SOME BIOLOGICAL ASSESSMENT OF *PLUCHEA INDICA* (L.) LESS ROOT (YAY-KHAYU) FROM THE TANINTHARYI REGION

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Abstract

Pluchea indica (L.) Less is naturally grown in mangroves along the Tanintharyi Coastal Region. It is locally known as "Yay-Khayu" and has been utilised as a source of food and medicine and for making herbal tea. This research has been carried out to investigates the total phenolic and flavonoid contents, antimicrobial, antioxidant, cytotoxicity, and anti-arthritic activities collected of *P.indica* root collected from the Tanintharyi Coastal Region. The total phenolic and flavonoid contents of ethanol and watery extracts from *P. indica* root were determined by the Folin-Ciocalteu Reagent (FCR) and aluminium chloride colourimetric methods. In addition, the antimicrobial efficacy of watery, ethanol, ethyl acetate, and petroleum ether crude extracts from *Pluchea indica* root samples was evaluated against eight microorganisms using the agar well diffusion method. The tested microorganisms included *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*. According to the analysis, all the crude extracts from the root sample exhibit significant antimicrobial activity on most of the microorganisms. The cytotoxicity of ethanol and watery extracts from the *P. indica* root sample was evaluated using the *Artemia salina* (brine shrimp) bioassay. The obtained results showed that neither the ethanol nor the watery extract of the root sample exhibited significant toxicity to a maximum dose of 1000 µg/mL. The antioxidant potential of the ethanolic and watery extracts from the root sample was assessed using the DPPH free radical scavenging assay. The antioxidant activity of the ethanol extract was found to be higher than that of the watery extract from the root sample. Furthermore, the egg albumin denaturation method was used to evaluate the anti-arthritic properties of both ethanol and watery extracts from the root sample. It was found that the ethanol extract showed significantly higher anti-arthritic activity than the watery extract of the root sample.

Keywords: *Pluchea indica* (L.) Less, antimicrobial activity, cytotoxicity, antioxidant activity, anti-arthritic activity

Introduction

The medicinal plants are vital species that, based on traditional medicine and modern scientific research, offer valuable health benefits and aid in treating various diseases. They are considered abundant sources of compounds that can be utilized in developing and producing pharmaceutical drugs to enhance human health (Oladeji *et al*., 2019).

Antioxidants are compounds that reduce oxidative damage to biological processes by donating electrons to free radicals, rendering them harmless. Free radicals are closely linked to oxidative stress, which occurs when oxygen interacts with certain chemicals, leading to the formation of free radicals. Once these are produced, the potential risk lies in the damage they may cause by interacting with vital cellular components such as DNA, proteins, and cell membranes. Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids, and phenolic compounds are more effective (Duh *et al*., 1999).

Arthritis includes various types of joint disorders, such as rheumatoid arthritis (RA) and osteoarthritis (OA), which can affect either a single joint or multiple joints. The typical signs of arthritis include joint swelling, tenderness, and stiffness, often accompanied by a reduced range of motion. These symptoms can vary in intensity, from mild discomfort to severe impairment. (Lespasio *et al*., 2017).

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P. indica known as Yay-Khayu in Myanmar, is a member of the *Asteraceae* family and a well-known medicinal plant that grows naturally in many Asian countries. It could be found in Thailand, India, China, the Philippines, Malaysia, and Myanmar (Wang *et al*., 2008; Panda and Luyten, 2018). It contains various biochemical compounds, including sesquiterpenes, flavonoids, tannins, saponins, sterols, alkaloids, and thiophenes (Ruan *et al*., 2018). It is also used to treat a wide range of conditions, including lumbago, kidney stones, leucorrhea, inflammation, gangrenous and atonic ulcers, haemorrhoids, dysentery, eye disorders, itchy skin, acid stomach, dysuria, abdominal pain, scabies, fever, muscle soreness, diabetes, and rheumatism, among others (Sabrin *et al*., 2022).

Therefore, the total phenolic and total flavonoid contents, antimicrobial activity, cytotoxic effects, antioxidant properties, and anti-arthritic potential of *P. indica* (Yay-Khayu) root extract were evaluated in this study.

