STUDY ON SOME BIOACTIVITIES OF SULAR-NA-PHAR (*OLDENLANDIA CORYMBOSA* L.) PLANT

Khaing Zar Lin[1], Myint Myint Htwe[2], Hnin Hnin Aye[3], Daw HlaNgwe[4]

Abstract

In the present work, some bioactivities such as antitumor, antimicrobial, radical scavenging and cytotoxic activities of Sular-na-phar (*Oldenlandiacorymbosa* L.) plant and its bioactive compound were investigated. The antitumor activity of the H_2O and EtOH extracts of Sular-na-phar was examined by using PCG (Potato Crown Gall) test with the isolated bacterium *A. tumefaciens* and only H_2O extract prevented the formation of tumor. The antimicrobial activity of the crude extracts from this plant sample was determined against six strains of microorganisms by agar well diffusion methods at PRD (Pharmaceutical Research Department). The pet ether extract of Sular-na-phar did not show the antimicrobial activity. However, the EtOH and H_2O extracts possessed medium and EtOAc extract possessed high antimicrobial activities. Radical scavenging activity of EtOH and H_2O extract samples was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Assay. EtOH extract showed stronger radical scavenging activity than H_2O extract of plant sample. Cytotoxic effect of EtOH and H_2O extracts of whole plant of Sular-na-pharwas investigated against *Artemiasalina*(Brine Shrimp). The cytotoxic effect of plant extracts was not found on Brine Shrimp up to maximum dose of 1000 µg/mL. The bioactive compound(white colour crystal, 0.0285%) was isolated from defatted MeOH extract of *O. corymbosa* (Sular-na-phar) on the silica gel column chromatographic separation. The isolated compound was identified asursolic acid by physico-chemical properties and modern spectroscopic techinques such as UV, FT IR, ^{1}HNMR, ^{13}C NMR spectrometry as well as by comparing with the reported data. Finally, *invitro* cytotoxicity was also screened by using MTS Cell Proliferation Colourimetric Assay, against H1299, Clone no. 9, A549 and MCF-7 cell lines. According to the results, cell viability cannot be inhibited by ursolic acid for H1299 and Clone no. 9 cell lines significantly. However, the cell viability of A549 and MCF-7 cell can be effectively inhibited by ursolic acid.

Keywords: Sular-na-phar (*Oldenlandia corymbosa* L.), antimicrobial activity, antitumor activity, radical scavenging activity, cytotoxic effect, anticancer activity

[1.] PhD Candidate, Department of Chemistry, University of Yangon
[2.] Dr,Associate Professor, Department of Chemistry, Taungoo University
[3.] Dr, Professor, Department of Chemistry, University of Yangon
[4.] Dr,Professor and Head, Department of Chemistry, University of Yangon

Introduction

The plant has been traditionally used as cooling medicine in treatment of fever caused by deranged bile and also used in viral infections, cancer, acne, skin ailments, hepatitis, eye diseases and bleeding. It also shows bitter, acrid, cooling, febrifugal, pectoral, anthelmintic diuretic, depurative, diaphoretic, expectorant, digestive and has stomachic properties (Agrawal, 2013).

Oldenlandia corymbosa L. contains oleanolic acid, ursolic acid and gamma sitosterol, alkaloids, tannins, flavonoids, biflorine, biflorone, r-sitosterol, oleanolic acid, asperulosidic acid, geniposide acid, asperulosidic, deacetylasperuloside and scandoside methyl ester, a polysaccharide composed of glucose, galactose and glucuronic acid. The air dried plantcontains 0.12 %, alkaloids bifloron and biflorin. It contains13.55% inorganic ash that is responsible for it's coolingeffect (Chakraborty *et al.,* 2011; The Wealth of India 1950).

Some bioactivities of Sular-na-phar plant has been investigated by (ThandarAung, 2006). This research work intended to further study for different activities of Sular-na-phar plant, particularly anticancer activity.

Current research has supported the potential of plant-derived natural compounds for the treatment and prevention of cancer and ursolic acid is one such compound. Triterpenoid is one of the important compounds used as an anti-inflammatory, anticancer and anti-microbial agents (Navin and Kim, 2016). One such important and highly investigated pentacyclic triterpenoid, ursolic acid has attracted great attention of late for its potential as a chemopreventive and chemotherapeutic agent in various types of cancer.

Materials and Methods

Sample Collection

Sular-na-phar (the whole plant) (*O. corymbosa* L.) was collected from Shwepyithar Township, Yangon Region. After collection, the air-dried samples were cut into small pieces and ground by using grinding machine. Then the sample was separately stored in the airtight container to prevent moisture and other contamination.

Extraction of Chemical Constituents from Sular-na-phar

Dried powdered samples (400g) were percolated in Pet ether (PE) (60-80°C) (1000mL) for one week and filtered. This procedure was repeated for three times. Then the filtrate was concentrated by a vacuum rotatory evaporator to get respective pet-ether extract.

Similarly, EtOAc, 70% EtOH and H_2O extracts of each dried powdered samples were prepared according to the above procedure.

