INVESTIGATION OF SOME BIOACTIVITIES OF *CURCUMA PETIOLATA* ROXB. (MALAR) RHIZOME

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Abstract

Curcuma petiolata, also known as the petiole turmeric and as referred to as Malar in Myanmar is primarily used in traditional medicine for its anti-inflammatory and antioxidant properties. Additionally, its rhizome is utilised as a culinary spice in various Southeast Asian dishes, adding flavour and colour. This study aims to explore the preliminary phytochemical tests, soluble matter content, antimicrobial, antioxidant, cytotoxic, and anti-arthritic properties of the rhizome of *C. petiolata*. In this study, the preliminary phytochemical investigation, it was found that alkaloids, flavonoids, glycosides, saponins, tannins, α-amino acids, carbohydrates, phenolic compounds, reducing sugars, organic acids, steroids, and terpenoids were present in the rhizome of *C. petiolata*. The extractable matter contents of the sample were determined by the WHO method with different solvents such as water, ethanol, methanol, chloroform, ethyl acetate, and petroleum ether. The resultant data indicated that the selected sample is more soluble in water. Additionally, the antimicrobial activity of crude extracts such as petroleum ether, ethyl acetate, ethanol, and watery extracts of *C. petiolata* was tested on eight microorganisms by agar well diffusion method. The antioxidant activity of ethanol and watery extracts of *C. petiolata* rhizome was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. A brine shrimp lethality bioassay investigated the cytotoxicity of the watery and ethanol extracts. The egg albumin denaturation method performed the anti-arthritic activity of watery and ethanol extracts. As a result, *C. petiolata* contains biologically active compounds and exhibits medicinal properties.

Keywords: *Curcuma petiolata*, antimicrobial activity, antioxidant activity, cytotoxicity, anti-arthritic activity

Introduction

Medicinal plants have been utilised for thousands of years in various cultures worldwide for their therapeutic properties. These plants contain bioactive compounds that can promote health and treat diseases, making them a cornerstone of traditional medicine (Wagner and Ulrich-Merzenich, 2009). Recent studies have highlighted the importance of these plants in modern pharmacology, with many contemporary drugs derived from their natural compounds (Newman and Cragg, 2016). Today, there is increasing scientific interest in exploring these plants for new medicines and treatments. Understanding their potential benefits helps researchers develop effective healthcare solutions. *Curcuma petiolata*, commonly known as a species of turmeric and referred to as Malar in Myanmar, is a flowering plant belonging to the ginger family, Zingiberaceae. This plant is native to the tropical regions of Southeast Asia, particularly found in countries like Thailand and Malaysia. It is recognised for its distinctive foliage and vibrant yellow flowers, contributing to its popularity in ornamental horticulture (Kress *et al*., 2002). *C. petiolata* rhizome is a botanical specimen renowned in traditional medicine for its diverse biological activities and phytochemical compositions. *C. petiolata* has been historically valued for its medicinal properties, including antioxidant (Jayaprakash *et al*., 2006), anti-inflammatory (Ammon *et al*., 1992), anticancer (Hatcher *et al*., 2008) and antimicrobial effects (Haddad *et al.*, 2011). This research aims to further explore its health benefits and potential applications in healthcare and drug development.

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Materials and Methods

Collection and Preparation of Plant Sample

The sample was collected from Inn Pyin Village, Kalaw Township, Southern Shan State in Myanmar. The selected sample was identified as *Curcuma petiolata* Roxb. (Malar) at the Botany Department of the University of Yangon. After collection, the rhizome was thoroughly washed with water to remove impurities. Then, it was sliced into smaller pieces and air-dried at room temperature. After drying, the sample was ground into a fine powder using a grinding machine, then sieved and stored in an airtight container for future use.

Preliminary Phytochemical Investigation of *C. petiolata* (Malar) Rhizome

A preliminary phytochemical analysis was carried out on a dried powdered sample of *C. petiolata* (Malar) rhizome using the appropriate reported methods. The aim was to determine the presence or absence of various phytochemicals such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, organic acids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, starch, and terpenoids.

Determination of the Soluble Matter Contents

The soluble matter content of the sample in various organic solvents, including petroleum ether, ethyl acetate, chloroform, methanol, ethanol, and water, was determined using the method outlined by the WHO.

