INVESTIGATION OF SOME BIOACTIVITIES AND PHYTOCONSTITUENTS FROM THE PEELS OF *LITCHI CHINENSIS* SONN. (LYCHEE)

Kay Khine Win Swe[1], Prema[2], Ni Ni Than[3]

Abstract

The aim of the present work is to investigate the phytoconstituents, the total phenolic contents, antioxidant activity, cytotoxicity, antiarthritic activity, and anti-diabetic activity of *Litchi chinensis* Sonn. (Lychee) peels. Preliminary phytochemical screening by test tube methods revealed that alkaloids, *α*-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids and flavonoids are present and cyanogenic glycosides is absent in the sample. The total phenolic content of ethanol and watery extracts from Lychee peels was determined by the Folin-Ciocalteu Reagent (FCR) method. Total phenolic content was found to be the highest in ethanol extract (114.86 ± 0.14) μg/mL. *In vitro* antioxidant activity of ethanol and watery extracts of Lychee peels was assessed by the DPPH free radical scavenging assay. IC_{50} values were found to be 86.21 μg/mL for the watery extract and 31.03 μg/mL for the ethanol extract of Lychee peels. The cytotoxicity of the watery and ethanol extracts was evaluated by a brine shrimp lethality bioassay. From these results, the LD_{50} values of watery and ethanol extracts were found to be non-toxic at the 1000 μg/mL concentration. *In vitro* antiarthritic activity of watery and ethanol extracts was screened by the egg albumin method. According to the data, ethanol extract (IC_{50} = 60.56 μg/mL) has more potent antiarthritic activity than the watery extract (IC_{50} = 86.39 μg/mL). *In vitro α*-amylase inhibitory effect was determined by the starch-iodine method. The IC_{50} values were found to be 6.66 μg/mL for the watery extract and 5.83 μg/mL for the EtOH extract of the lychee peels.

Keywords: *Litchi chinensis* Sonn., phytochemicals, total phenolic contents, antioxidant activity, cytotoxicity, antiarthritic activity, antidiabetic activity

Introduction

Over 60 % of the world's population, including about 80 % in developing nations, still rely exclusively on medicinal plants for their health requirements, making traditional medicine the preferred main healthcare system in many communities. This is because of a variety of factors, including cost effectiveness, accessibility, and affordability. There is a long history of using plants to treat many human ailments. Different plant parts, including leaf, stem, bark, root, and others, are utilized to prevent, alleviate symptoms, or restore abnormalities to normal. Approximately 80 % of the active chemicals used in modern medicine today, which include active substances separated from higher plants, show a positive association between their current therapeutic uses and their historic usage (Shehri *et al*., 2022). One of the significant fruit plants, lychee, contains a number of bioactive chemicals with pharmacological effects. The lychee (*Litchi chinensis*), a fruit belonging to the Sapindaceae family, originated in China and is now a common sight across the tropical and subtropical parts of the world. In the subtropics, one of the best fruit trees is the lychee. It comes with numerous health advantages, including anticancer,

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

hepatoprotective, antioxidant, antiplatelet, antiviral, antimutagenic, antimicrobial, antihyperlipidemic, antipyretic, and anti-inflammatory effects (Deshwal *et al*., 2022).

Materials and Methods

Plant materials

Lychee fruits were collected from July to August, 2021 in Myitkyina township, Kachin State, Myanmar. The collected sample was identified as *Litchi chinensis* Sonn. (Lychee) fruits at the Botany Department, University of Yangon. The sample was cleaned by washing thoroughly with water and peeled off. Then the fresh peel was cut into small pieces and air-dried at room temperature. The dried sample was ground into powder by a grinding machine, sieved and stored in an airtight container for further use.

Phytochemical Investigation of Lychee Peels

Phytochemical tests of lychee peels were carried out according to the standard methods (Geetha *et al*., 2014 and Harbone, 1984) which were investigate the presence or 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 watery 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.

Determination of Total Phenolic Contents by Folin-Ciocalteu Method

The total phenolic content was determined by the Folin-Ciocalteu (FC) method. 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 reaction mixture was read at λ_{max} 765 nm. Total phenolic content as μg GAE/mg of crude extract was estimated from the gallic acid standard calibration curve (Hishamuddin *et al*., 2020).

