SCREENING SOME BIOLOGICAL ACTIVITIES OF *SPATHOLOBUS SUBERECTUS* (KYATHWAY-PANNWE-PIN) STEM

Chue Shwesin Kyaw[1], Prema[2], Thandar Aung[3]

Abstract

Spatholobus suberectus (Kyathway-pannwe-pin) stem was chosen to evaluate some biological activities such as antioxidant, antimicrobial, cytotoxic, anti-arthritic, and anti-diabetic. The DPPH assay revealed that both watery and ethanol extracts exhibited antioxidant activity, with IC_{50} values of 2.01 μg/mL and 2.66 μg/mL, respectively. The antimicrobial activity of pet-ether, ethyl acetate, ethanol, and watery extracts was tested on eight microorganisms by the agar-well diffusion method. Among all these extracts, the ethanol extract exhibited potent activity, with inhibition zone diameters ranging from about 20 mm and above. The cytotoxicity of the watery and ethanol extracts was evaluated through a brine shrimp lethality bioassay. The results did not indicate any cytotoxic effects for either extract at concentrations up to 1000 μg/mL. *In vitro* anti-arthritic activity determined by the egg albumin denaturation method showed that the IC_{50} values of ethanol and watery extracts were found to be 35.92 μg/mL and 108.89 μg/mL. The anti-diabetic activity of ethanol and watery extracts was screened with an *in vitro* α-amylase inhibition assay. The IC_{50} values of ethanol extract (23.97 μg/mL) have higher inhibition than that of the watery extract (81.64 μg/mL).

Keywords: *Spatholobus suberectus*, antioxidant, antimicrobial, cytotoxic, anti-arthritic, and anti-diabetic activities

Introduction

Natural products are derived from plants and play an essential role in the maintenance of human health. In early times, plants were the main source of medicines. According to recent facts, the plant kingdom comprises approximately 250,000 plant species, of which only about 15% have been investigated for the treatment of several illnesses. The phytochemical properties of diverse plants and their biological activities were studied to know their pharmacological roles in disease prevention. Medicinal plants play a vital role in various diseases, and their medicinal values lie in some bioactive constituents that produce a definite physiological action on the human body (Noureddine, 2024). The dried vine stem of *Spatholobus suberectus* (SSD), chiefly distributed in Fujian Province and the Guangxi Zhuang autonomous region of China. *Spatholobus suberectus* (SSD) has been extensively employed in traditional Chinese medicine to treat several ailments. The stem of *S. suberectus* has been used as a traditional Chinese medicine for the treatment of anaemia, menoxenia, and rheumatism (Hieu, 2019). SSD has been frequently attributed to having antioxidant, anti-diabetic, anti-inflammatory, haematopoietic, neuroprotective, antimicrobial, and anticancer properties. SSD and its active compounds are effective therapeutic agents for treating a variety of diseases with negligible side effects (Feng, 2022). *Spatholobus suberectus* has been known as a Korean folk medicine for promoting blood circulation and relieving blood stasis as anaemia. The antioxidant activities of *S. suberectus* were found to be present in the antioxidant substances from natural products (Lee, 2005). The chemical constituents of *Spatholobus* include flavonoids, terpenes, sterols, anthraquinones, lactones, and volatile oils. Many of these ingredients are found to possess anti-oxidant,

[1] Department of Chemistry, University of Yangon
[2] Department of Chemistry, University of Yangon
[3] Department of Chemistry, University of Yangon

antimicrobial, and anti-inflammatory effects (Yehau, 2023). The extracts of *S. suberectus* have been reported to display diverse biological activities, such as tyrosinase inhibition, anti-inflammation, HIV-1-protease inhibition and immunomodulatory activities. The isolation of a series of flavonoids, quinones and steroids from the plant have been reported. Biological assay has indicated that the flavonoids, the main constituents of the plant, show a variety of activities, such as topoisomerase II inhibitory, skin whitening, cytotoxic, anti-virus and anti-oxidation activities (Liu, 2018). A recent study demonstrated the antioxidant, antimicrobial, cytotoxic, anti-arthritic, and anti-diabetic activities of the various crude extracts from *S. suberectus* stem.

