INVESTIGATION OF SOME BIOACTIVITIES AND PHYTOCONSTITUENTS FROM THE PEELS OF *DIMOCARPUS LONGAN* LOUR. (LONGAN)

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Abstract

The aim of the present work is to investigate the total phenolic contents, antioxidant, cytotoxic, and anti-arthritic activities, and phytoconstituents of *Dimocarpus longan* Lour. (Longan) peel (LGP). The total phenolic content of ethanol and watery extracts from LGP was determined by the Folin-Ciocalteu Reagent (FCR) method. Total phenolic content was found to be the highest in ethanol extract (176.12 ± 0.15) μg/mL. The *in vitro* antioxidant activity of ethanol and watery extracts of LGP was assessed by the DPPH free radical scavenging assay. The IC_{50} values were found to be 70.52 μg/mL for the watery extract and 8.77 μg/mL for the ethanol extract of LGP. 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. The anti-arthritic activity of watery and ethanol extracts screened by the egg albumin method. According to the data, the watery extract (IC_{50} = 97.77 μg/mL) is more potent than standard diclofenac sodium (IC_{50} = 57.57 μg/mL) in anti-arthritic activity. By silica gel column chromatographic separation technique, two steroids, one terpenoid and a steroidal carboxylic acid were isolated from petroleum ether extract of LGP and characterized by physico-chemical properties and the FT IR spectroscopic method.

Keywords: *Dimocarpus longan* Lour., antioxidant activity, cytotoxicity, anti-arthritic activity

Introduction

Medicinal plants play a crucial role in the development of potent therapeutic agents. Natural products from plants, animals and minerals are the basis of the treatment of human diseases. Today, about 80 % of individuals in developing countries still rely on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. Currently, 80 % of the world population depends on plant-derived medicine for the primary line of primary health care and human alleviation because it has no side effects (Dipak et al., 2012). Longan (Dimocarpus longan lour.), commonly known as dragon's eye is an important evergreen fruit crop grown in tropical and subtropical regions. It belongs to the sapindaceae family and widely distributed in Myanmar, China, Vietnam, Thailand, India, Philippines, and other Southeast Asian countries. The main world longan-producing countries are China, Vietnam, and Thailand. This fruit has been used as traditional medicine in other countries. It is rich in carbohydrates, protein, fiber, fat, vitamin C, amino acids, and minerals. Different parts can be used to treat or prevent different types of diseases. It is a good source of polyphenolic compounds. Its active compounds possess anti-arthritic, antioxidant, anti-hyperglycemic, anticancer, antidote, and other activities (Shahrajabian et al., 2019). Therefore, the present work aimed to investigate some bioactivities and phytoconstituents from the peels of Dimocarpus longan Lour. (Longan).

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Materials and Methods

Plant materials

Longan fruits were collected from July to August, 2021 in Sagaing Township, Sagaing Region, Myanmar. The collected sample was identified as Dimocarpus longan Lour. (Longan) 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 samples were ground into powder by a grinding machine, sieved, and stored in an airtight container for further use.

Preparation of crude extracts for biological activities

About 20 g of dried powder sample was extracted three times with 95 % ethanol (each time with 100 mL). Extraction time was allowed for six hours and then filtered. Then, the solvent was removed by the rotary evaporator, providing the ethanol extract. To obtain watery extract, 20 g of dried powder samples was separately soaked in 100 mL of distilled water. It was then concentrated by evaporating the solvent on a water bath to get a watery extract. In this way, crude extracts were obtained. The crude extracts were dried and kept in a refrigerator for a few weeks.

Determination of total phenol contents (TPC) as gallic acid equivalent

The total phenolic content was determined by the Folin-Ciocalteu method. Each extracted sample solution (0.5 mL) was added 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. The blank solution was prepared as in the above procedure by using distilled water instead of the sample solution. Total phenolic content was estimated as micrograms of gallic acid equivalent per milligram (μg GAE/mg) of crude extract (Hishamuddin *et al*., 2020).

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

Preparation solutions

DPPH (2 mg) was thoroughly dissolved in 100 mL of 95 % ethanol. This solution was freshly prepared in the brown-coloured reagent bottle and stored in the fridge for no longer than 24 h. Each of the tested samples (ethanol and watery extracts) (10 mg) was thoroughly mixed with 10 mL of ethanol. The stock solution was obtained. This stock solution was twofold serially diluted with ethanol to obtain the desired concentrations: 3.91, 7.81, 15.63, 31.25, 62.5, and 125 μg/mL.