Materials and Methods

Location of Sampling Sites

P. indica is one of the coastal flowering plants in Tanintharyi Coastal Region. It is an evergreen shrub and is found abundantly in salt marshes and mangrove swamps with a 1 to 2 m height. It plays a significant part in preserving the ecological balance along the Tanintharyi coastal areas. The selected sample also occurred in Palaw Township, Tanintharyi Region of Myanmar. For the current study, a *P. indica* root sample was chosen. The location of the sampling site and the collected root sample are illustrated in Figure 1.

Figure 1. Map showing the collection site of *P. indica* root (Yay-Khayu) in Palaw Township, Tanintharyi Region

Collection and Preparation of Sample

P. indica root sample was collected from Palaw Township during June 2024. This sample was identified by an authorized botanist in the Department of Botany, at Myeik University. The root sample was cleaned with tap water and then distilled water to remove the dirt on the surface of the root, and air-dried. The air-dried sample was made into powder with an electric grinder and stored in airtight containers to prevent moisture changes and other contamination.

Determination of Total Phenol Contents (TPC) as Gallic Acid Equivalent

The total phenolic content was determined by the Folin-Ciocalteu method (Saxena *et al*., 2013). Each extracted sample solution (0.5 mL) was added into 5 mL of FC reagent (1:10) and incubated for 5 min. To each tube, 4 mL of 1 M sodium carbonate solution was added, the tubes were kept at room temperature for 15 min, and the UV absorbance of the reaction mixture was read at λ_{max} 765 nm. Total phenolic content was estimated as micrograms of gallic acid equivalent per milligram (μg GAE/mg) of crude extract.

Determination of Total Flavonoid Contents (TFC) by Aluminium Chloride Colorimetric (ACC) Assay

The total flavonoid content was determined using an aluminium chloride colorimetric assay (Basma *et al*., 2011). Each extracted sample solution (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 1 % aluminium chloride solution, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water, the tubes were allowed to stand for 30 min at room temperature. The absorbance of the solution was measured at 415 nm using a UV spectrophotometer. Results were expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract).

Determination of Antimicrobial Efficacy

The antimicrobial efficacy of watery, ethanol, ethyl acetate, and pet-ether extracts of *P. indica* root was investigated by using the agar well diffusion method at the Department of Botany, Pathein University.

Evaluation of Cytotoxicity Bioassay

Preparation of artificial seawater: Artificial seawater (3.8 % (w/v) NaCl) was prepared by dissolving (38 g) of sodium chloride in 1 L of distilled water.

Hatching of brine shrimp (*Artemia salina*): Brine shrimp cysts (0.5 g) were put into 1 L of artificial seawater in a bottle. This bottle was placed near a lamp. Light is essential for the cysts to hatch. Brine shrimp cysts are required to hatch with constant supplied oxygen and 24 h incubation at room temperature.

Determination of cytotoxicity test: The cytotoxicity test of crude extracts was assessed by using brine shrimp lethality bioassay. The sample solution was prepared with 9 mL of artificial seawater, 1 mL of various concentrations of samples, and alive brine shrimp (10 nauplii) with a Pasteur pipette. Potassium dichromate was used as a standard positive control and caffeine was also used as a standard negative control. They were incubated at room temperature for 24 h. After 24 h, the number of survivals of nauplii was counted and the percentage of mortality was determined using the following equation (Singh *et al*., 2015).

% mortality = (no. of dead nauplii/initial no. of live nauplii) × 100

Determination of Antioxidant Capacity by DPPH Free Radical Scavenging Assay

The effect on DPPH radical was determined using the method by Hishamuddin *et al*. (2020). Antioxidant activities of 95 % ethanol and watery extracts were carried out in a DPPH free radical scavenging assay, and the absorbance of these solutions was measured at 517 nm by using a UV-visible spectrophotometer. Absorbance measurements were done in triplicate for each solution, and then the mean values obtained were used to calculate the percent inhibition of oxidation by the following formula. Then, IC_{50} (50 % inhibitory concentration) values were also calculated by a linear regressive Excel program.