Botanical Aspect of *Spatholobus suberectus*

Family name	Leguminosae
Botanical name	*Spatholobus suberectus*
Genus	*Spatholobus*
Species	*S.suberectus*
English name	Chicken blood vein
Myanmar name	Kyathway-pannwe-pin
Part used	Stem

Figure 1. Photographs of *S. suberectus* plant and stem

Materials and Methods

Collection and Preparation of Plant Sample

Spatholobus suberectus (Kyathway-pannwe-pin) stems were collected from Waimaw Township, Kachin State, and identified at the Department of Botany, Myitkyina University. The fresh stems of *S. suberectus* were collected and dried in the open air and then under shade at room temperature **(**Figure 1). Stems were placed at 40 ˚C in an oven for 18 h to remove the excess moisture. The dried stems were then chopped and ground to a powder using a mechanical blender.

Preparation of Crude Extracts for Biological Activities

Dried powdered samples were percolated with different solvents such as petroleum ether, ethyl acetate, ethanol, and water, respectively. After one week, the extracts were filtered through Whatman filter paper Grade 1, and the collected filtrate each was placed in a rotary evaporator under reduced pressure and controlled different temperatures for evaporation of the different solvents. Finally, the volume of extracts will be reduced, and the crude extracts were obtained.

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The antioxidant activity of the ethanol and watery extracts was determined based on radical scavenging activity by using a 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The control solution was prepared by mixing 1.5 mL of 0.002% (w/v) DPPH solution and 1.5 mL of 95% ethanol in the brown bottles. The sample solution was also prepared by mixing thoroughly 1.5 mL of 0.002% (w/v) DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and shaken on a shaker for 30 min. After shaking, the absorbance of each solution was measured at 517 nm using a UV-visible spectrophotometer. The absorbance measurements were done in triplicate for each concentration, and the mean values obtained were used to calculate the percentage of radical scavenging activity (RSA) by the following equation.

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

Where,

% RSA = % radical scavenging activity of test sample

$A_{control}$ = absorbance of the control solution (DPPH + ethanol)

A_{blank} = absorbance of the blank solution (sample + ethanol)

A_{sample} = absorbance of the sample solution (sample + DPPH)

The antioxidant activity expressed as IC_{50} is the concentration (μg/mL) for 50% oxidative inhibition of the substance. The IC_{50} values were calculated by linear regressive excel program (Brand-Williams *et al.,* 1995).

Determination of Antimicrobial Activity by Agar Well Diffusion Method

The antimicrobial activity of various crude extracts from *S. suberectus* was screened by the agar well diffusion method on eight microorganisms (*Agrobacterium tumefaciens, Bacillus subtilis, Bacillus pumilus, Candida albicans, Escherichia coli, Micrococcus luteus, Pseudomonas fluorescens***,** *and Staphylococcus aureus*) at the Department of Chemistry, Pathein University. In this method, peptone (0.5 g) and sodium chloride (0.25 g) were mixed with distilled water and made up to 100 mL with distilled water. The nutrient agar was boiled, and 20-25 mL of the medium was poured into a test tube and plugged with cotton wool and autoclaved at 121°C for 15 min. Then the tubes were cooled down to 60 °C and poured into sterilized Petri dishes and 0.1 mL of spore suspension was also added into the dishes. The agar was allowed to set for 30 min after which, an 8 mm plate agar-well was made with the help of a sterilized cork border. After that, about 0.1 mL each of extract was introduced into the agar well and incubated at 37 °C for 24 h, and the results were recorded. The inhibition zone (clear zone) appeared around the agar well, indicating the presence of antimicrobial activity. The inhibition zone diameter indicates that ≤ 10 mm was low activity, 10–15 mm was moderate activity, and ≥ 15 mm was good activity. The greater the inhibition zone diameters, the higher the antimicrobial activity.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

The brine shrimp (*Artemia salina*) was used in this study for a cytotoxicity bioassay. Artificial seawater [3.8 % (w/v) NaCl] was prepared by dissolving 38 g of sodium chloride in 1 L

of distilled water. A 0.5 g portion of brine shrimp cysts was added to a 1 L of artificial seawater flask, and placed close to a lamp. The cysts must have light in order to hatch. For brine shrimp cysts to hatch, continuous oxygen supply and a 24 h incubation period at room temperature are needed. The test solution of 1 mL was mixed with 9 mL of artificial seawater and placed in the ice cup chamber. Alive brine shrimp (10 nauplii) were taken with a Pasteur pipette and placed into each chamber, which was kept at room temperature. After 24 h incubation, the number of surviving brine shrimp was counted and a 50 % lethality concentration (LC_{50}) was calculated. The control solution was prepared as in the above procedure by using distilled water instead of the sample solution (Dockery and Tomkins, 2020).