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

DPPH free radical scavenging activity was determined by a spectrophotometric method. The control solution was prepared by mixing 1.5 mL of DPPH solution and 1.5 mL of ethanol in the brown bottles. The sample solution was also prepared by mixing thoroughly 1.5 mL of DPPH solution and 1.5 mL of different concentrations of each test sample solution (3.91, 7.81, 15.63, 31.25, 62.50, and 125.00 μg/mL). These bottles were incubated at room temperature and were

shaken on the shaker for 30 min. After 30 min, the absorbance of each solution was measured at 517 nm by spectrophotometer (Hishamuddin *et al*., 2020). Absorbance measurements were done in triplicate for each solution.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

The brine shrimp (*Artemia salina*) was used in this study for the cytotoxicity bioassay. Artificial sea water [3.8 % (w/v) NaCl] was prepared by dissolving (38 g) of sodium chloride in 1 L of distilled water. Brine shrimp cysts (0.5 g) were put into 1 L of artificial sea water in a bottle. This bottle was placed near a lamp. Light is essential for the cysts to hatch. Brine shrimp cysts required to hatch constant supplied oxygen and 24 h incubation at room temperature. 9 mL of artificial seawater and 1 mL of different concentrations of each sample (1000, 100, 10, 1 µg/ mL) and each standard solution (caffeine and $K_2Cr_2O_7$) were added to each chamber. Alive brine shrimp (10 nauplii) were then taken with a pasteur pipette and placed into each chamber. They were incubated at room temperature for about 24 h. After 24 h, the number of dead or survival brine shrimps were counted and the estimation of cytotoxicity was done by 50 % lethality dose (LD_{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 Antiarthritic Activity by Egg Albumin Method

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin from fresh hen's egg, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of different (100, 200, 400, 800, and 1000 µg/mL) concentrations of each crude extract of lychee. Similar volume of double-distilled water served as control. 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 values were measured at 660 nm. Diclofenac sodium was used as a reference drug (Sunmathi *et al*., 2016).

Determination of Antidiabetic Activity by *α*-Amylase Inhibition Assay

α-Amylase inhibitory activity was determined by starch-iodine method. 10 µL of *α* amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentrations (1.96, 3.91, 7.81, 15.63, 31.25, 62.50, and 125.00 µg/mL) of each extract. After incubation at 37 °C for 10 min, 10 µL of starch solution (1 %) was added, and the mixture was reincubated for 15 min. Next, 0.1 mL of 1 % iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm (Ganapaty *et al*., 2013).

Results and Discussion

Phytochemical Investigation of Lychee Peels

The preliminary phytochemical investigation was carried out to know the different types of phytoconstituents present in the sample. The investigation results revealed that the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids but cyanogenic glycoside was found to be absent (Table 1). The lychee peels can be employed as antioxidant, antidiabetic, and

anti-inflammatory drugs due to the presence of phenolic compounds, steroids, tannins, terpenoids, and flavonoids components.

Table 1. Results of Preliminary Phytochemical Investigation of the Lychee Peels

No.	Test	Extracts	Test reagents	Observation	Results
1	alkaloids	1 % HCl	(i) Dragendorff's reagent	orange ppt	+
			(ii)Mayer's regent	white ppt	+
			(iii)sodium picrate	yellow ppt	+
			(iiii)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 colour	+
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

Total Phenolic Content of Crude extracts of Lychee Peels

In the present investigation, the total phenolic contents (TPC) of watery and ethanol extracts were estimated by the Folin-Ciocalteu method. Gallic acid (3,4,5-trihydroxybenzoic acid) was used to construct a standard calibration curve for total phenol estimation. TPC was expressed as microgram of gallic acid equivalent (GAE) per milligram of crude extract (μg GAE/mg).

According to the results, the TPC of ethanol extract (114.86 ± 0.14) μg GAE/mg was found to be higher than that of watery extract (71.66 ± 0.00) μg GAE/mg (Figure 1).