$$\% \text{ Inhibition} = [A_{control} - (A_{sample} - A_{blank}) / A_{control}] \times 100$$

where, $A_{control}$ = absorbance of DPPH in EtOH solution

A_{sample} = absorbance of the test sample and DPPH solution

A_{blank} = absorbance of the test sample and EtOH solution

Screening of Anti-arthritic Activity

The anti-arthritic activity was evaluated using the egg albumin denaturation method by Madhuranga (2023)**.** Diclofenac sodium was used as the standard drug. The control solution was created by 2 mL of distilled water, 0.2 mL of 1 % egg albumin, and 2.8 mL of phosphate-buffered saline (pH 7.4). The sample solution was also prepared with 0.2 mL of 1 % egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of different concentrations of crude extracts of root. Then the mixtures were incubated at 37 °C in an incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 280 nm by a UV-visible spectrophotometer. The whole test procedure was done in triplicate.

Results and Discussion

Total Phenolic Contents of Crude Extracts Root of *P. indica* (Yay-Khayu)

In the present investigation, the total phenolic contents of the *P. indica* root sample were estimated by the Folin-Ciocalteu method. Gallic acid (3, 4, 5-trihydraxybenzoic acid) was used to construct a standard calibration curve for total phenol estimation. Total phenolic content (TPC) was expressed as micrograms of gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/mg) (Table 1 and Figure 2). According to the results, the total phenolic content was found to be ethanol extract (104.69 mg) of GAE/g which is higher than the watery extract (52.86 mg) of GAE/g of the extract (Table 2 and Figure 3).

Table 1. The absorbance of Different Concentrations of Standard Gallic Acid at λ_{max} 765 nm

Concentration (µg/mL)	Absorbance (mean value)
0.625	0.094
1.25	0.105
2.5	0.134
5	0.183
10	0.283
20	0.472

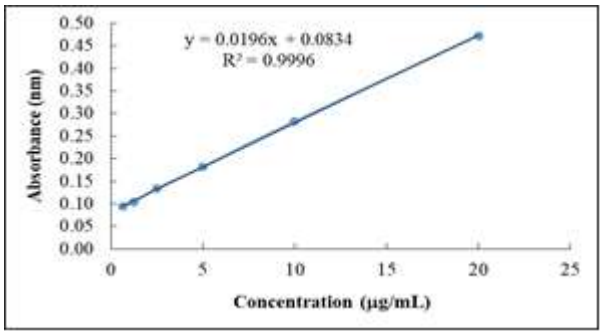

Figure 2. Calibration curve of standard gallic acid at 765 nm

Table 2. Total Phenolic Contents of Crude Extracts of the Root of *P. indica*

Extracts	TPC (mg GAE/g ± SD)
Ethanol	104.69 ± 0.001
Watery	52.86 ± 0.003

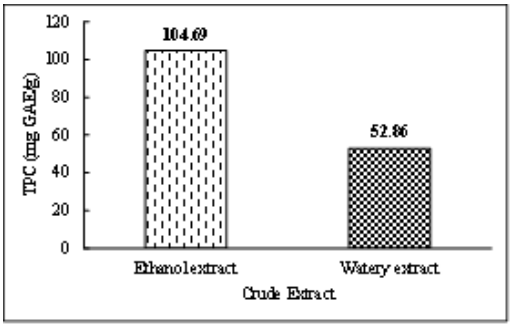

Figure 3. A bar graph of total phenolic contents of crude extracts of the root of *P. indica*

Total Flavonoid Contents of Crude extracts Root of *P. indica* (Yay-Khayu)

The total flavonoid contents of the *P. indica* root sample were investigated by the aluminium chloride colourimetric method. Quercetin was used as a standard compound for the

quantification of the standard calibration curve for total flavonoid estimation as shown in Table 3 and Figure 4. The total flavonoid contents (TFC) were expressed as micrograms of quercetin equivalent (QE) per milligram of crude extract (µg QE/mg). From the results, the total flavonoid contents in ethanol and ethanol extracts were found to be 24.36 and 13.45 mg QE/g, respectively. (Table 4 and Figure 5).