Determination of antioxidant activity

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 with 1.5 mL of the test sample solution. 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 using a spectrophotometer (Hishamuddin *et al*., 2020). Absorbance measurements were done in triplicate for each solution, and the percent inhibition of oxidation was calculated by the following equation.

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

where

% RSA = % radical scavenging activity
$A_{Control}$ = Absorbance of control solution
A_{Sample} = Absorbance of sample solution

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

Preparation of solutions

Accurately weighed 5 mg of each sample (ethanol extract, watery extract, potassium dichromate and caffeine) was separately dissolved in 5 mL of distilled water to obtain a respective stock solution (1000 µg/mL). Desired concentrations (1000, 100, 10, 1 µg/mL) of each solution were prepared from this stock solution by tenfold serial dilution with distilled water.

Procedure

The brine shrimp (*Artemia salina*) were used in this study for a 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 samples and standard solutions 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 Anti-arthritic 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 varying concentrations of crude extracts of Longan so that final concentrations became 100, 200, 400, 800 and 1000 µg/ mL. 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 was measured at 660 nm. Diclofenac sodium was used as a reference drug (Sunmathi *et al*., 2016). The percentage inhibition of protein denaturation was calculated by using the following formula:

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

where

$A_{Control}$ = Absorbance of control solution
A_{Sample} = Absorbance of sample solution

Extraction and Isolation of Compounds

The air-dried powder sample of LGP (1 kg) was macerated with ethanol (3 × 1 L) at room temperature for about two weeks by sonication. method. The extract was concentrated under reduced pressure at 60 ˚C. The extract was partitioned with PE and EtOAc successively. The PE

fraction (5 g) was separated by a column chromatographic method, eluting successively with PE only, PE: EtOAc (9:1 to 1:5 v/v), EtOAc only, and EtOAc: MeOH (9:1 to 1:1 v/v) as a solvent system. Then, ten fractions were obtained. The collected fractions were monitored by TLC, and similar fractions were combined into 10 fractions: F-1 (f_{1-19}), F-2 (f_{20-125}), F-3 ($f_{126-216}$), F-4 ($f_{217-250}$), F-5 ($f_{251-324}$), F-6 ($f_{325-337}$), F-7 ($f_{-338-356}$), F-8 ($f_{357-365}$), F-9 ($f_{366-372}$), and F-10 ($f_{366-372}$) based on TLC analysis. From these fractions, four compounds were isolated. Fractions F-2, F-3, F-4, and F-5 were further chromatographed on silica gel columns by using PE: EtOAc (19:1 to 5:1, v/v) to get compound **A** (30.40 mg), compound **B** (20.32 mg), compound **C (**55.65 mg), and compound **D** (18.76 mg), respectively. The isolated compounds **A**–**D** were characterized by their physico-chemical properties and FT IR spectroscopic method.

Results and Discussion

Total Phenolic Content of Crude extracts of Longan Peels

In the present investigation, the total phenolic contents of crude extracts of Longan peels 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 content estimation. Total phenolic content (TPC) was expressed as microgram of gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/mg) (Table 1 and Figure 1).

According to the results, the total phenolic content (TPC) (µg GAE/mg) of ethanol extract (176.12 ± 0.15) µg GAE/mg was found to be higher than that of watery extract (76.22 ± 0.45) µg GAE/mg (Table 2 and Figure 2).

Table 1. The Absorbance of Standard Gallic Acid Solution at λ_{max} 765 nm

No.	Concentration (µg/mL)	Absorbance at λ_{max} 765 nm
1	3.125	0.603
2	6.25	0.373
3	12.5	0.252
4	25	0.194
5	50	0.164
6	100	0.147