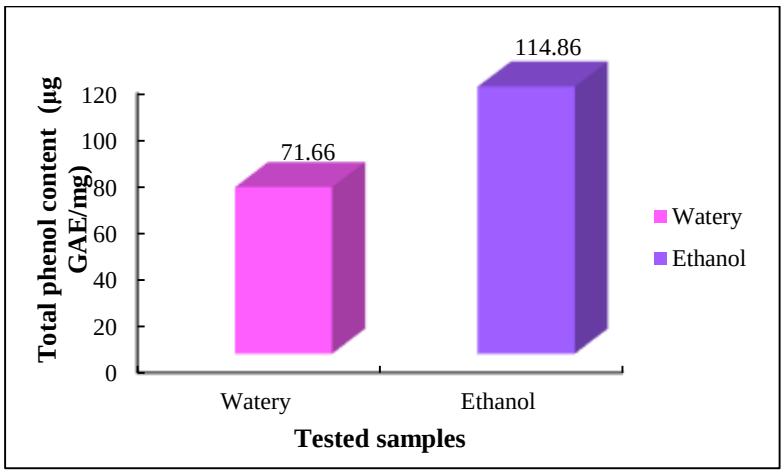

Figure 1. Histogram of total phenolic content of ethanol and watery extracts of lychee peels

Antioxidant Activity of Crude Extracts of Lychee Peels by DPPH Free Radical Scavenging Assay

Lychee peels are a good source of phenolic compounds as their potential antioxidant and antioxidant activity depend on the total phenolic contents. The antioxidant activity of ethanol and watery extracts of lychee peels was studied by the DPPH free radical scavenging assay. IC_{50} values were observed to be 131.48 µg/mL for watery extract and 60.99 µg/mL for ethanol extract. The largest scavenging activity to scavenge the DPPH radical was observed in ethanol extract, which inhibited 50 % of free radicals at the concentration (IC_{50}) of 60.99 µg/mL. Because of the higher phenolic content, the ethanol extract is more effective than watery extract. The results are shown in Table 2 and Figures 2 and 3. The ethanol extract possessed the antioxidant activity less than the standard ascorbic acid.

Table 2. Average % Inhibition and IC_{50} Values of Crude Extracts of Lychee Peels

Tested samples	% RSA ± SD of different concentrations (µg/mL)							IC_{50} (µg/mL)
	3.91	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	
watery extract	8.90 ±0.27	13.29 ±0.00	26.58 ±0.33	35.55 ±0.44	40.85 ±0.65	48.66 ±0.55	74.47 ±0.03	131.48
ethanol extract	17.65 ±0.27	19.46 ±0.41	20.81 ±0.41	21.46 ±0.60	51.46 ±0.04	74.98 ±0.00	83.99 ±0.24	60.99
Std. ascorbic acid	46.15 ±0.03	75.81 ±0.28	79.12 ±0.16	85.93 ±0.32	87.37 ±0.37	87.62 ±0.00	87.75 ±0.07	4.41

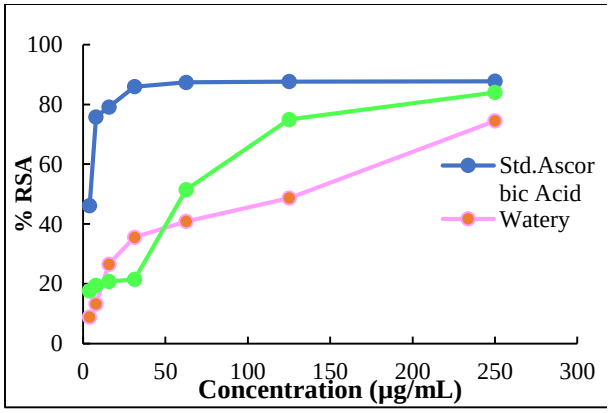

Figure 2. % RSA versus concentration of watery and ethanol extracts of lychee peels

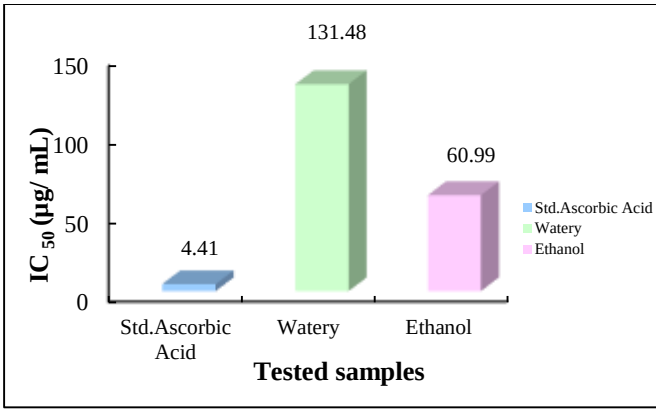

Figure 3. IC_{50} values of standard ascorbic acid and watery and ethanol extracts of lychee peels