Preparation of Crude Extracts for Biological Activities

Approximately 20 g of dried powder was subjected to successive extractions with 95 % ethanol for six hours, followed by filtration. The filtrate was then concentrated using a rotary evaporator to obtain the ethanol extract. For the watery extract, another 20 g of dried powder was boiled in 100 mL of distilled water and filtered. The resulting solution was concentrated by evaporating the water using a water bath to yield the watery extract. Both crude extracts were subsequently dried and stored in a refrigerator for a few weeks.

Determination of Antimicrobial Activity by Agar Well Diffusion Method

The antimicrobial activity of various crude extracts, such as ethanol, petroleum ether, ethyl acetate, and watery extract, was investigated by the agar-well diffusion method at the Department of Chemistry, Pathein University. Chloramphenicol and nystatin were used as positive controls for antibacterial and antifungal tests, respectively. Test organisms are *Escherichia coli*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Agrobacterium tumefaciens*, and *Candida albicans*.

The test agar plates containing test organisms (0.1 mL) were punched to make the wells (10 mm in diameter) using a sterilised cork borer and filled with the extract solutions (0.1 mL). Then these plates were incubated at 37 °C for 24 h. After incubation, the diameters of the growth inhibition zones surrounding the agar wells were measured in mm. These zones indicated the presence of antimicrobial activities, selectively inhibiting the growth of test organisms.

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The antioxidant activity of *C. petiolata* extracts was investigated using the DPPH free radical scavenging assay with a spectrophotometric method. For the control solution, 1.5 mL of

DPPH solution was combined with 1.5 mL of ethanol in the brown bottles. The sample solutions were prepared by thoroughly mixing 1.5 mL of DPPH solution with 1.5 mL of various concentrations of the tested sample (3.91, 7.81, 15.63, 31.25, 62.50, and 125.00 µg/mL). These mixtures were incubated at room temperature and shaken on a shaker for 30 minutes. Following this incubation period, the absorbance of each solution was measured at 517 nm using a spectrophotometer (Baliyan *et al*., 2022). Absorbance measurements were conducted in triplicate for each solution, and the percent radical scavenging activity was calculated according to the following equation:

$$\% \text{ RSA} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100$$

where, % RSA = percent radical scavenging activity
$A_{control}$ = absorbance of DPPH in EtOH solution
A_{sample} = absorbance of tested sample + DPPH solution
A_{blank} = absorbance of tested sample + EtOH solution

Cytotoxicity Determination Using the Brine Shrimp Lethality Bioassay

In this study, brine shrimp (*Artemia salina*) was utilised for a cytotoxicity bioassay. Artificial seawater was prepared by dissolving 38 grams of sodium chloride in 1 litre of distilled water. Brine shrimp cysts (0.5 grams) were added to 1 litre of artificial seawater in a bottle and were then placed under a lamp to provide the light necessary for hatching. The cysts required a constant oxygen supply and were incubated at room temperature for 24 h. Next, 9 mL of artificial seawater and 1 mL of sample solutions at different concentrations (1000, 100, 10, and 1 µg/mL) were added to individual chambers. Using a Pasteur pipette, 10 live brine shrimp nauplii were transferred into each chamber. They were incubated at room temperature for approximately 24 h. After this period, the number of dead or surviving brine shrimps was counted to determine cytotoxicity, and the 50 % lethal concentration (LC_{50}) was calculated as described by Baravalia *et al*. (2012). The control solution was prepared similarly, using distilled water instead of the sample solution. Caffeine and potassium dichromate were used as standards.

Determination of Anti-arthritic Activity by Egg Albumin Method

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of various concentrations of crude extracts from Malar rhizome, resulting in final concentrations of 7.81, 15.62, 31.25, 62.5, and 125 µg/mL. A similar volume of double-distilled water was used as a control. The mixtures were incubated at 37 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. After cooling, the absorbance was measured at 660 nm. Diclofenac sodium was used as a reference drug (Sunmathi *et al*., 2016).

$$\% \text{ Protein denaturation} = \frac{A_{sample} - A_{control}}{A_{sample}} \times 100$$

$A_{control}$ = absorbance of control solution
A_{sample} = absorbance of tested sample solution

Results and Discussion

Phytochemicals Present in *C. petiolata* (Malar) Rhizome

To know the types of phytoorganic constituents present in the rhizome of *C. petiolata* (Malar), the phytochemical investigation was preliminarily carried out according to conventional methods. The results obtained from these experiments are summarised in Table 1. The

phytochemical tests revealed the presence of secondary metabolites such as alkaloids, *α*-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponins, phenolic compounds, flavonoids, tannins, steroids, starch, and terpenoids in the sample except cyanogenic glycosides.

