SOD, CAT, and GSH to normal [57].

Akrom *et al*. (2021) conducted research on the hepatoprotective activity of chewable tablets containing *C. asiatica* extract in rats induced by a high-fat diet. Rats were given chewable tablets containing *C. asiatica* extract with doses of 100, 200, and 300 mg/kg BW. For 5 weeks, the rats were induced on a high-fat diet, and in the last week, they were given chewable tablets containing *C. asiatica* extract, then blood triglyceride, SGPT, and SGOT levels were observed. The results showed that chewable tablets containing *C. asiatica* extract could reduce triglyceride and SGPT levels in rats induced by a high-fat diet [58].

Hong *et al*., (2021) conducted research on the hepatoprotective activity of 50% ethanol extract of *C. asiatica* in rats with acute liver failure induced by lipopolysaccharide or galactosamine, then compared it with prophylactic drugs such as dimethyl diphenyl bicarboxylate, silymarin, ursodeoxycholic acid, and Lentinus edodes extract. mycelia. The results showed that 50% ethanol extract of *C. asiatica* given to rats with acute liver failure could improve liver tissue damage and liver function [59].

Park *et al*. (2021) conduct research on the hepatoprotective activity of 50% ethanol extract of *C. asiatica* in acetaminophen-induced acute liver injury mice. The results showed that 50% ethanol extract of *C. asiatica* could inhibit liver necrosis and pro-inflammatory cytokines and help prevent acetaminophen-induced liver tissue injury in mice. Still, the results of its containment depended on the dose of 50% ethanol extract of *C. asiatica* given [60].

3.4.7. Anticancer activity.

Aizad *et al*. (2020) conducted research on the 3-D composite structure study enhanced with polyhydroxybutyrate-co-hydroxyvalerate and carboxymethylcellulose (PHBV-co-CMC) on lung cancer cells to determine the anticancer effect of *C. asiatica* extract. The 3-D scaffolds were infused with CMC gel; the observations were using fluorescent microscopy and scanning electron microscopy. The results showed that *C. asiatica* extract could cure 70% higher with the 3-D PHBV scaffold co-CMC composite structure than with the 2-D model [61].

Huang *et al*. (2022) conducted research on asiatic acid compounds from *C. asiatica* to test their anti-invasive ability in human kidney cancer cells using ERK/p38MAPK-mediated modulation of MMP15 expression. This study detected cell proliferation and cell cycle distribution using the PI staining method with flow cytometry, colony formation test, and MTT. The results showed that the asiatic acid compound from *C. asiatica* could inhibit the metastatic properties of RCC cells by inhibiting p-ERK/p-p38MAPK and could downregulate MMP-15 [62].

Zhu *et al*., (2021) research asiatic acid compounds from *C. asiatica* to test anticancer effects on doxorubicin MCF-7 resistant breast cancer cells. In this study, cells were incubated with asiatic acid from *C. asiatica* for 2-24 hours, then cell viability and cytotoxicity were evaluated. This anticancer study used the (MTT) and lactate dehydrogenase (LDH) methods, Confocal Microscopy, Flow Cytometry, and the Caspase-Glo Assay System. This research shows that the asiatic acid compound from *C. asiatica* can induce cell death, soit has an anticancer activity to reverse multidrug resistance [63].

3.4.8. Antidiabetic and hyperglycemic activity.

Macalalad and Gonzales (2022) research the silicon screening study to identify the potential for inhibition of sodium-glucose co-transporter 2 from *Centella asiatica* for treating diabetes. The results of the study showed that there were five *C. asiatica* phytochemical compounds that had strong activity against sodium-glucose co-transporter 2 inhibition, but only three compounds had better oral bioavailability for diabetes therapy [64].

Yadav and Upasani (2022) research three combinations of extracts of *C. asiatica*, *Asparagus racemosus*, and *Plumeria rubra* in glibenclamide-induced diabetic nephropathy rats. The study results showed that the three combinations of extracts can repair the kidney, and improve kidney morphology abnormalities [65].

Liu *et al*. (2022) researched the combination of *C. asiatica* with polymer hydroxyethyl cellulose as a matrix for healing diabetic skin ulcers. The research results show that this combination can accelerate the healing of ulcers, relieve inflammatory reactions, and inhibit the growth of bacteria on the wound surface [66].

Setyaningsih *et al*. (2021) conduct research on the ethanol extract of *C. asiatica* on glomerular inhibition and vascular remodeling in diabetic rats. The results showed that the ethanol extract of *C. asiatica* given to diabetic rats could improve glomerulosclerosis and vascular injury [67].

Hayati *et al*. (2021) research to test the antihyperglycemic effectiveness of the Self Nano-Emulsifying Drug *C. asiatica* ethanol extract in zebrafish. The study results showed that Self Nano-Emulsifying Drug 100 mg/2 L could reduce fasting blood glucose levels by 69.90% and Self Nano-Emulsifying Drug 200 mg/2 L by 72.20% [68].

Putri *et al*. (2022) conducted a study using a combination of *C. asiatica* extract and Sappan wood extract on malondialdehyde levels and insulin resistance in rats induced with Streptozotocin and Nicotinamide. The study results show that the combination of the two extracts can reduce insulin resistance so that it can be used as a health drink for diabetics [69].

4. Conclusions

Centella asiatica contains chemical compounds such as terpenoids, flavonoids, alkaloids, tannins, steroids, and phenolic compounds. Terpenoid compounds are the main components of gotu kola, including asiaticoside, madecassoside, asiatic acid, and madecassic acid. Several extraction methods have been reviewed by comparing conventional and non-conventional extraction methods to increase the recovery of the bioactive compounds asiaticoside, asiatic acid, madecassoside and madecassic acid. From the comparison of the two methods, non-conventional extraction methods can be recommended to extract *C. asiatica* for higher compound recovery. Although currently, there is a trend of non-conventional extraction methods, generally, the extraction of bioactive compounds from *C. asiatica* is still carried out by conventional methods. The non-conventional extraction method approach can be used to develop extraction methods and can produce higher compound recoveries. Then the compound was purified using HPLC, HPLC-UV, and RP-HPLC. Terpenoids from *Centella asiatica* have pharmacological activity as antimicrobial, wound healing, antioxidant, neuroprotective, anti-inflammatory, hepatoprotective, anticancer, antidiabetic, and hyperglycemic.

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Conflicts of Interest

The authors declare no conflict of interest.

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