Cytotoxicity of Crude Extracts of Lychee Peels

The cytotoxicity of watery and ethanol extracts of lychee peels were evaluated by the brine shrimp lethality bioassay. The organisms used were brine shrimp, *Artemia salina*. Potassium dichromate and caffeine were used as the positive and negative controls. The cytotoxicity of watery and ethanol extracts of selected plant is shown in Table 3. From the results, LD_{50} values of watery and ethanol extracts were found to be greater than 1000 µg/mL. LD_{50} values of crude extracts, less than 1000 µg/mL was toxic (active) and greater than 1000 µg/mL was non-toxic (inactive). Therefore, watery and ethanol extracts have no cytotoxic effect.

Table 3. Cytotoxicity of Different Concentrations of Crude Extracts of Lychee Peels against *Artemia salina* (Brine Shrimp)

Tested samples	D of dead brine shrimp Presents in different concentrations of sample (µg/mL)				LD_{50} (µg/mL)
	1	10	100	1000	
watery extract	23.33±0.33	30.00±0.00	33.33±0.58	36.67±0.00	> 1000
ethanol extract	16.67±0.00	26.67±0.33	36.67±0.00	43.33±0.00	> 1000
*Std. $K_2Cr_2O_7$	43.33±0.58	46.67±0.58	76.67±0.58	100±0.00	19.99
**Std. Caffeine	0.00±0.00	13.33±0.58	23.33±0.58	33.33±0.58	>1000

*Std. $K_2Cr_2O_7$ = Positive control
**Std. Caffeine = Negative control

Antiarthritic Activity of Crude Extracts of Lychee Peels

One of the well-documented causes of inflammatory and arthritic diseases is denaturation of tissue proteins. The effect of watery extract (58.81 µg/mL) and ethanol extract (31.31 µg/mL) of lychee peels was evaluated against denaturation of egg albumin (Table 4). According to the results, the IC_{50} value of the ethanol extract of lychee peels is lower than those of watery and standard diclofenac sodium. Since, the lower the IC_{50} value, the higher antiarthritic activity, the ethanol extract of lychee peels has more antiarthritic potency than standard and watery extract (Figures 4 and 5). So, it can be concluded that ethanol extract of lychee peels can serve as an antiarthritic agent.

Table 4. Average % Protein Denaturation and IC_{50} Values of Crude Extracts of Lychee Peels

Tested samples	% Protein denaturation of different concentrations (µg/mL)						IC_{50} (µg/mL)
	31.25	62.5	125	250	500	1000	
watery extract	38.88 ± 0.27	51.49 ±0.00	62.59 ±0.33	77.03 ±0.00	77.90 ±0.11	82.83 ±0.04	58.81
ethanol extract	49.99 ±0.06	56.31 ±0.09	56.34 ±0.06	61.25 ±0.02	63.30 ±0.07	79.09 ±0.00	31.31
*Std. diclofenac sodium	34.87 ±0.03	52.84 ±0.34	54.65 ±0.10	60.57 ±0.03	65.20 ±0.07	78.83 ±0.00	57.57

*Std. diclofenac sodium = positive control

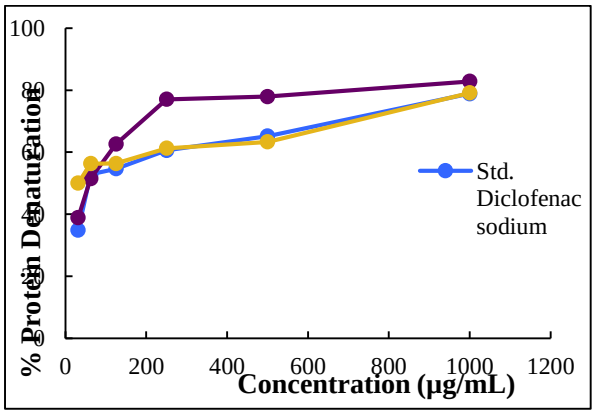

Figure 4. % Protein denaturation versus concentration of watery and ethanol extracts of lychee peels

