INVESTIGATION OF PRELIMINARY PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *CRATEVA MAGNA* (LOUR.) DC. (KADET) LEAF

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Abstract

This study investigated the antimicrobial, antioxidant, cytotoxic, and anti-arthritic activities of *Crateva magna* (LOUR.) DC. (Kadet) leaf. Preliminary phytochemical screening confirmed the presence of alkaloids, *α*-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids, while cyanogenic glycosides were not detected. The antimicrobial activity of aqueous, ethanol, ethyl acetate, and petroleum ether extracts was evaluated using the agar well diffusion method. Ethanol and ethyl acetate extracts demonstrated strong antimicrobial activity, with inhibition zones measuring 25 to 26 mm. In antioxidant activity accessed via the DPPH free radical scavenging assay, the water extract had an IC_{50} of 345.58 μg/mL, while the ethanol extract showed a significantly lower IC_{50} of 24.81 μg/mL. Cytotoxicity was tested using the brine shrimp lethality bioassay, indicating both extracts were non-toxic at a concentration of 1000 μg/mL, with LC_{50} values suggesting safety. Anti-arthritic activity was evaluated using the egg albumin denaturation method, revealing that the water extract (IC_{50} = 66.26 μg/mL) had stronger anti-arthritic effects than the ethanol extract (IC_{50} = 68.39 μg/mL).

Keywords: *Crateva magna*, antimicrobial activity, antioxidant activity, cytotoxicity, anti-arthritic activity

Introduction

Medicinal plants are valuable for healing and treating human diseases due to their rich content of phytochemicals. Phytochemicals such as primary and secondary metabolites are naturally occurring in the medicinal plants, leaves, vegetables and roots that have been defences mechanism and protect from various diseases. According to the World Health Organization (WHO), over 80 percent of the global population relies on traditional herbal medicine for their primary health care needs. This is the significant role that herbal remedies play in health systems, especially in regions with limited access to conventional medical services. Plants are a rich source of secondary metabolite such as terpenoid, steroids, flavonoid, alkaloids and phenolic compounds, which serves as an important source for the treatment various diseases (Abirami *et al.*, 2017). *Crateva magna* (Lour.) DC. is an evergreen medicinal plant belong to the family Capparaceae and it also known as three-leaved capper. This plant is distributed in India, Indo-Malaysian, Myanmar and China (Mohsina *et al.*, 2022). *C. magna* is a medium-sized, deciduous tree and approximately 30 m height. About 70 species of genus *Crateva* are scattered mainly in different parts of the world (Kumar *et al.*, 2020). The roots, bark, and leaf of *C. magna* are utilized in the treatment of a variety of ailments, including fever, snake bites, poisoning, ulcers, skin eruptions, cancer, diabetes, arthritis, hepatitis, and both acute and chronic inflammation. The leaf paste is applied externally to relieve hemorrhoids, while the juice is ingested to provide relief from bleeding piles (Behera *et al.*, 2016). Additionally, the bark juice is administered orally to prevent common childhood diseases.

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A comprehensive literature review identifies numerous bioactive compounds in *C. magna*, such as friedelin, diosgenin, sitosterol, saponins, flavonoids, sterols, glucosinolates, lupeol, betulinic acid, rutin, quercetin, varunol, glucocapparin, and kaempferol-3-*O*-*α*-D-glucoside (Meera *et al.*, 2017). These compounds contribute to the plant's notable biological activities, including anti-inflammatory, anticancer, antioxidant, antimicrobial, and antidiabetic effects, primarily attributed to the presence of alkaloids, flavonoids, terpenes, phenolic

Botanical Aspect of *Crateva magna* (Lour.) DC. (Kadet) Leaf

Family	Capparaceae
Botanical name	*Crateva magna* (Lour.) DC.
Myanmar name	Kadet, Kadet-kha, Kon-kadet
Part used	Leaf

Figure 1. Photograph of *C. magna* leaf compounds, and steroids (Bhattacharjee *et al.*, 2012).