Determination of Anti-arthritic Activity by Egg Albumin Method

The reaction mixture consisted of 0.2 mL of fresh hen's egg albumin, 2.8 mL of phosphate buffered saline (pH 6.3), and test solutions of different concentrations were mixed to form a total volume of 5 mL. An equal volume of distilled water served as the control solution. The test samples and standard were incubated at 37 ± 2 °C in an incubator for 15 min, followed by heating at 70 °C for 5 min. The absorbance of different concentrations of the tested sample was measured at 660 nm on a UV-visible spectrophotometer. The percent inhibition of protein denaturation was determined by the following formula:

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

Where,

$A_{control}$ = absorbance of control solution

A_{sample} = absorbance of tested sample solution

Determination of Anti-diabetic Activity by *α*-Amylase Inhibition Method

Alpha-amylase activity was carried out by the starch-iodine method. 10 µL of *α*-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M of PBS containing 0.006 M NaCl, pH 7.0) containing different concentrations of extracts. After incubation at 37 °C for 10 min, 10 µL of 1 % starch solution was added, and the mixture was re-incubated for 15 min. Next, 0.1 mL of 1 % iodine solution was added, and after adding 5 mL of distilled water, the absorbance was taken at 565 nm (Ganapaty *et al*., 2013). All experiments were done in triplicate, and the % inhibition was calculated by using the following equation:

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

Where,

$A_{control}$ = absorbance of control solution

A_{sample} = absorbance of tested sample solution.

Results and Discussion

Antioxidant Activity of Extracts of *S. suberectus* by DPPH Free Radical Scavenging Assay

The antioxidant activity of EtOH and watery extracts from the stem of *S. suberectus* was determined with six different concentrations (62.5, 31.25, 15.62, 7.81, 3.9, and 1.95 µg/mL)**.** According to the result of antioxidant activity, the IC_{50} values of ethanol, watery extracts, and standard ascorbic acid were found to be 2.01 µg/mL, 2.66 µg/mL, and 1.99 µg/mL, respectively. Based on the results, the stem of *S. suberectus* possesses antioxidant activity as standard as ascorbic acid. Therefore, the stem of *S. suberectus* can be used as a natural antioxidant and may be useful for the treatment of various diseases caused by oxidative stress. The resulting data are given in Table 1 and Figures 2 and 3.

Table 1. Average % Inhibition and IC_{50} Values of Crude Extracts of *S. suberectus* Stem

Tested samples	% RSA ± SD of different concentrations (µg/mL)						IC_{50} (µg/mL)
	1.95	3.9	7.81	15.62	31.25	62.5	
Ethanol extract	49.91 ±0.33	52.96 ±0.37	60.28 ±1.36	68.45 ±0.28	78.59 ±0.37	80.94 ±0.43	2.01
Watery extract	47.75 ±0.24	53.94 ±0.61	55.54 ±0.81	59.86 ±0.73	61.83 ±0.24	63.94 ±0.73	2.66
Std. Ascorbic Acid	49.91 ±0.69	54.79 ±1.36	59.86 ±0.73	61.83 ±0.24	82.77 ±0.71	85.45 ±0.45	1.99