The main constituents of *C. petiolata* such as phenolic compounds, tannins, flavonoids, and steroids, may contribute to its bioactivities, including antimicrobial, antioxidant, and antiarthritic properties.

Table 1. Preliminary Phytochemical Screening Results of *C. petiolata* Rhizome

Tests	Extract	Reagent used	Observation	Remarks
		Dragendorff's reagent	orange ppt.	+
alkaloids	1 % HCl	Wagner's reagent	reddish brown ppt.	+
		sodium picrate	yellow ppt.	+
flavonoids	EtOH	conc. HCl and Mg ribbon	pale yellow	+
terpenoids	$CHCl_3$	acetic anhydride and conc. H_2SO_4	yellow	+
steroids	PE	acetic anhydride and conc. H_2SO_4	green	+
α-amino acids	H_2O	ninhydrin reagent	purple	+
glycosides	H_2O	10 % lead acetate	white ppt.	+
saponins	H_2O	H_2O	frothing	+
tannins	EtOH	1 % gelatin and 1 % $FeCl_3$	brown ppt.	+
carbohydrates	H_2O	5 % *α*-naphthol and conc: H_2SO_4	reddish brown ppt.	+
phenolic compounds	H_2O	10 % $FeCl_3$	deep blue ppt.	+
starch	H_2O	I_2, KI	dark-blue ppt.	+
reducing sugars	H_2O	Benedict's solution	brick-red	+
organic acids	H_2O	bromocresol green	green	+
cyanogenic glycosides	H_2O	conc. H_2SO_4, sodium picrate paper	no brick red	-

(+) = presence (-) = absence (ppt.) = precipitate

The Soluble Matter Contents of *C. petiolata* Rhizome

The soluble matter contents in some organic solvents and water were determined by the WHO method. In these experiments, the organic solvents used were ethanol, methanol, ethyl acetate, chloroform, and pet-ether. In the determination of water-soluble matter, the addition of a small amount of chloroform was necessary to prevent the fermentation of the extract. The soluble matter contents in some organic solvents such as PE, $CHCl_3$, EtOAc, EtOH, MeOH, and water calculated on dried weight of the samples were found to be 5.73 %, 2.68 %, 3.42 %, 2.83 %, 3.16

%, and 18.85 % (w/w) in rhizome, respectively (Table 2). The resulting data suggested that the soluble matter contents increase with increasing polarity of the solvents. It also indicated that the sample may contain more polar compounds.

Table 2. Soluble Matter Contents of *C. petiolata* Rhizome

Solvent	Soluble matter contents (%)
petroleum ether	5.73
chloroform	2.68
ethyl acetate	3.42
ethanol	2.83
methanol	3.16
water	18.85

Antimicrobial Activity of Crude Extracts of Malar Rhizome

The assessment of antimicrobial activity using the agar well diffusion method involves measuring the clear zone diameter around the wells. This diameter, which includes the size of the well, indicates the degree of antimicrobial activity. The results of antimicrobial activity are shown in Table 3 and Figure 1. It was found that all crude extracts have antimicrobial activity. All extracts of Malar rhizome exhibited potent activity against all tested microorganisms with inhibition zones ranging from 20 mm and above. Therefore, Malar rhizomes can be effective against a variety of diseases caused by bacteria, viruses, fungi, and parasites. Moreover, it can be used in natural medicine, food preservation, and as an environmentally friendly alternative to synthetic antibiotics.