Table 3. The absorbance of Different Concentrations of Standard Quercetin at λ_{max} 415 nm

Concentration (µg/mL)	Absorbance (mean value)
0.625	0.003
1.25	0.006
2.5	0.013
5	0.027
10	0.057
20	0.111

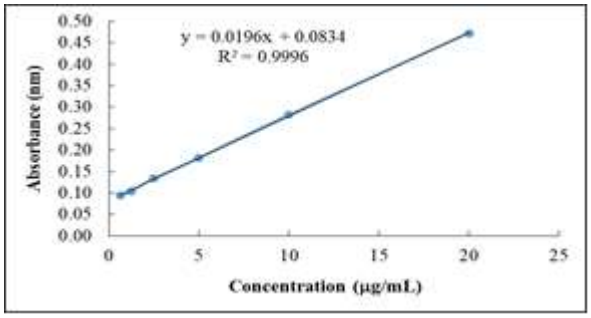

Figure 4. Calibration curve of standard quercetin at 415 nm

Table 4. Total Flavonoid Contents of Crude Extracts of the Root of *P. indica*

Extracts	TFC (mg QE/g ± SD)
Ethanol	45.00 ± 0.014
Watery	23.57 ± 0.001

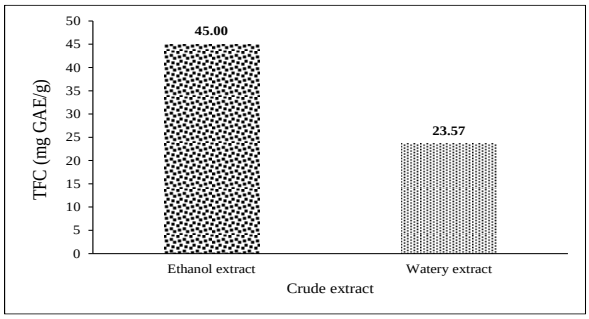

Figure 5. A bar graph of total flavonoid contents of crude extracts of the root of P. indica

Antimicrobial Efficacy of *P. indica* Root (Yay-Khayu) Sample

The antimicrobial efficacy was determined by the agar well diffusion method. The antimicrobial efficacy of the different extracts (watery, ethanol, ethyl acetate, and pet-ether) against eight microorganisms such as *Agrobacterium tumefaciens, Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Staphylococcus aureus, Micrococcus luteus,* and *Pseudomonas fluorescens* are listed in Table 5 and Figure 6.

A larger inhibition zone indicates stronger antimicrobial activity of the tested substances. Based on these findings, all crude extracts from root samples demonstrated significant antimicrobial effects against most microorganisms. Among them, the ethyl acetate extracts showed the highest antimicrobial activity against all tested microorganisms, with inhibition zones measuring between 19 mm and 23 mm, followed by ethanol, watery, and pet-ether extracts. Moreover, ethanol extract has higher antimicrobial activity than watery and pet-ether extract within inhibition zones ranging from 17 mm to 18 mm. While the watery extract had weaker antimicrobial activity compared to ethyl acetate and ethanol extracts, it was more effective than the pet-ether extract, with inhibition zones from 15 mm to 17 mm. Among all extracts, the pet-ether extract displayed the weakest antimicrobial activity against both bacterial and fungal pathogens. From

overall observation, the root extracts may be applied in the development of antimicrobial agents, especially in the treatment of infections caused by both gram-positive and gram-negative bacteria, as well as fungal pathogens.

Table 5. Antimicrobial Efficacy of *P. indica* Root (Yay-Khayu) Sample

Test organisms	Inhibition zone diameter (mm)				
	Watery extract	Ethanol extract	Ethyl acetate extract	Pet-ether extract	Standard
A. tumefaciens	17	18	23	12	27
B. pumilus	16	18	19	12	27
B. subtilis	16	17	19	12	27
C. albicans	16	18	20	11	27
E. coli	15	17	19	11	27
M. luteus	16	17	20	11	28
P. fluorescens	16	17	19	11	28
S. aureus	16	17	19	11	28

For bacteria*STD = Chloramphenicol
For fungus *STD = Nystatin

10-12 mm = weak activity, 13-18 mm = moderate activity, > 18 mm = high activity; well size = 8 mm