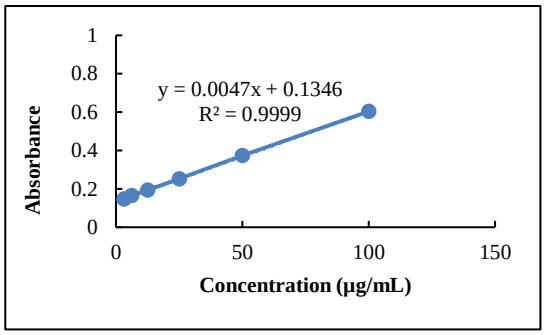

Figure 1. A calibration curve of gallic acid standard curve

Table 2. Total Phenol Contents of Crude Extracts of Longan Peels

Extracts	TPC (µg GAE/mg ±SD)
Watery	76.22 ± 0.45
Ethanol	176.12 ± 0.15

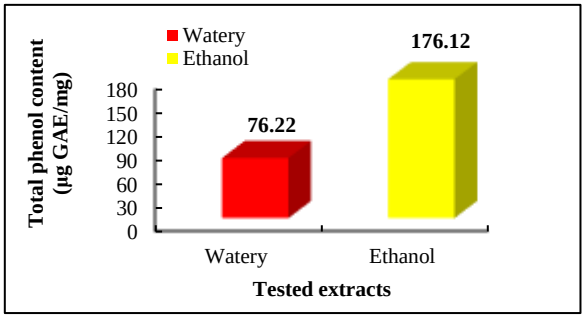

Figure 2. Histogram of total phenolic content of ethanol and watery extracts of longan peels

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

Longan peels are a good source of phenolic compounds, as their potential antioxidants and antioxidants activity depend on the content of phenolic compounds. The antioxidant activity of ethanol and watery extracts of LGP was studied by the DPPH free radical scavenging assay. IC_{50} values were observed to be 70.52 µg/mL for watery extract and 8.77 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 8.77 µg/mL. Because of the phenolic content, the ethanol extract is more effective than watery extract. The results are shown in Table 3 and Figures 3 and 4. The ethanol extract possessed less antioxidant activity than standard ascorbic acid (4.41 µg/mL).

Table 3. Average % RSA Inhibition and IC_{50} Values of Crude Extracts of Longan 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	
Watery extract	11.42 ±0.11	17.26 ±0.19	21.22 ±0.26	26.36 ±0.45	37.03 ±0.31	55.91 ±0.05	70.52
Ethanol extract	20.43 ±0.06	47.41 ±0.21	64.97 ±0.38	80.74 ±0.41	82.06 ±0.51	83.33 ±0.29	8.77
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	4.41

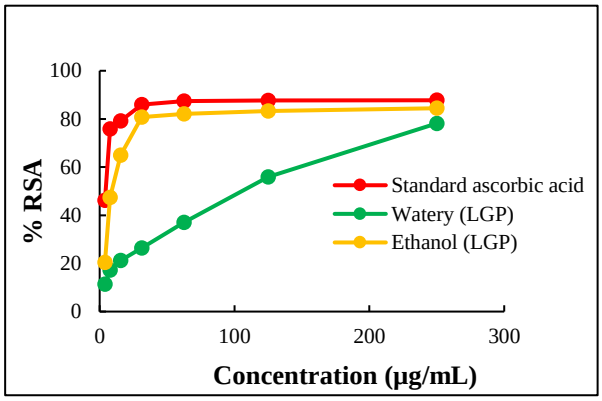

Figure 3. Mean % inhibition versus concentration of different extracts of longan peels

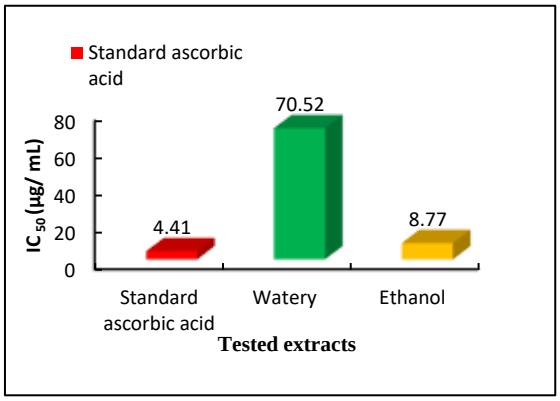

Figure 4. IC_{50} values of standard ascorbic acid and crude extracts of longan peels

Cytotoxicity of Crude Extracts of Longan Peels

The cytotoxicity of watery and ethanol extracts of LGP was evaluated by the brine shrimp lethality bioassay. The organisms used were brine shrimp (*Artemia salina*). The cytotoxicity of watery and ethanol extracts of selected plants is shown in Table 4. From these results, the 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 4. Cytotoxicity of Different Concentrations of Crude Extracts of Longan Peels against *Artemia salina* (Brine Shrimp)