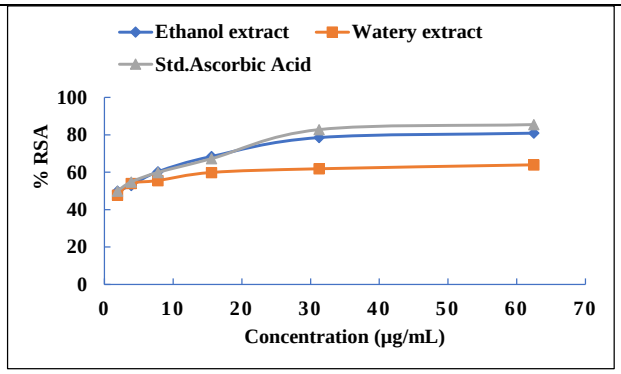

Figure 2. A plot of % RSA of different concentrations of crude extracts of *S. suberectus* stem

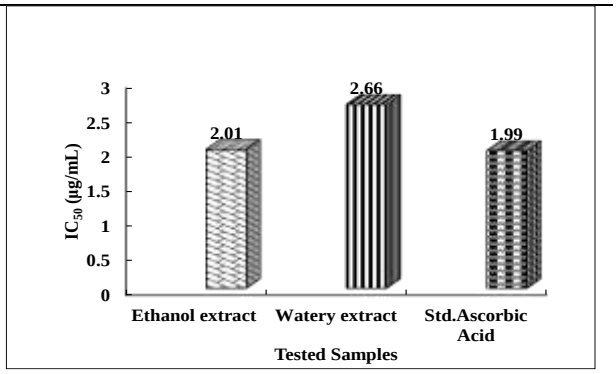

Figure 3. A bar graph of IC_{50} values of *S. suberectus* stem and standard ascorbic acid

Antimicrobial Activity of *S. suberectus* Stem

The antimicrobial activity of various crude extracts was screened by an agar well diffusion method on eight microorganisms as compared to the standard chloramphenicol for bacteria and nystatin is used for the fungus. The larger the inhibition zone diameter, the higher the antimicrobial activity observed in Table 2 and Figure 4.

According to the results, ethanol and ethyl acetate extracts inhibited all species of tested microorganisms with inhibition zone diameters in the range between 17–28 mm. However, watery and pet-ether extracts of the sample showed a 12–14 mm inhibition zone, indicating mild antimicrobial activity. Therefore, ethanol and ethyl acetate extracts may be used for the treatment of ear and sinus infections, skin infections, meningitis, strep throat, bacterial pneumonias and whooping cough.

Table 2. Inhibition Zones Diameters of some Crude Extracts against Eight Different Microorganisms by Agar Well Diffusion Method

Organisms	Inhibition zone diameter (mm)				
	PE	EtOAc	EtOH	H_2O	STD
B. subtilis	13	26	27	13	27
S. aureus	12	25	17	12	28
P. fluorescens	13	28	28	13	28
B. pumilus	13	26	27	13	27
C. albicans	13	25	25	13	27
E. coli	12	26	27	12	27
A. tumefaciens	13	26	26	13	27
M. luteus	13	26	27	14	28

(-) = no activity
10–14 mm = low activity
15–19 mm = medium activity
20 mm and above = high activity