Table 3. Inhibition Zone Diameter of Crude Extracts from *C. petiolata* by Agar Well Diffusion Method

Microorganisms	Diameter of inhibition zone (mm)				
	Watery extract	Ethanol extract	Pet-ether extract	Ethyl acetate extract	*STD
A. tumefaciens	37	28	21	20	27
B. pumilus	37	29	21	21	27
B. subtilis	38	28	21	21	27
C. albicans	38	28	21	20	27
E. coli	37	29	20	20	27
M. luteus	37	29	21	21	28
P. fluorescens	38	28	20	20	28
S. aureus	37	28	20	20	28

*STD = Chloramphenicol (for bacteria) and Nystatin (for fungus)

Agar well diameter = 8 mm 10–14 mm = weak activity

15–19 mm = moderate activity 20 mm and above = high activity

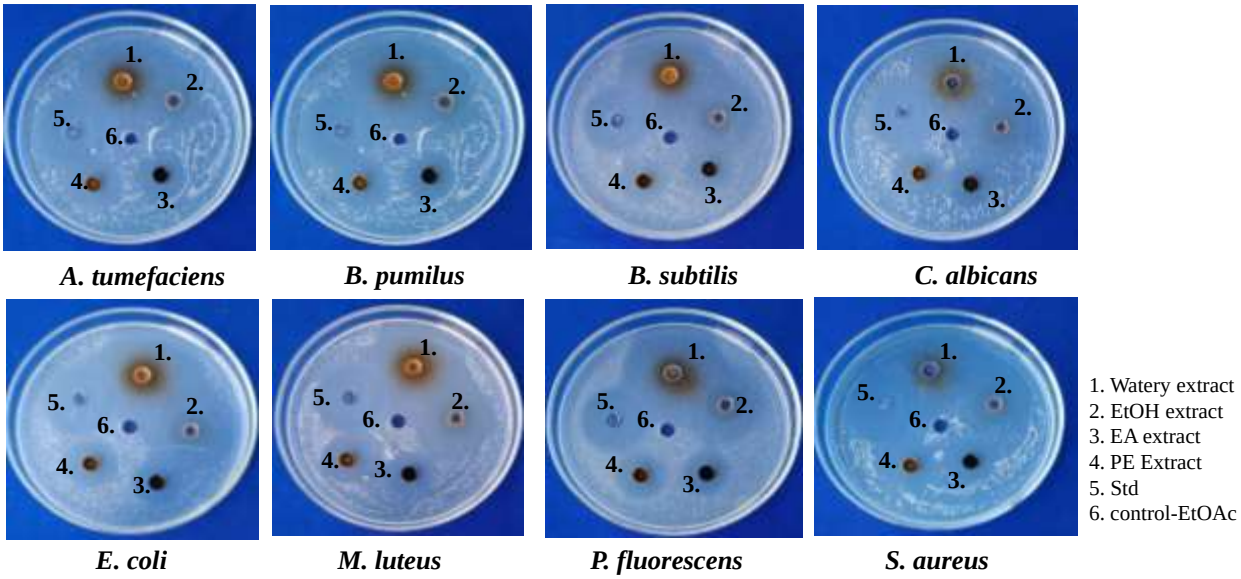

Figure 1. Effect of watery, ethanol, petroleum ether, and ethyl acetate extracts from Malar rhizome on some microorganisms

Antioxidant Activity of Extracts of *C. petiolata* Rhizome by DPPH Free Radical Scavenging Assay

The antioxidant activity of ethanol and watery extracts of the Malar rhizome was assessed by the DPPH free radical scavenging assay. DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical-scavenging activities of antioxidants. It was found that the larger % RSA indicates the higher antioxidant activity. In contrast, the lower IC_{50} value indicates the more effective antioxidant activity. The inhibition percentage of DPPH in the presence of watery and ethanol extracts of *C. petiolata* and their IC_{50} values are shown in Table 4 and Figure 2. The IC_{50} values were observed to be 8.89 µg/mL for the watery extract and 18.34 µg/mL for ethanol extract. The watery extract was found to have higher antioxidant activity than the ethanol extract but lower than the positive control ascorbic acid (IC_{50} = 4.68 µg/mL) (Figure 3). The watery extract of *C. petiolata* has potent activity. Therefore, Malar rhizome could be used for the treatment of many diseases caused by oxidative stress.