Tested Samples	% of Dead Brine Shrimp in Different Concentrations of Sample (µg/mL)				LD_{50} (µg/mL)
	1	10	100	1000	
Watery extract	20.00±0.00	23.33±0.58	40.00±0.58	40.00±0.00	> 1000
Ethanol extract	13.33±0.00	13.33±0.00	16.67±0.58	46.67±0.58	> 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

Anti-arthritic Activity of Crude Extracts of Longan Peels

One of the well-documented causes of inflammatory and arthritic diseases is denaturation of tissue proteins. The effect of watery and ethanol extracts of LGP was evaluated against denaturation of egg albumin, showed 97.77 µg/mL and 347.41 µg/mL respectively (Table 5). According to the results, the IC_{50} value of the watery extract of LGP is lower than ethanol but higher than standard diclofenac sodium, 57.57 µg/mL. The lower the IC_{50} value, the higher anti-arthritic activity. Therefore, the watery extract of LGP has more anti-arthritic potency than ethanol extract. So, it can be concluded that a watery extract of LGP can serve as an anti-arthritic agent.

Table 5. Average % Protein Denaturation and IC_{50} Values of Crude Extracts of Longan Peels

Tested Samples	% Protein Denaturation ± SD of different concentrations (µg/mL)						IC_{50} (µg/mL)
	31.25	62.5	125	250	500	1000	
Watery extract	34.98 ±0.10	41.29 ±0.09	56.75 ±0.00	61.67 ±0.09	86.09 ±0.02	90.20 ±0.23	97.77
Ethanol extract	27.10 ±0.04	44.62 ±0.21	43.92 ±0.00	47.38 ±0.01	54.14 ±0.00	74.40 ±0.29	347.41
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

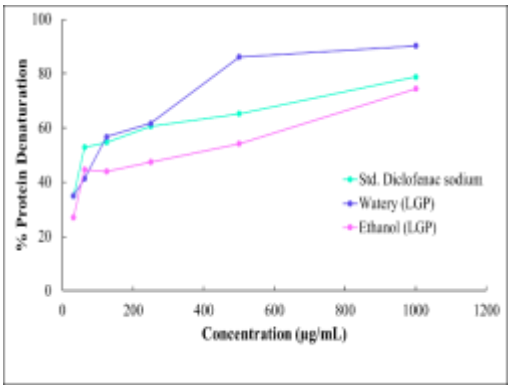

Figure 5. Mean % inhibition versus concentration of different extracts of longan peel

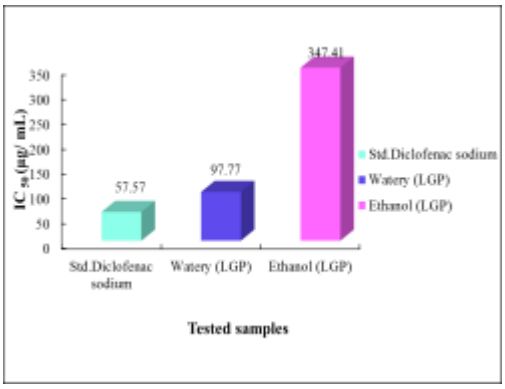

Figure 6. IC_{50} values of crude extracts of longan peels

Some Physico-chemical Properties of the Isolated Compounds

By silica gel column chromatographic separation, four compounds were isolated from the PE extract of LGP. They were characterized by melting point, FT IR, comparison with reported data. Compound **A** was synthesized as a colourless needle crystal PE: EtOAc (19:1 v/v) (R_f = 0.4, Hex: CH_2Cl_2, 4:1 v/v, m.pt 255-260 ˚C, 0.002 % yield). It is UV inactive. It is soluble in PE, $CHCl_3$ and CH_3OCH_3 but insoluble in EtOAc, MeOH, EtOH, and H_2O. In the chemical test, it gave a green colour when treated with Libermann-Burchard solution due to the presence of steroids and a brown spot appeared when tested with iodine vapour because of the C=C present. In the FT IR data, Figure 7(a) indicated the presence of asymmetric and symmetric C-H stretching and bending vibration of sp^3 hydrocarbons at 2925, 2858 cm^{-1} and 1460, 1389 cm^{-1}, respectively. The carbonyl group of C=O stretching vibration was observed at 1714 cm^{-1}. The cyclic ether of C-O stretching bands was displayed at 1190, 1109, and 1073 cm^{-1} and C-H out of plane bending vibration was displayed at 924, 794 cm^{-1}. According to the results, compound **A** may be considered a steroid compound.