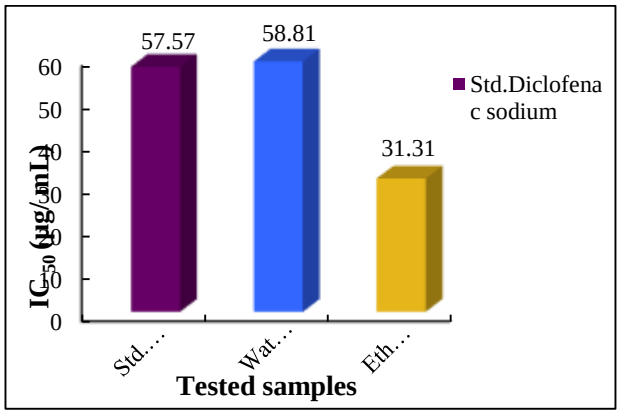

Figure 5. IC_{50} values of watery and ethanol extracts of lychee peels

Antidiabetic Activity of Crude Extracts of Lychee Peels

The antidiabetic effect of ethanol and water extracts of lychee peels was studied by the *α*-amylase inhibitory assay. Acarbose was used as a standard. According to the results, the IC_{50} values were found to be 6.66 µg/mL for the watery extract and 5.83 µg/mL for the EtOH extract of the lychee peels. From this observation, the EtOH extract was more potent than the watery extract in antidiabetic activity, comparable with standard acarbose (IC_{50} = 5.22 µg/mL). The results are shown in Table 5 and Figures 6 and 7

Table 5. Average % Inhibition and IC_{50} Values of Crude Extracts of Lychee Peels

Tested samples	% RSA ± SD of different concentrations (µg/mL)							IC_{50} (µg/mL)
	1.96	3.91	7.81	15.63	31.25	62.5	125	
watery	41.28 ±0.08	41.66 ± 0.04	53.49 ±0.00	58.99 ±0.00	64.31 ±0.04	67.56 ±0.09	75.49 ±0.21	6.66
ethanol	39.83 ±0.04	41.32 ±0.06	58.99 ±0.17	72.21 ±0.42	79.69 ±0.20	81.22 ±0.03	85.98 ±0.21	5.83
*Std. acarbose	45.48 ±0.00	47.49 ±0.03	54.99 ±0.58	62.03 ±0.00	64.40 ±0.09	71.29 ±0.10	74.01 ±0.00	5.22

*Std. Acarbose = positive control

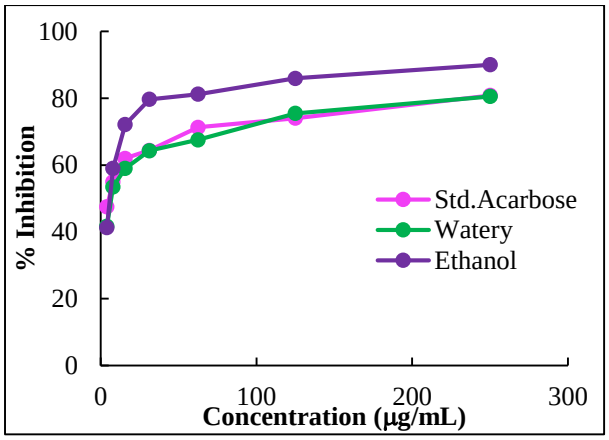

Figure 6. Mean % inhibition versus concentration of watery and ethanol extracts lychee peels