Table 4. Average % Inhibition and IC_{50} Values of Crude Extracts of Malar Rhizome

Tested samples	% RSA ± SD of different concentrations (µg/mL)						IC_{50}
	3.91	7.81	15.63	31.25	62.5	125	(µg/mL)
watery extract	44.91 ± 0.44	49.37 ± 0.21	53.91 ± 0.53	59.62 ± 0.42	67.71 ± 0.60	71.76 ± 0.42	8.89
ethanol extract	37.59 ± 1.78	41.21 ± 1.04	47.42 ± 1.78	62.27 ± 1.92	77.55 ± 0.09	86.33 ± 0.32	18.34
Std. ascorbic acid	48.99 ± 0.53	54.03 ± 1.36	56.64 ± 0.44	63.83 ± 1.06	66.61 ± 0.35	87.13 ± 0.76	4.68

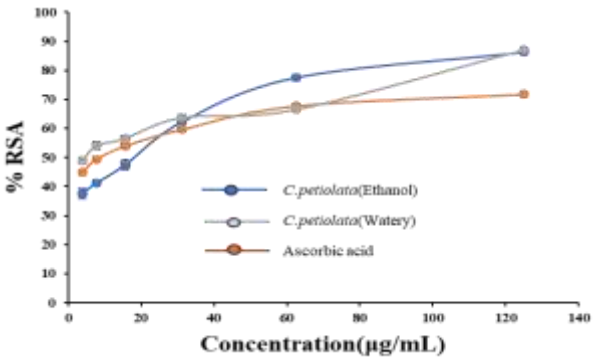

Figure 2. A plot of % RSA of different concentrations of crude extracts from *C. petiolata* rhizome

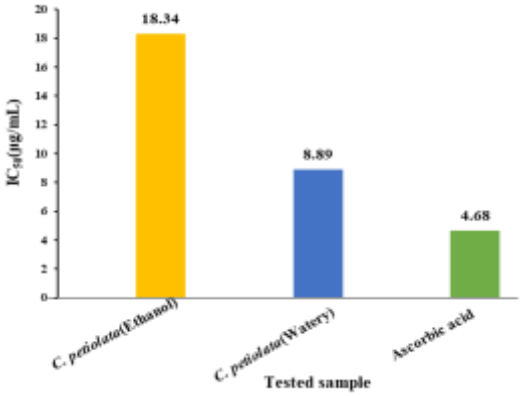

Figure 3. A bar graph of IC_{50} of crude extracts from *C. petiolata* rhizome

Cytotoxicity of Crude Extracts of Malar Rhizome

While certain plants exhibit beneficial biological properties, they can also pose risks to human health. A cytotoxicity test determines whether a product or compound will have any toxic effect on living cells. The brine shrimp lethality bioassay investigated the cytotoxicity of watery and ethanol extracts of Malar rhizome, and the results are shown in Table 5. From the results, the LC_{50} values of watery and ethanol extracts were found to be greater than 1000 µg/mL. If the LC_{50} values of samples are less than 1000 µg/mL these samples are toxic (active) and greater than 1000 µg/mL are non-toxic (inactive). The positive control ($K_2Cr_2O_7$) exhibited a significantly lower LC_{50} value of 43.75 µg/mL, classifying it as highly toxic. The negative control (caffeine) also showed an LC_{50} value greater than 1000 µg/mL, confirming no inherent cytotoxicity. Since the LC_{50} values of watery and ethanol extracts are less than 1000 µg/mL they have no cytotoxic effects and therefore, Malar is safe for use in various purposes.

Table 5. Cytotoxicity of Different Concentrations of Crude Extracts of Malar Rhizome against *Artemia salina* (Brine Shrimp)

Tested samples	% of dead brine shrimp in different concentrations of sample (µg/mL)				LC_{50} (µg/mL)
	1	10	100	1000	
watery extract	30 ± 0.00	30 ± 1.41	35 ± 2.12	35 ± 0.71	> 1000
ethanol extract	20 ± 1.41	30 ± 1.41	40 ± 1.41	47 ± 0.71	> 1000
Std. $K_2Cr_2O_7$	7 ± 1.00	20 ± 0.00	100 ± 0.00	100 ± 0.00	43.75
Std. caffeine	0 ± 0.00	23 ± 1.20	30 ± 0.58	40 ± 1.16	>1000

(caffeine – negative control and $K_2Cr_2O_7$ - positive control)

Anti-arthritic Activity of Crude Extracts of Malar Rhizome

Anti-arthritic activity of crude extracts of Malar rhizome was determined by the protein denaturation method. The effect of watery and ethanol extracts of the Malar rhizome was evaluated against denaturation of egg albumin and showed 12.73 µg/mL and 19.18 µg/mL (Table 6 and Figure 4), respectively. From the results shown in Figure 5, the IC_{50} value of the ethanol extract of the malar rhizome is lower than that of the watery extract. The IC_{50} value of the watery extract is close to standard and it has more anti-arthritic potency than the ethanol extract since the lower the IC_{50} value, the higher the anti-arthritic activity. So, the watery extract of malar rhizome can serve as a potent anti-arthritic agent and it could be used for treatments such as skin inflammation, gout, osteoarthritis, and rheumatoid arthritis.