Materials and Methods

Plant materials

Crateva magna leaf was collected from Hinthada Township, Ayeyarwady Region, Myanmar. The collected sample was identified as *Crateva magna* (Lour.) DC. (Kadet) leaf at the Botany Department, University of Yangon. The sample was cleaned by washing thoroughly with water. Then the fresh leaf sample was cut into small pieces and air-dried at room temperature. The dried samples were ground into powder by a grinding machine and stored in an airtight container for further use.

Phytochemical Investigation of *C. magna* Leaf

Phytochemical tests for the *C. magna* leaf were carried out according to the test tube methods (Geetha *et al*., 2014, and Harbone, 1984) to investigate the presence and absence of alkaloids, *α*-amino acids, carbohydrates, cyanogenic glycosides, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids. The observed results are shown in Table 1.

Preparation of Crude Extracts for Biological Activities

About 20 g of dried powder sample was extracted three times with 95 % ethanol for six hours and then filtered, reduced the volume by rotary evaporator to obtain ethanol extract. To obtain water extract, 20 g of dried powder samples was boiled in 100 mL of distilled water and filtered. It was then concentrated by evaporating the solvent on a water bath to get a watery extract. The crude extracts were dried and kept in a refrigerator for a few weeks.

Screening of Antimicrobial Activity of the Samples by Agar well Diffusion Method

The antimicrobial activity of four crude extracts, such as petroleum ether, ethyl acetate, ethanol, and water extracts from the *C. magna* leaf was determined against eight strains of microorganisms, such as *Bacillus subtilis, Staphylococcus* aureus, *Pseudomonas fluorescens, Bacillus pumilus, Escherichia coli, Agrobacterium tumefaciens, Micrococcus luteus, and Candida albicans,* as standard chloramphenicol used for bacteria and nystatin for fungus by employing the agar well diffusion method. According to the method described by Selvamohan *et al.* (2012), these tests were screened at Pathein University. The tested microorganisms were inoculated and plated with nutrient agar and incubated at 37°C for 24 h. Then, a hole with a diameter of 8 mm was punched aseptically with a sterile cork or a tip on the nutrient agar plates with the respective microorganisms, and each 0.1 mL of different extracts (1 mg extract in 1 mL) was introduced into the well. Then, the plates were incubated for 24 h to allow the extract to diffuse through the agar media to form zones of inhibition. The extent of antimicrobial activity was measured by the diameter of the inhibition zone.

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The control solution was prepared by combining 1.5 mL of DPPH solution with 1.5 mL of ethanol in brown bottles to protect from light. The sample solutions were prepared by mixing 1.5 mL of DPPH solution with 1.5 mL of ethanol containing different concentrations of the test samples (15.62, 31.25, 62.50, 125.00, 250.00, and 500.00 μg/mL). The IC_{50} value of ascorbic acid was determined for use as a standard. The test mixtures and the standard were incubated at room temperature and shaken for 30 minutes. After incubation, the absorbance of each solution was measured at 517 nm using a spectrophotometer (Hishamuddin *et al*., 2020). Absorbance measurements were conducted in triplicate for each solution to ensure reliability.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

The brine shrimp (*Artemia salina*) were used in this study for a cytotoxicity bioassay. The brine shrimp cysts (0.5 g) were hatched in a hatching tank containing 1 L of artificial seawater (3.8% (w/v) NaCl in distilled water). The tank was well aerated with the aid of an air pump, and the proper light source was also provided. The cysts were hatched at room temperature for 24 h. Alive brine shrimp (10 nauplii) were inoculated into each chamber containing 9 mL of artificial seawater and 1 mL of different concentrations of samples (1000, 100, 10, 1 μg/mL) and standard solutions (caffeine and potassium dichromate) and incubated at room temperature for about 24 h. Then, the number of dead or surviving brine shrimps was counted, and the estimation of cytotoxicity was done by 50% lethality dose (LC_{50}) (Singh *et al*., 2015). The control solution was prepared as in the above procedure by using distilled water instead of the sample solution.

Determination of Anti-arthritic Activity by Egg Albumin Method

The reaction mixture (5 mL) consisted of 0.2 mL of 1% egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 7.4), and 2 mL of different concentrations (62.5, 125, 250, 500, and 1000 μg/mL) of crude extracts of *C. magna* leaf. A similar volume of mixture without a sample served as a control. Then the mixtures were incubated at 37˚C in an incubator for 30 min and then heated at 70˚C for 15 min. After cooling, the absorbance was measured at 280 nm. Diclofenac sodium was used as a reference drug (Madhuranga and Samarakoon, 2023).