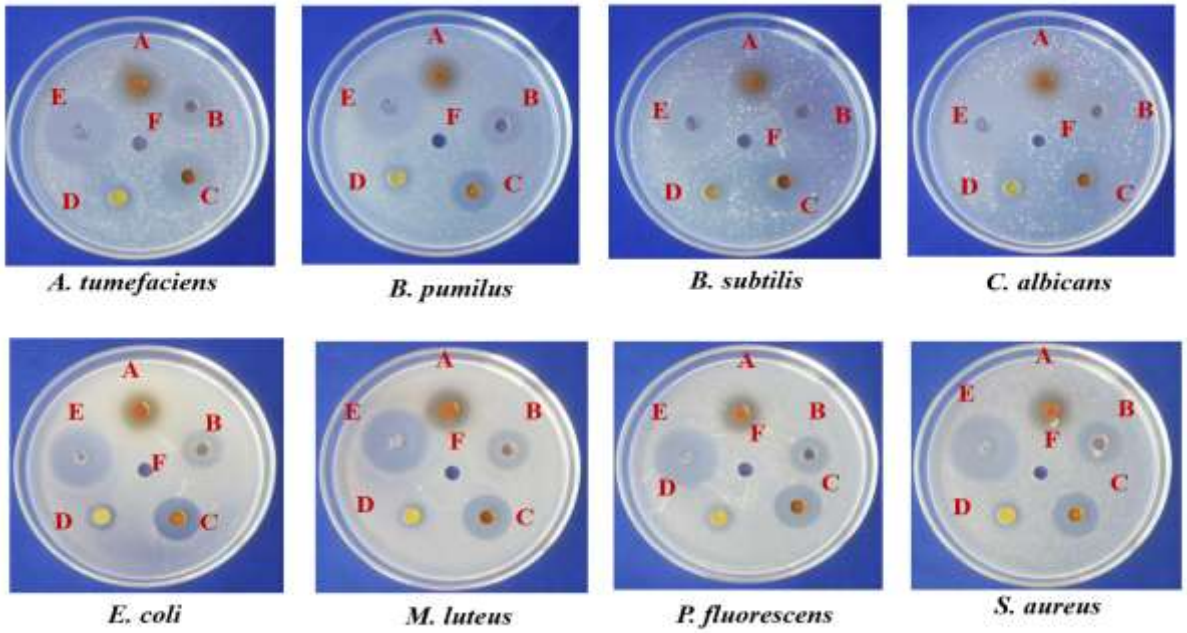

Figure 6. Inhibition well diameters of *P. indica* (Yay-Khayu) root against eight microorganisms (A) Watery extract, (B) 95 % Ethanol extract, (C) Ethyl acetate extract (D) Pet-ether extract, (E) Standard (F) Control

Cytotoxicity of Watery and Ethanol Extracts of *P. indica* Root (Yay-Khayu) Sample

A brine shrimp lethality bioassay, using the *Artemia salina* (Brine shrimp), was a simple and cost-effective bioassay that was used to assess the cytotoxic effects of the watery and ethanol extracts of the root sample. The cytotoxicity was expressed as 50 % lethal dose concentration LC_{50}. A lower LC_{50} value indicates greater toxicity, while a higher LC_{50} indicates that the substance is

less toxic or non-toxic. Substances with LC_{50} values below 1000 µg/mL are typically regarded as cytotoxic. Watery and ethanol extracts of the *P. indica* (Yay-Kha-Yu) root sample were dissolved in distilled water to get 2000 µg/mL concentrations, used as a stock solution, and then it was diluted with 9 mL of artificial seawater and alive brine shrimp (10 nauplii) to obtain 1000, 100, 10, and 1 µg/mL concentrations. In this experiment, potassium dichromate was used as a cytotoxic agent and effectively induced mortality in the brine shrimp, serving as the standard positive control. In contrast, caffeine served as the standard negative control and demonstrated no cytotoxicity. The results of the cytotoxicity test for watery and ethanol extracts of *P. indica* (Yay-Khayu) root are shown in Table 6.