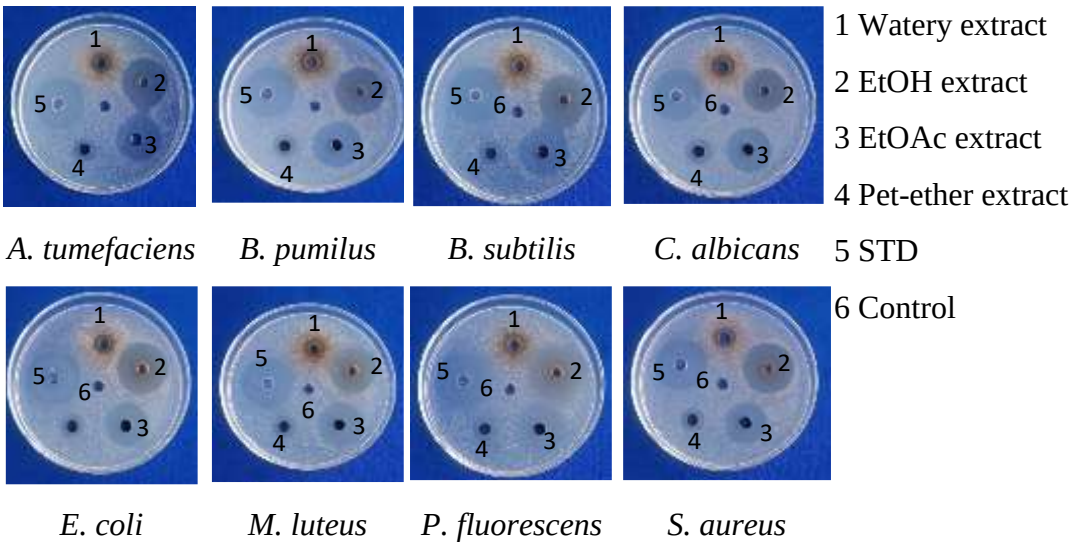

Figure 4. Photographs showing the antimicrobial activity of crude extracts of *S. suberectus* stem

Cytotoxicity of Crude Extracts of *S. suberectus* Stem

The cytotoxicity effects of crude extracts of *S. suberectus* stem were determined by brine shrimp lethality bioassay. The percent mortality of brine shrimp in different concentrations and the LC_{50} values of crude extracts and those of standards, potassium dichromate and caffeine, are shown in Table 3. According to experiments, both crude extracts were not toxic at 1000 µg/mL for 24 h. For positive control, the standard potassium dichromate was 5.50 µg/mL, and the negative control as standard caffeine was found to be greater than 1000 µg/mL. It was concluded that both extracts did not exhibit cytotoxicity effects in lethal concentrations of 1000 µg/mL.

Table 3. Cytotoxicity of Different Concentrations of Crude Extracts of *S. suberectus* Stem against *Artemia salina* (Brine Shrimp)

Tested Samples	Dead % by using Different Concentrations (µg/mL) of Sample (Mean ± SEM)				LC_{50} (µg/mL)
	1	10	100	1000	
Ethanol extract	13.33 ± 5.77	23.33 ± 5.77	30.00 ± 0.00	43.33 ± 5.77	> 1000
Watery extract	6.67 ± 5.77	16.67 ± 11.54	36.67 ± 5.77	43.33 ± 5.77	> 1000
Std. $K_2Cr_2O_7$	46.67 ± 5.77	53.33 ± 11.55	86.67 ± 5.77	96.67 ± 5.77	5.50
Std. Caffeine	0.00 ± 0.00	13.33 ± 0.58	23.33 ± 0.58	33.33 ± 0.58	> 1000

Anti-arthritic Activity of Ethanol and Watery Extracts of *S. suberectus* Stem by Using Egg albumin

In vitro anti-arthritic activity, ethanol and watery extracts with different concentrations provide protection against denaturation of proteins. The IC_{50} values of inhibition of protein denaturation of ethanol and watery extracts of *S. suberectus* stem were found to be 35.92 µg/mL and 108.89 µg/mL. Based on the resulting data, the IC_{50} value of ethanol extract showed more significant anti-arthritic activity than that of the watery extract. The results are summarized in Table 4 and Figures 5 and 6.

Table 4 Average % Protein Denaturation and IC_{50} Values of Crude Extracts of *S. suberectus* Stem

Tested Samples	% Protein Denaturation ± SD of different concentrations (µg/mL)						IC_{50} (µg/mL)
	15.625	31.25	62.5	125	250	500	
Ethanol extract	26.83 ± 0.87	48.51 ± 1.01	58.49 ± 1.47	63.19 ± 0.19	64.99 ± 0.51	71.87 ± 1.02	35.92
Watery extract	22.89 ± 0.18	34.63 ± 0.53	46.54 ± 1.07	51.19 ± 0.33	59.87 ± 0.18	64.78 ± 0.27	108.89
Std. Diclofenac	43.52 ± 0.00	50.43 ± 0.30	64.77 ± 0.90	65.28 ± 0.90	66.11 ± 0.30	67.53 ± 1.08	30.27

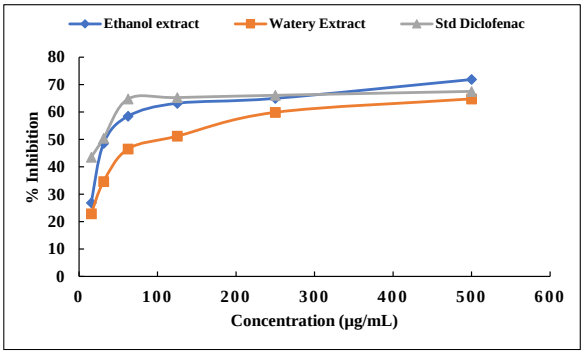

Figure 5. A plot of % protein denaturation of crude extracts of *S. suberectus* stem