Results and Discussion

Phytochemical Investigation of *C. magna* Leaf

The preliminary phytochemical test was carried out to identify the various phytoconstituents present in the sample. The phytochemical test revealed the presence of alkaloids, *α*-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, organic acids, terpenoids, and flavonoids were found to be present in the sample whereas, cyanogenic glycoside were found to be absent according to the test tube method. The *C. magna* leaf can be employed as antioxidant, antidiabetic, antibacterial and anticancer drugs due to the presence of secondary metabolites such as phenolic compounds, steroids, tannins, terpenoids, and flavonoids components.

Table 1 Results of Preliminary Phytochemical Investigation of the *C. magna* Leaf

No.	Test	Extract	Test Reagents	Observation	Results
1	alkaloids	1% HCl	(i)Dragendorff's reagent	Orange ppt	+
			(ii)Mayer's regent	White ppt	+
			(iii)Wagner's reagent	Brown ppt	+
2	*α*-Amino acids	H_2O	Ninhydrin reagent	Purple spot	+
3	carbohydrates	H_2O	10 % α-naphthol and H_2SO_4	Red ring	+
4	cyanogenic glycosides	H_2O	H_2SO_4 + sodium picrate solution	No brick red	-
5	glycosides	H_2O	10 % lead acetate	White ppt.	+
6	organic acids	H_2O	Bromocresol green	Yellow ppt	+
7	phenolic compounds	H_2O	$K_3Fe(CN)_6 + FeCl_3$	Deep blue colour	+
8	reducing Sugars	H_2O	Benedict's solution	Yellow ppt.	+
9	saponins	H_2O	Distilled water	Frothing	+
10	starch	H_2O	Iodine solution	Deep blue colour	+
11	steroids	PE	Acetic anhydride + conc: H_2SO_4	Greenish blue color	+
12	tannins	H_2O	5% $FeCl_3$ solution	Bluish black ppt	+
13	Terpenoids	$CHCl_3$	Acetic anhydride + Conc: H_2SO_4	Red colour	+
14	Flavonoids	EtOH	Conc: HCl + Mg turning	Pink colour	+

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

Antimicrobial Activity of Crude Extracts of *C. magna* Leaf by Agar well Diffusion Method

The antimicrobial activity of four extracts (petroleum ether, ethanol, ethyl acetate, and water) from the leaf of *C. magna* was evaluated by measuring the inhibition zone diameters against eight tested microorganisms, as detailed in Table 2 and Figure 2. The results indicate that

both the ethyl acetate and ethanol extracts demonstrated significant antimicrobial activity against all eight microorganisms, with inhibition zone diameters ranging from 24 to 26 mm. In contrast, the water and petroleum ether extracts exhibited minimal activity, with inhibition zones measuring between 13 and 14 mm. These findings suggest that ethyl acetate and ethanol extracts are particularly effective, showing their potential for further development in antimicrobial applications.