Table 6. Average % Protein Denaturation and IC_{50} Values of Crude Extracts of Malar Rhizome

Tested samples	% protein denaturation ± SD of different concentrations (µg/mL)						IC_{50} (µg/mL)
	7.81	15.62	31.25	62.5	125	250	
watery extract	38.95 ± 0.40	56.55 ± 1.34	64.81 ± 0.35	74.28 ± 0.13	79.26 ± 0.10	85.30 ± 0.18	12.73
ethanol extract	7.64 ± 0.00	20.83 ± 0.00	52.64 ± 0.64	64.80 ± 0.89	69.59 ± 0.75	79.18 ± 0.21	19.18
St. diclofenac sodium	45.69 ± 0.85	53.23 ± 0.00	60.26 ± 0.66	70.28 ± 1.14	79.39 ± 0.75	85.96 ± 0.24	12.28

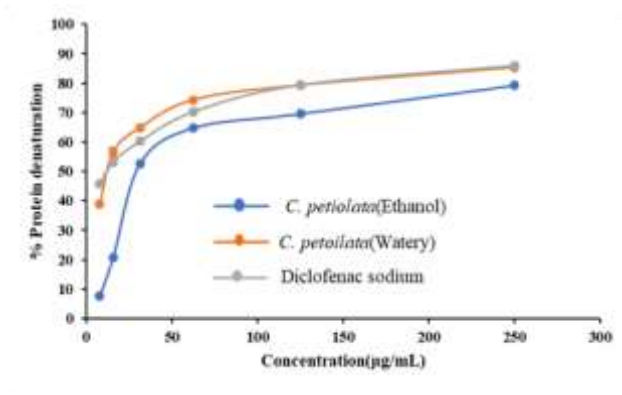

Figure 4. A plot of % protein denaturation of different concentrations of crude extracts from *C. petiolata* rhizome

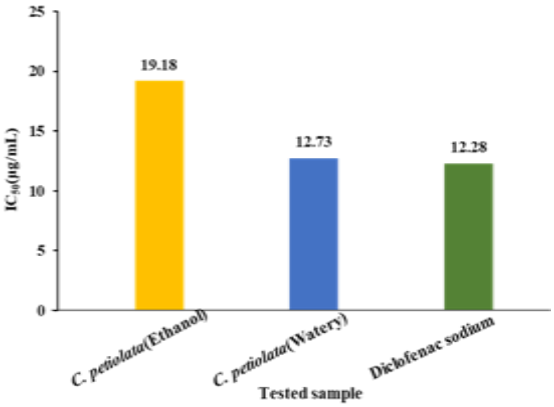

Figure 5. A bar graph of IC_{50} of crude extracts from *C. petiolata* rhizome

Conclusion

From the research on the bioactivity of *Curcuma petiolata* (Malar) rhizome, the inferences could be deduced as follows. Based on the data, the antimicrobial activity of watery and ethanol extracts is potent. The antioxidant activity of the watery extract (IC_{50} = 8.89 μg/mL) is more effective than that of the watery extract (IC_{50} = 18.34 μg/mL). According to the cytotoxic effect, ethanol and watery extracts from the *C. petiolata* rhizome were observed to be free from toxic effects up to 1000 μg/mL. In terms of anti-arthritic activity, the ethanol extract (IC_{50} = 12.7 μg/mL) was more effective than the watery extract (IC_{50} = 19.18 μg/mL). Malar rhizome reveals its significant potential as a natural therapeutic agent, showing strong antioxidant properties that may help migrate oxidative stress-related diseases. Its antimicrobial effect indicates against bacterial and fungal infections. Cytotoxicity results exhibit further safety evaluation, and its anti-arthritic activity highlights its potential for managing inflammatory conditions. Studying the biological activities of *C. petiolata* helps us understand its traditional medicinal uses and highlights its potential as a source of new medicines.

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