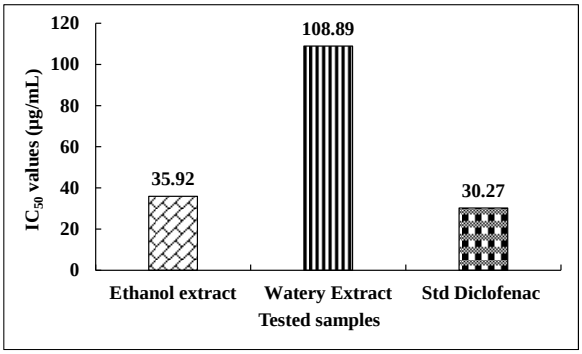

Figure 6. IC_{50} values of crude extracts of *S. suberectus* stem

Anti-diabetic Activity of Crude Extracts of *S. suberectus* Stem

The anti-diabetic effect of ethanol and watery extracts of *S. suberectus* stem was screened *in vitro* as an alpha-amylase assay. According to the results, the IC_{50} value of *S. suberectus* stem was found to be 23.97 µg/mL for the EtOH extract, indicating the more active inhibition, whereas 81.64 µg/mL for the watery extract on the α-amylase enzyme. The smaller IC_{50} value indicates the highest inhibition. The IC_{50} values of extracts compared with the standard acarbose are shown in Table 5 and Figures 7 and 8.

Table 5. Average % Inhibition and IC_{50} Values of Crude Extracts of *S. suberectus* Stem

Tested Samples	% Inhibition in different concentration (µg/mL)							IC_{50} (µg/mL)
	1.95	3.91	7.81	15.62	31.25	62.5	125	
Ethanol extract	23.25 ± 0.59	27.36 ± 1.56	32.17 ± 1.39	38.62 ± 1.17	59.90 ± 1.00	64.91 ± 0.00	69.44 ± 0.41	23.97
Watery extract	19.72 ± 0.99	21.01 ± 0.73	26.62 ± 1.49	30.75 ± 1.27	40.95 ± 0.41	46.76 ± 0.86	57.33 ± 0.14	81.64
Std. Acarbose	42.99 ± 0.19	50.00 ± 0.25	59.58 ± 0.66	63.91 ± 0.20	66.51 ± 0.37	68.40 ± 0.21	69.78 ± 0.39	3.91

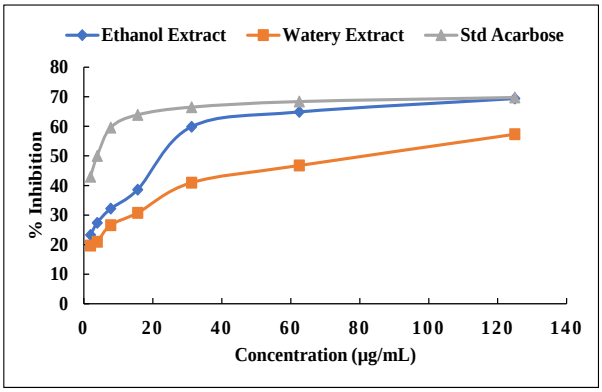

Figure 7. Mean % inhibition versus concentration of crude extracts of *S. suberectus* stem