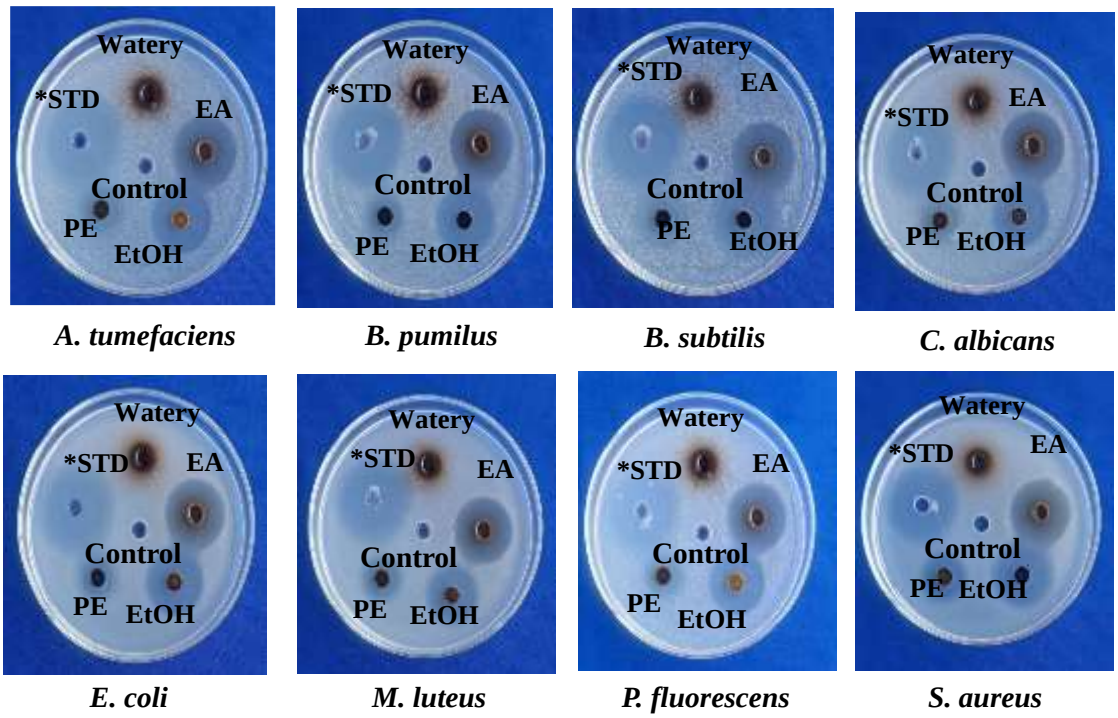

Figure 2 Photographs of antimicrobial activity of different crude extracts of *C. magna* leaf on eight microorganisms

Table 2 Inhibition Zone Diameter of the *C. magna* Leaf Extracts Against Eight Different Microorganisms

Microorganisms	Inhibition Zone Diameters of Extracts (mm)				
	Water	Ethanol	Ethyl acetate	Pet-ether	STD
A. tumefaciens	14	26	24	14	27
B. pumilus	13	25	25	14	27
B. subtilis	13	25	24	13	27
C. albicans	13	26	24	14	27
E. coli	13	25	25	14	27
M. luteus	13	26	24	13	28
P. fluorescens	13	25	25	13	28
S. aureus	13	25	25	13	28

diameter of agar well = 8 mm
9 mm-14 mm = weak activity
15 mm- 20 mm = medium activity
21 mm above = potent activity

For Bacterial STD = Chloramphenicol
For Fungus STD = Nystatin

Antioxidant Activity of Crude Extracts of *C. magna* Leaf by DPPH Free Radical Scavenging Assay

The antioxidant activity of the water and ethanol extracts of *C. magna* leaf was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with IC_{50} values representing the concentration needed to inhibit 50 % of DPPH radicals. These results were compared to the antioxidant activity of standard ascorbic acid. As shown in Tables 3 and Figures 3, 4, and 5, a significant difference emerged between the two extracts: the water extract had an IC_{50} value of 345.58 µg/mL, while the ethanol extract demonstrated a much lower IC_{50} value of 24.81 µg/mL. This indicates that the ethanol extract possesses a considerably greater capacity for scavenging DPPH radicals than the water extract. The enhanced scavenging activity of the ethanol extract may be linked to the higher solubility of bioactive compounds in ethanol, which likely increases their availability and effectiveness in radical scavenging.

Cytotoxicity of Crude Extracts of *C. magna* Leaf

The cytotoxicity of water and ethanol extracts of *C. magna* leaf was evaluated using the brine shrimp lethality bioassay Artemia salina as the test organism. The results presented in Table 4 indicated that the LC_{50} values for both extracts were greater than 1000 µg/mL. According to the criteria, extracts with LC_{50} values less than 1000 µg/mL are considered toxic (active), while those greater than 1000 µg/mL are regarded as non-toxic (inactive). Thus, both the water and ethanol extracts showed no cytotoxic effects.