Compound **B** (R_f = 0.6, PE: EtOAc (9:1) v/v, m.pt 290 ˚C, 0.001% yield) was obtained as a colourless crystal. It is UV inactive. It is soluble in PE, $CHCl_3$, and CH_3OCH_3 but insoluble in EtOAc, MeOH, EtOH, and H_2O. According to the pink colouration that was observed when treated with acetic anhydride and conc: H_2SO_4. Therefore, compound **B** may be considered a terpenoid. The FT IR spectrum of compound **B** (Figure 6 (b)) showed absorption bands at 3620, 3466 cm^{-1} and 1360, 1256 cm^{-1} due to the presence of alcoholic -OH stretching and bending bands (Silverstein *et al*., 2015), respectively. The bands at 2929, 2872cm^{-1} and 1449, 1385 cm^{-1} were appeared due to the asymmetric and symmetric C-H stretching and bending vibrations of sp^3 hydrocarbons, respectively. The bands at 1174, 1049, and 1020 cm^{-1} could be presented as C-O stretching vibration of alcohol groups, and C-H out of plane bending vibration was observed at 981, 920, 791, and 620 cm^{-1}, respectively. Therefore, compound **B** may be considered a terpenoid.

Compound **C** (R_f = 0.6, PE: EtOAc (5:1) v/v, m.pt 139-140 ˚C, 0.003 % yield) was obtained as a colourless needle crystal. It is soluble in PE, $CHCl_3$ and CH_3OCH_3, EtOAc but insoluble in MeOH, EtOH, and H_2O. Compound **C** visualized green colouration with Libermann-Burchard solution due to the presence of steroid. Moreover, the TLC behaviour of compound **C** was found to be identical with that of *β*-sitosterol in any solvent system. Therefore, compound **C** may be *β*-sitosterol. In the FT IR spectrum of compound **C** Figure 6 (c) due to O-H stretching of the alcoholic group, broadband can be seen at 3473 cm^{-1} and bending vibration at 1367 cm^{-1}. The asymmetrical and symmetrical C-H stretching vibrations of $–CH_2$ and $–CH_3$ groups may be assigned to 2933, 2865 cm^{-1} and bending vibrations at 1464 cm^{-1}. The olefinic group of C=C stretching vibration is at 1687 cm^{-1}. At 1385 cm^{-1}, it appeared due to the CH_3 deformation of the isopropyl group. The cyclic alcohol of C−O stretching vibration occurred at 1023, 1049, 1174 cm^{-1} and the bands of 920, 959 cm^{-1} were designated as the C-H deformation of out of plane bending in benzene. All the results, such as melting point, R_f value, chemical properties, and FT IR spectral data of compound **C** were found to be similar to those of reported *β*-sitosterol (Azeez *et al*., 2018). As a result, the isolated compound C could be assigned as *β*-sitosterol.

Compound **D** (R_f = 0.6, PE: EtOAc, 5:1 v/v, m.pt 130-140 ˚C, 0.001% yield) was obtained as a colourless needle crystal PE: EtOAc (9:1 v/v). It is soluble in PE, $CHCl_3$ CH_3OCH_3 and EtOAc but insoluble in MeOH, EtOH, and H_2O. Compound **D** gave a green colour with Libermann-Burchard solution and a yellow colour with Bromocresol green because it is the steroid compound. Therefore, compound **D** may be considered as steroid acid. In the FT IR spectrum of compound **D** Figure 6 (d), the carboxylic acid of O-H stretching vibration for the COOH group indicated the absorption band at 3473 cm^{-1} and 1360 cm^{-1}, respectively. The

asymmetrical and symmetrical C-H stretching and bending vibrations of $–CH_2$ and $–CH_3$ groups were assigned at 2933, 2869 cm^{-1} and 1442, 1385 cm^{-1}, respectively. The C=O of carboxylic acid for the COOH group was displayed at 1710 cm^{-1}. The C-O stretching of the alcoholic O-H group was observed at 1220, 1049, and 1020 cm^{-1}, respectively. C-H out of plane bending vibration was shown at 981, 920, 709, and 530 cm^{-1}, respectively. According to the results, compound **D** may be considered a steroidal carboxylic acid.