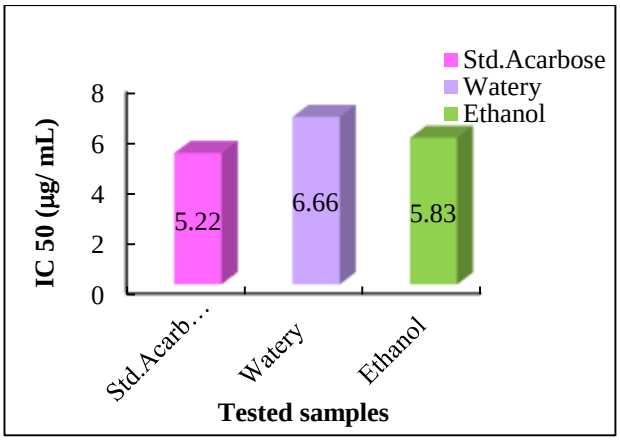

Figure 7. IC_{50} values of crude extracts of crude of lychee peels

Conclusion

The present research work deals with the first report for the investigation of phytoconstituents, total phenol contents, antioxidant, cytotoxicity, and antiarthritic and antidiabetic activities of *Litchi chinensis* Sonn. (Lychee) peels. From the preliminary phytochemical test, the secondary metabolites are present in the lychee samples. Based on the data, EtOH extract (114.86 ± 0.14 μg GAE/mg) was found to possess a higher total phenol content than watery extract (71.66 ± 0.00 μg GAE/mg). The antioxidant activity of the ethanol extract (IC_{50} = 60.99 μg/mL) was more effective than that of the watery extract. According to the cytotoxic effect, ethanol and watery extracts from lychee peels were observed to be free from toxic effect up to 1000 μg/mL dose. In antiarthritic activity, ethanol extract (IC_{50} = 31.31 μg/mL) was more effective than the watery extract and the standard diclofenac sodium (IC_{50} = 57.57 μg/mL). In antidiabetic activity, watery (IC_{50} = 5.83 μg/mL) and ethanol (IC_{50} = 6.66 μg/mL) extracts effectively inhibited *α*-amylase. As the results, this sample could be useful in the treatment of anti-aging, antiarthritic, and diabetes.

Acknowledgements

The authors would like to express their profound gratitude to the Ministry of Education, the Department of Higher Education, for providing the opportunity to do this research, and the Myanmar Academy of Arts and Science for allowing them to present this paper.

References

Deshwal, V. K., K. Vig, S. B. Singh, and P. D. Devi (2012). Evaluation of the Antibacterial Activity of Bark of *Litchi chinensis* against *Escherichia Coli*, A UTI Causing Organism". *Journal of Plant Development Sciences*, vol. **4**, pp. 101–103

Dipak, G., P. Axay, C. Manodeep, and K. Jagdish. (2012). "Phytochemical and Pharmacological Profile of *Punica granatum*: An Overview". *International Research Journal of Pharmacy*, vol. **3**(2), pp. 65–68

Ganapaty, S., R. Nandeesh, V. P. Veerapur, B. S. Thippeswaamhy, 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. 282–290

Geetha, T. S and N. Geetha. (2014). "Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) Stapf, Leaves from Kodaikanal Hills, Tamilnadu". *International Journal of PharmTech Research*, vol. **6**(2), pp. 521–529

Harbone, J. B. (1984). "*Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*", New York: 2nd Ed., Chapman and Hall, 120–126

Hishamuddin, S. N. A., Q. Q. Amir, A. H. Mohamed, and N. R. Said. (2020). "Extraction of Bioactive Compounds from Longan Peel by Using Solvent Extraction Method and its Antioxidant Activity". *ASM Science journal*, vol. **13**(6), pp. 60–65

Shehri, S. H. A., R. A. Alhadlaq, K. I. BinMuhanna, N. S. Aldosri, and M. A. Alghamdi. (2022). "A Review of Medicinal Plants, their Definition, Uses, Active Ingredients and Prevalence in the Kingdom of Saudi Arabia". *International Journal of Science and Research*, vol. **11**(12), pp. 1183–1188

Singh, M. P., J. Singh, and R. Rajesh. (2015). "Brine Shrimp Cytotoxic Assay for Polyherbal Formulation of *Moringa oleifera, Viola odorata, Allium sativum*". *International Journal of Innovative Pharmaceutical Sciences and Research*, vol. **3**(12), pp. 1639–1643

Sunmathi, D., R. Sivakumar, and K. Ravikumar. (2016). "*In Vitro* Anti-inflammatory and Antiarthritic Activity of Ethanolic Leaf Extract of *Alternanthera sessilis* (L.) R.BR. ex DC and *Alternanthera philoxeroides* (Mart.) Griseb". *International Journal of Advances in Pharmacy, Biology and Chemistry*, vol. **5**(2), pp. 109–115