Table 3. %RSA and IC_{50} Values of Crude Extracts of *C. magna* Leaf and Standard Ascorbic Acid

Extract	**% RSA (mean ±SD) in different concentration (µg/mL)**						**IC_{50} (µg/mL)**
	15.625	**31.25**	**62.5**	**125**	**250**	**500**	
Water	13.87 ±1.53	23.14 ±1.23	29.99 ±1.43	35.03 ±1.25	45.05 ±1.95	58.06 ±0.57	345.58
Ethanol	48.34 ±1.27	51.17 ±1.29	55.87 ±1.81	59.78 ±1.61	73.57 ±1.20	77.12 ±0.73	24.81
Standard	% RSA (mean ±SD) in different concentration (µg/mL)						IC_{50} (µg/mL)
	1.95	3.91	7.81	15.625	31.25	62.5	
Ascorbic acid	48.92 ±3.19	72.42 ±3.1	96.02 ±0.18	96.99 ±0.21	97.11 ±0.42	97.23 ±0.10	2.04

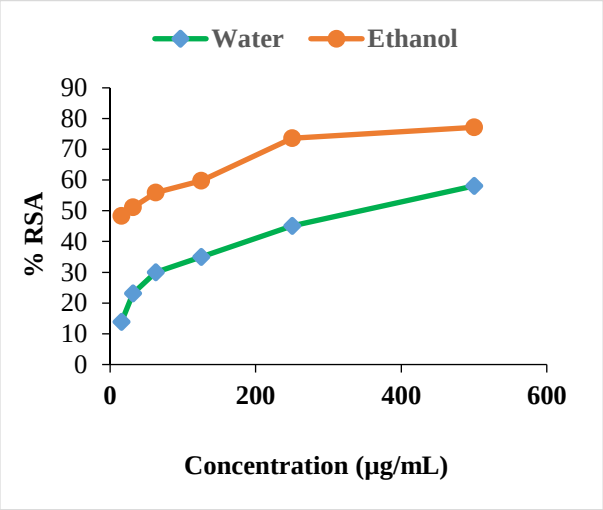

Figure 3. A plot of % RSA of different concentrations of crude extracts of *C. magna* leaf

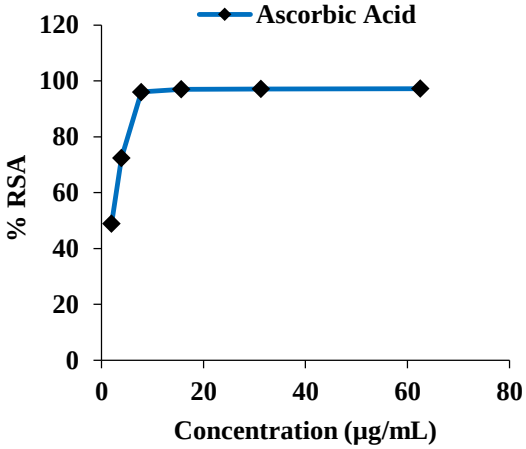

Figure 4. A plot of % RSA of different concentrations of standard ascorbic acid

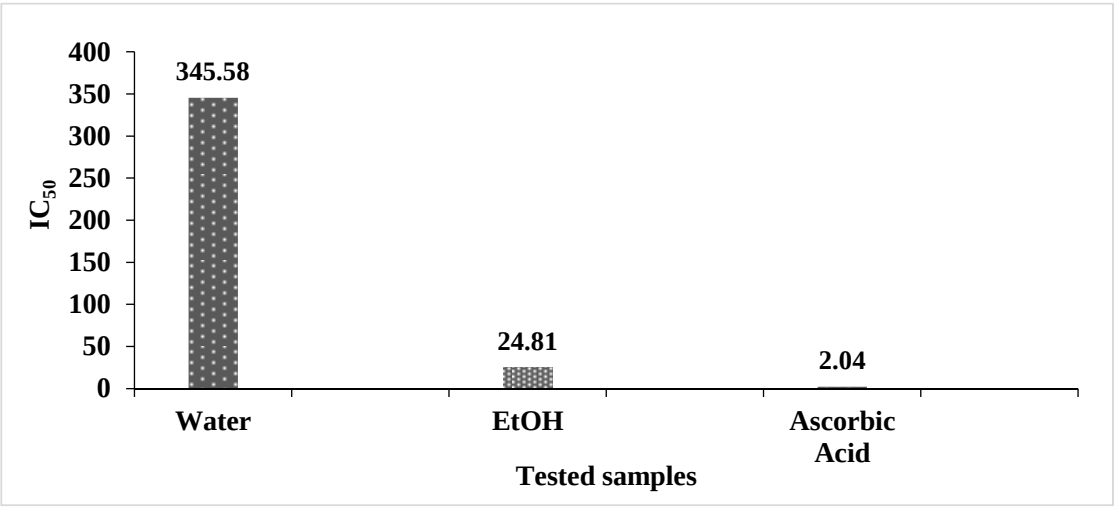

Figure 5. A bar graph of IC_{50} values of crude extracts of leaf of *C. magna* and standard ascorbic acid