Both the watery and ethanol extracts of *P. indica* root showed LC_{50} values of 1000 µg/mL, indicating that these extracts are non-toxic at the tested concentration of 1000 µg/mL. Overall, the findings suggest that *P. indica* root extracts are non-toxic at the tested dosages, which could be indicative of their potential safety in further biological application.

Table 6. Results of Cytotoxicity Test for Four Different Doses of Watery and Ethanol Extracts of *P. indica* Root (Yay-Khayu) Sample

Tested sample	Extracts	% of Dead Brine Shrimp (Mean ± SEM) in Various Concentrations (µg/mL)				LC_{50} (µg/ mL)
		1	10	100	1000	
Root	watery	10 ± 1.00	20 ± 0.00	23 ± 0.58	33 ± 0.58	>1000
	ethanol	13 ± 0.57	23 ± 0.57	33 ± 0.58	50 ± 1.15	1000
standard	$K_2Cr_2O_7$	7 ± 1.00	20 ± 0.00	100 ± 0.00	100 ± 0.00	43.75
	caffeine	0 ± 0.00	23 ± 1.20	30 ± 0.58	40 ± 1.16	>1000

Antioxidant Activity of Watery and Ethanol Extracts from *P. indica* Root (Yay-Khayu) Sample

The present work shows the antioxidant activity of watery and ethanol extracts from *P. indica* (Yay-Khayu) root was studied by the DPPH assay. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration IC_{50}. The DPPH assay acts as a free radical scavenger or hydrogen donor to evaluate antioxidant capacity. Watery and ethanol extracts of the selected *P. indica* (L.) Less. (Yay-Khayu) root samples were dissolved in ethanol and then it was diluted with ethanol to obtain 125, 62.5, 31.3, 15.62, 7.81, and 3.91 µg/mL concentrations. After mixing these solutions with DPPH solution, their absorbance was measured at 517 nm. Ascorbic acid was used as a standard reference in this experiment. The IC_{50} values for each sample were determined using a linear regression analysis in Excel. The results are described in Table 7 and Figures 7 and 8.

The results of this experiment showed that IC_{50} was 42.46 µg/mL for watery extract and 26.27 µg/mL for ethanol extract. Since the lower the IC_{50} values, the higher the free radical scavenging activity, i.e., the higher the antioxidant capacity, the antioxidant activity of ethanol extract (IC_{50} = 20.67 µg/mL) was found to be higher than that of watery extract (IC_{50} = 42.46 µg/mL) in the root sample. Compared to the standard ascorbic acid (IC_{50} = 4.68 µg/mL), all crude extracts of the root samples demonstrated less potent antioxidant activity. Thus, the high content of antioxidants in the ethanol extract from the Yay-Khayu root sample suggested its potential as a strong antioxidant material and its ability to neutralize and reduce many oxidizing compounds.

Table 7. Oxidative Percent Inhibitions and IC_{50} Values of Ethanol and Watery Extract of *P. indica* Root (Yay-Khayu) Sample and Standard Ascorbic Acid

Tested sample	Extracts	% Inhibitions (Means ± SD) in various concentrations (µg/mL)						IC_{50} (µg/mL)
		3.91	7.81	15.625	31.25	62.5	125	
Root	ethanol	12.63 ± 0.20	22.85 ± 2.28	44.69 ± 0.40	61.12 ± 0.00	72.95 ± 0.40	87.58 ± 0.53	20.67
	watery	34.94 ± 0.12	38.41 ± 0.50	40.81 ± 0.95	47.03 ± 0.70	55.31 ± 0.40	63.19 ± 0.12	42.46
Standard	ascorbic acid	48.99 ± 0.53	54.09 ± 1.36	56.64 ± 0.44	63.83 ± 1.06	66.61 ± 0.35	87.13 ± 0.76	4.68

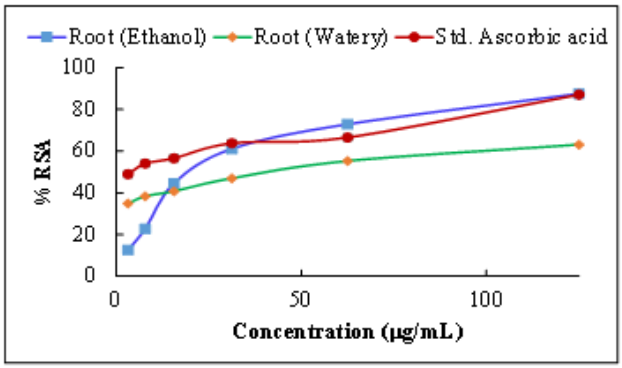

Figure 7. A plot of % RSA vs concentration of crude extracts of *P. indica* root and standard ascorbic acid