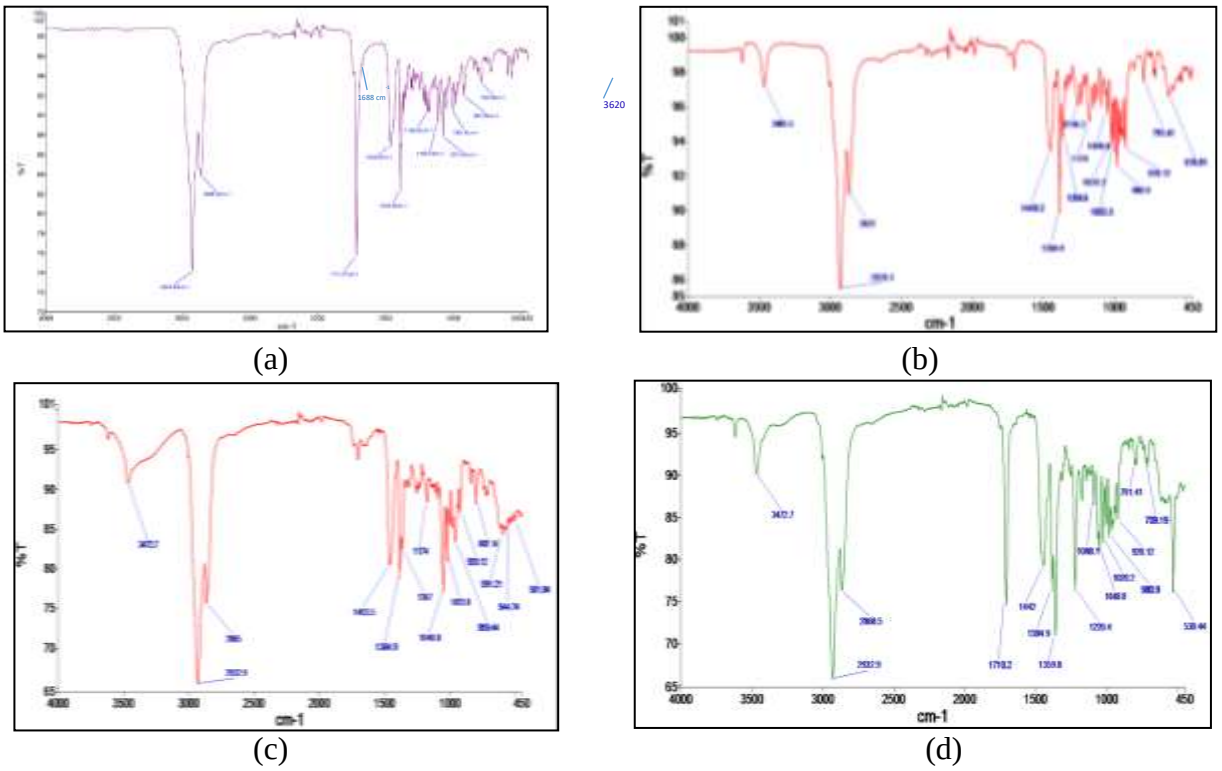

Figure 7. FT IR spectra of isolated (a) compound A, (b) compound B, (c) compound C and (d) compound D from PE extract of longan peels

Conclusion

The present research work deals with the first report for the investigation of total phenol contents, antioxidant, cytotoxicity, and anti-arthritic activities and phytoconstituents of *Dimocarpus longan* Lour. (Longan) peels. Based on the data, EtOH extract (176.12 ± 0.15 µg GAE/mg) was found to possess a higher total phenol content than watery extract (76.22 ± 0.45) µg GAE/mg). The antioxidant activity of the ethanol extract (IC_{50} = 8.77 µg/mL) is more effective than that of the watery extract. According to the cytotoxic effect, ethanol and watery extracts from LGP were observed to be free from toxic effects up to 1000 µg/mL dose. In terms of anti-arthritic activity, the watery extract (IC_{50} = 97.77 µg/mL) was more effective than the ethanol extract but slightly lower than the standard diclofenac sodium (IC_{50} = 57.57 µg/mL). On silica gel column chromatographic separation, four compounds were isolated from the PE crude extract of the LGP. Four compounds such as a steroid (**A**, m.pt 255-260 ˚C, 0.002 % yield), a terpenoid (**B**, mpt 290 ˚C, 0.001% yield), β-sitosterol (**C**, mpt 138-140 ˚C, 0.003% yield), and a steroidal carboxylic acid (**D**, mpt. 130-140 ˚C, 0.001% yield) were isolated. The information about the chemical composition and biological investigation described in this report might be useful in the formulation of corresponding traditional medicines.

Acknowledgements

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

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