Table 4 Cytotoxicity of Different Concentrations of Crude Extracts of *C. magna* Leaf and Standard by Brine Shrimp Assay

Tested Samples	% of Dead Brine Shrimp in Different Concentrations of Sample (µg/mL) ± SEM				LC_{50} (µg/mL)
	1	10	100	1000	
Water extract	0.00 ± 0.00	10.00 ± 1.00	30.00 ± 1.00	33.33 ± 0.57	> 1000
Ethanol extract	10.00 ± 1.00	13.33 ± 0.58	16.67 ± 1.15	20.00 ± 1.00	> 1000
Std. $K_2Cr_2O_7$	7.00 ± 1.50	20.00 ± 0.00	100.00 ± 0.00	100 ± 0.00	43.75
Std. Caffeine	3.33 ± 0.57	13.33 ± 1.00	13.33 ± 0.58	23.33 ± 1.15	>1000

Anti-arthritic Activity of Crude Extracts of *C. magna* Leaf and Standard Diclofenac Sodium

One of the causes of inflammation and arthritic diseases is assumed to be protein denaturation. Agents that significantly reduce the denaturation of egg albumin in this assay may possess potential anti-arthritic properties. The effects of water and ethanol extracts of *C. magna* leaf on the denaturation of egg albumin were evaluated, resulting in IC_{50} values of 66.26 µg/mL for the water extract and 68.39 µg/mL for the ethanol extract in Table 5 and figure 8. These findings indicate that the water extract has a lower IC_{50} value, correlating with higher anti-arthritic activity. Thus, the water extract demonstrates greater anti-arthritic potency than the ethanol extract, suggesting that it could serve as an effective anti-arthritic agent.

Table 5 Average % Protein Denaturation and IC_{50} Values of Crude Extracts of *C. magna* Leaf and Standard Diclofenac Sodium

Extract	Inhibition (mean ±SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	62.5	125	250	500	1000	
Water	48.55 ± 0.00	72.59 ± 0.18	85.33 ± 0.10	92.78 ± 0.29	96.52 ± 0.12	66.26
Ethanol	47.75 ± 0.17	75.09 ± 1.67	87.11 ± 0.77	93.49 ± 0.04	96.41 ± 0.08	68.39
Standard	Inhibition (mean ±SD) in different concentration (µg/mL)					IC_{50} (µg/mL)
	3.91	7.81	15.625	31.25	62.5	
Diclofenac sodium	14.43 ± 1.81	48.15 ± 1.29	66.28 ± 1.88	81.06 ± 0.54	88.75 ± 0.16	8.61

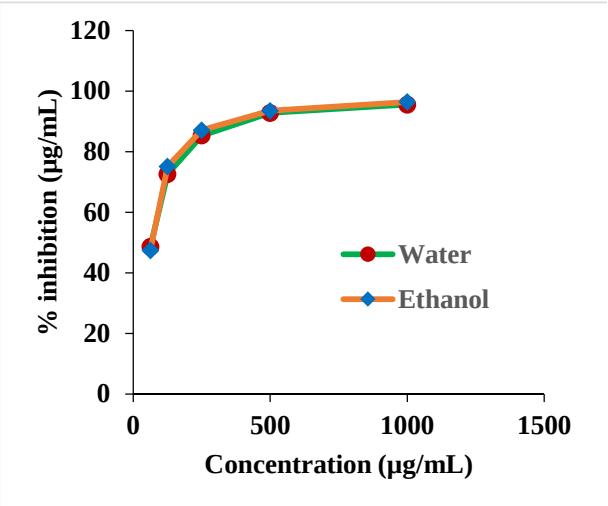

Figure 6. A plot of anti-arthritic activity of difference concentrations of crude extracts of *C. magna* leaf