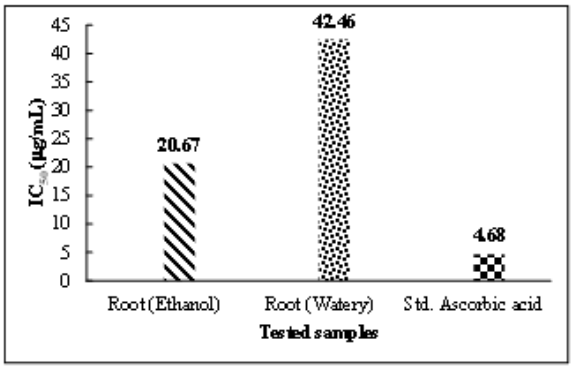

Figure 8. A bar graph of IC_{50} values of crude extracts of *P. indica* root and standard ascorbic acid

Anti-arthritic Activity of *P. indica* Root (Yay-Khayu) Sample

This study investigates the anti-arthritic properties of watery and ethanol extracts from the root of *P. indica* (L.) Less. (Yay-Khayu) using the egg albumin denaturation assay. Protein denaturation is linked to inflammation. A substance's potential anti-arthritic action is demonstrated by its capacity to prevent protein denaturation. Using a fresh hen's egg, the *in vitro* egg albumin denaturation method is used to screen for anti-arthritic properties. In this experimental work, diclofenac sodium was used as a standard. Watery and ethanol extracts from the root sample of the selected *P. indica* (Yay-Khayu) were dissolved in water and used as a stock solution. These were then further diluted with water to get concentrations of 500, 250, 125, 62.5, 31.3, 15.62, and 7.81 µg/mL. Afterward, each solution was mixed with 1 % egg albumin and phosphate-buffered saline (pH 6.4), and their absorbance was measured at 280 nm. Based on absorbance values, the obtained results of % inhibition of each sample in different concentrations were calculated and tabulated in Table 8, Figures 9 and 10.

In conditions like rheumatoid arthritis, cancer, and diabetes, which involve inflammation, protein denaturation leads to the formation of autoantigens. Therefore, by preventing protein denaturation, it is possible to reduce inflammatory activity. In the investigation of anti-arthritic

properties using the egg albumin denaturation method on *P. indica* (Yay-Khayu) root from Palaw Township, the IC_{50} values were determined to be 34.00 µg/mL for the watery extract and 25.35 µg/mL for the ethanol extract. From the experimental work, all the crude extracts from the root sample exhibited weaker anti-arthritic activity compared to the standard diclofenac sodium, which has an IC_{50} value of 18.39 µg/mL. The results show that the IC_{50} value of the ethanol extract from the root sample is lower than that of the watery extract. Since a lower IC_{50} value indicates greater anti-arthritic activity, this suggests that the ethanol extract has a stronger anti-arthritic potential compared to the watery extract. Thus, the ethanol extract of the Yay-Khayu root sample can serve as an anti-arthritic agent to reduce inflammation, joint pain, and the progression of arthritis symptoms.

Table 8. Results of Anti-arthritic Activity of Ethanol and Watery Extracts of *P. indica* Root (Yay-Khayu) Sample and Standard Diclofenac Sodium

Tested sample	Extracts	% Inhibition (Mean ± SD) in different concentrations (µg/mL)						IC_{50} (µg/ mL)
		15.62	31.3	62.5	125	250	500	
Root	ethanol	15.92 ± 0.00	70.67 ± 1.96	79.67 ± 0.44	83.21 ± 1.73	88.32 ± 0.48	93.04 ± 0.49	25.35
	watery	14.88 ± 1.33	49.43 ± 0.49	55.89 ± 1.62	76.01 ± 0.69	77.28 ± 0.56	87.14 ± 0.13	34.00
Standard	diclofenac sodium	46.36 ± 0.00	66.93 ± 1.45	78.20 ± 0.84	84.88 ± 0.55	89.70 ± 0.18	93.60 ± 0.12	18.39