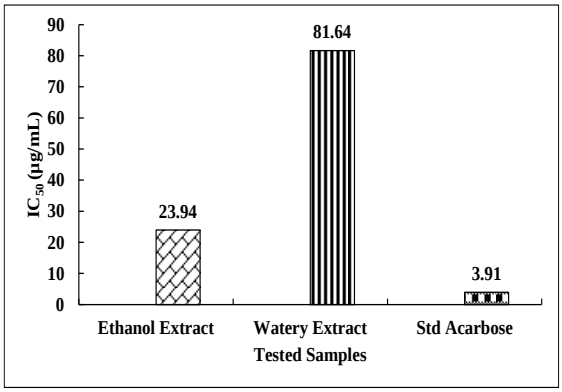

Figure 8. IC_{50} values of crude extracts of *S. suberectus* stem

Conclusion

From the overall research work concerning the evaluation of some biological activities present in the stem of *Spatholobus suberectus* (Kyatthway-pannwe-pin), the following inferences can be concluded. The antioxidant activity screening of ethanol and watery extracts of the stem of *S. suberectus* was evaluated using the DPPH assay. The IC_{50} values of ethanol and watery extracts and standard ascorbic acid were found to be 2.01 μg/mL, 2.66 μg/mL, and 1.99 μg/mL, indicating the stem of *S. suberectus* stem possesses antioxidant activity. According to the antimicrobial activity of various crude extracts, the ethanol and ethyl acetate extracts inhibited all species of tested microorganisms with inhibition zone diameters in the range between 17–28 mm. However, watery and pet-ether extracts showed an 12–14 mm inhibition zone, indicating mild antimicrobial activity. The cytotoxicity of ethanol and watery extracts determined by brine shrimp lethality bioassay were observed to be free from toxic effects up to 1000 μg/mL. In terms of anti-arthritic activity, the ethanol extract (IC_{50} = 35.92 μg/mL) was more effective than the watery extract (IC_{50} = 108.89 μg/mL). From the investigation of anti-diabetic activity of crude extracts, the IC_{50} value of the ethanol extract (23.97 μg/mL) was observed to be higher than those of the watery extract (81.64 μg/mL) of *S. suberectus* stem. These results will help to promote the development of Myanmar traditional medicine, particularly in the areas related to microbial infection, free radicals, arthritic, and diabetes mellitus.

Acknowledgements

The authors wish to extend their heartfelt thanks to the Ministry of Education and the Department of Higher Education (Yangon) for facilitating this research opportunity. And also appreciate the Myanmar Academy of Arts and Science for granting the chance to present this paper.

References

Brand-Williams, W., M. E. Cuvelier and C. Berset. (1995). "Use of a Free Radical Method to Evaluate Antioxidant Activity". *LWT- Food Science and Technology*, vol. **28** (1), pp. 25–30.

Dockery, M. and S. Tomkins. (2000). *Brine Shrimp Ecology*. London**:** 1st Edn., The British Ecology Society, pp.92–93.

Feng, Z., K. Ganesan, Q. Liu and J. Chen. (2022). "A Review of the Pharmacological Potential of *Spatholobus suberectus* Dunn on Cancer". *Cells*, vol. **11** (18), pp. 2885–2890.

Ganapaty, S., R. Nandeesh, V. P. Veerapur, B. S. Thippeswamy and D. Shivasharan. (2013). "*In vivo* and *In vitro* Anti-Diabetic Effects of Madhuca indica Roxb., in Alloxan-Induced Diabetic rats". *International Journal of Advances in Pharmacy, Biology and Chemistry*, vol. **2** (2), pp. 2277–2283.

Hieu, N. N., T. Vuvan and N. Huu. (2022). "Ethnopharmacology, Phytochemistry, and Pharmacology Activities of *Spatholobus suberectus* Vine Stem". *Natural Product Communication,* vol. **3**, pp. 28–37.

Lee, E. H., B. C. Chan and M. A. Noh. (2005). "Antioxidant Activity of *Spatholobus suberectus* Dunn". *Korean Journal of Pharmacognosy*, vol. **36** (1), pp. 50–55.

Liu, R., Y. Xu and Z. Zhan. (2018). "A New Flavanone from *Spatholobus suberectus* Dunn". *Journal of Chemical Research*, vol. **42**, pp. 529–530.

Noureddine, C and L. Zidane. (2024). "Plant-Derived Natural Products: A Source for Drug Discovery and Development". *Drugs Drug Candidates*, vol. **3** (1), pp.184–207.

Yehau, P., X. Lou and P. Gong. (2023). "*Spatholobi* caulis: A Systematic Review of its Traditional Uses, Chemical Constituents, Biological Activities and Clinical Applications". *J. of Ethnopharmacol*, vol. **10,** pp**.**10–16.