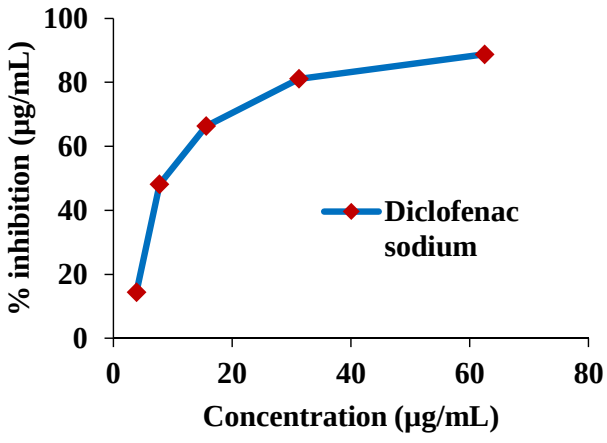

Figure 7. A plot of anti-arthritic activity of difference concentrations of diclofenac sodium

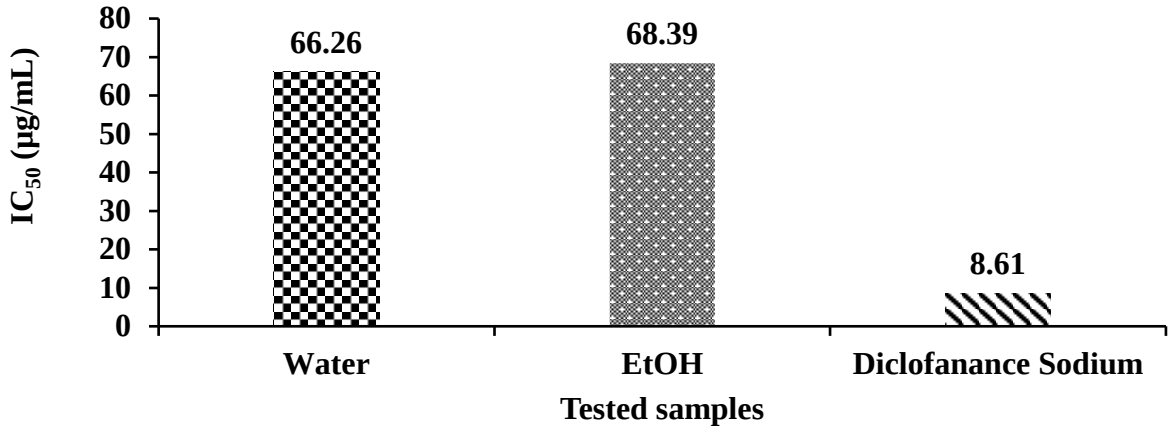

Figure 8. A bar graph of IC50 values of crude extracts of C. magna leaf and standard diclofenac Sodium

Conclusion

This research investigated the phytochemical constituents and their as well as some activities of *C. magna* leaf. Preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, α-amino acids, reducing sugars, steroids, carbohydrates, phenolic compounds, saponins, tannins, starch, organic acids, terpenoids, and flavonoids, while cyanogenic glycosides were absent. The antimicrobial activity of the test samples was determined by the agar well diffusion method. Based on the data, the ethanol and ethyl acetate extracts exhibited the highest antimicrobial activity, with inhibition zone diameters ranging from 24 to 26 mm, compared to the petroleum ether and water extracts because they contained flavonoids, phenols, and tannin. Antioxidant activity, assessed by the DPPH free radical scavenging assay, showed that the ethanol extract (IC_{50} = 24.81 µg/mL) was more effective than that of the water extract (IC_{50} = 345.58 µg/mL). Cytotoxicity testing indicated that both ethanol and water extracts were non-toxic up to 1000 µg/mL. Anti-arthritic activity, determined using egg albumin denaturation method, demonstrated that the water extract (IC_{50} = 66.26 µg/mL) was slightly effective than the ethanol extract (IC_{50} = 68.39 µg/mL). Overall, these findings suggest that *C. magna* possesses significant pharmacological potential, and the observed data may contribute to the development of novel therapeutic agents.

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