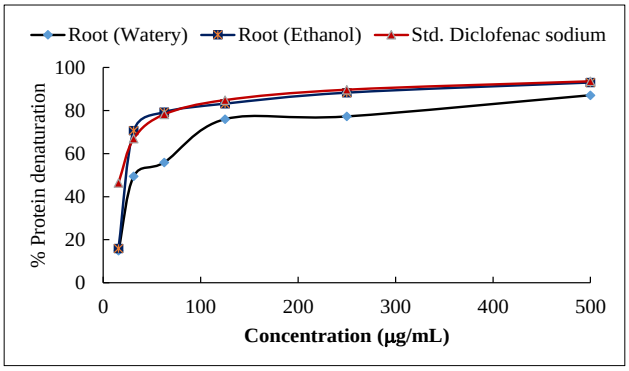

Figure 9. A plot of % protein denaturation vs concentration of crude extracts of *P. indica* root and standard diclofenac sodium

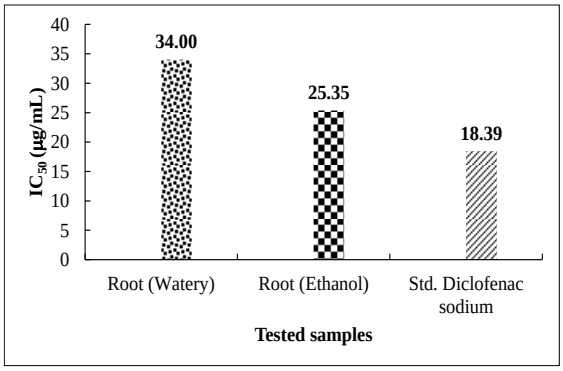

Figure 10. A bar graph of IC_{50} values of crude extracts of *P. indica* root and standard diclofenac sodium

Conclusion

The key objectives of this study were to evaluate the total phenolic and flavonoid contents, antimicrobial efficacy, cytotoxicity, antioxidant capacity, and anti-arthritic activity of *P. indica* (Yay-Khayu) root sample from the Palaw Township, Tanintharyi Region. Based on the data, the ethanol extract of *P. indica* root was observed to contain higher phenol and flavonoid contents than the watery extract. The finding suggests that *P. indica* root extracts, particularly the ethanol and ethyl acetate extracts, may serve as effective antimicrobial agents against bacterial and fungal pathogens. The toxicity assessment showed that both watery and ethanol extracts had LC_{50} values of 1000 µg/mL, indicating that they are non-toxic at this concentration. Therefore, *P. indica* root extracts are safe for potential biological applications at the tested doses, making them suitable for further research and development in therapeutic applications. The antioxidant potential of the root extracts was evaluated based on free radical scavenging activity, with lower IC_{50} values indicating stronger antioxidant capacity. The ethanol extract showed high antioxidant activity (IC_{50} = 20.67 µg/mL) compared to the watery extract (IC_{50} = 42.46 µg/mL). This highlights the ethanol extract as a potency antioxidant agent, which may protect against oxidative damage. This study demonstrated that both the watery and ethanol extracts of *P. indica* root have significant anti-arthritic potential, as indicated by their ability to prevent protein denaturation such as rheumatoid arthritis.

The IC_{50} value for the ethanol extract (25.35 µg/mL) was lower than that of the watery extract (34.00 µg/mL), suggesting that the ethanol extract has stronger anti-arthritic activity. This indicates that ethanol extract can be more effective in reducing inflammation, joint pain, and the progression of arthritis symptoms. Thus, the overall findings indicate that *P. indica* root extract samples have significant potential as antimicrobial agents, antioxidants, and anti-arthritic for the treatment of microbial infections, oxidative stress, and inflammation-related diseases.

Acknowledgement

The author would like to express our sincere thanks to Professor Dr Ni Ni Than allowing us to present this research topic.

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