Screening of Pharmacological Activities of the Whole Plant of Sular-na-phar

This section involves screening of some pharmacological activities such as antitumor activity, antimicrobial activity, antioxidant activity, cytotoxic activity of some crude extracts and anticancer activity of isolated compound of Sular-na-phar.

Screening of Anti-tumor Activity by Potato Crown Gall (PCG) (or) Potato Disc Assay (PDA) method

Anti-tumor activity of EtOH and H_2O extracts of Sular-na-phar was examined by Potato Crown Gall (PCG) (or) Potato Disc Assay (PDA) method (Ferrigni *et al.,* 1982) at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon, Myanmar.

Fresh, disease-free potatoes were purchased from a local market. Tubers of moderate size were surface sterilized by immersion in 0.1 % sodium hypochlorite for 20 min. Ends were removed and the potatoes were then soaked in sodium hypochlorite solution for an additional 10 min. A core of the tissue was extracted from each end and discarded. The remainder of the cylinder was cut into 1.0 cm thick discs with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL deionized distilled water, autoclaved for 20 minat 121˚C, 20 mL poured into each Petridish). Each plate contained four potato discs and 4 plates, used for each sample dilution.

Samples (0.05, 0.1, 0.2 g) were separately dissolved in DMSO (1 mL) and filtered through Millipore filters (0.22 μm) into sterile tube. This solution

(0.5 mL) was added to sterile distilled water (1.5 mL), and broth culture of *A. tumefaciens* in PBS (2 mL) was added. Controls were made in this way; DMSO (0.5 mL) and sterile DW (1.5 mL) were added to the tube containing 2 mL of broth culture of *A. tumefaciens*. Using a sterile disposable pipette, 1 drop (0.05 mL) each from these tubes was used to inoculate each potato disc, spreading it over the disc surface. After inoculation, Petri dishes were sealed by film and incubated at 27-30 ˚C for 3 days. Tumors were observed on potato discs after 3 days under stereo-microscope followed by staining with Lugol's iodine (10 % KI and 5 % I_2) after 30 min and compared with control. The antitumor activity was examined by observation of tumor produced or not

Screening of Antimicrobial Activity by Agar Well Diffusion Method

The screening of antimicrobial activity of various crude extracts such as PE, EtOAc, 70 % EtOH, H_2O extracts of Sular-na-phar were carried out by agar disc diffusion method at Pharmaceutical Research Department (PRD), Ministry of Industry, Yangon, Myanmar. Six microorganisms namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* , *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* were used for this test.

Screening of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of EtOH and H_2O extracts from Sular-na-phar was screened by using DPPH Free Radical Scavenging Assay (Marinova and Batchvarov, 2011).

The control solution was prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of ethanol in the brown bottle. The blank solution sample solution was also prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance values of these solutions were measured at 517 nm and the percentage of radical scavenging activity (% RSA) was calculated by the following equation.

$$\% \text{ RSA} = [(A_{DPPH} - A_{Sample}) - A_{Blank} / A_{DPPH}] \times 100$$

where, % RSA = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

$$\% \text{ Inhibition} = \frac{A_{DPPH\ alone} - A_{Sample}}{A_{DPPH\ alone}} \times 100$$

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50% inhibition concentration) values were calculated by linear regressive excel program.

Cytotoxicity Test by Brine Shrimp Lethality Bioassay

Cytotoxic effect of EtOH and H_2O extracts from the whole plant of Sular-na-phar was investigated by brine shrimp lethality bioassay according to the procedure described by Dockery and Tomkins, (2000). Artificial sea water (9 mL) and 1 mL of different concentrations of samples and standard solutions were added to each chamber. Alive brine shrimps (10 nauplii) were then taken with 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 was counted and 50% lethality dose (LD_{50}) was calculated (Sahgal *et al.,* 2010).

The control solution was prepared as the above procedure by using distilled water instead of sample solution.

Isolation and Identification of Chemical Constituents from the Sular-na-phar

Methanol extract was then dissolved in methanol and water (9:1) and then washed with hexane. This procedure repeated for three times. The total filtrate were concentrated under rotatory evaporator to obtain defatted

methanol extract. Defatted methanol extracts (7g) was separated by column chromatographic method and eluting with PE : EtOAc (10:1, 9:1, 7:1, 5:1, 3:1, 1:1, 1:3 and 1:5) solvent system. On chromatographic separation, 120 fractions were collected. From the inspection of TLC chromatograms viewed under UV lamp, the fractions having the same appearance on TLC chromatograms were combined to give (VII) fractions. According to the inspection of TLC chromatograms, fraction (FIII) and (FIV) were combined and washed with PE/EtOAc to remove impurities. Then it was recrystallized with MeOH until to get white crystal as compound SL 1.

Structural Identification of Isolated Compound.

The isolated compound was identified by determining their structures using UV-visible, FT IR and NMR spectroscopic data. The Fourier transform infrared spectra of isolated compound (SL 1**)** were recorded by FT IR (8400) spectrophotometer (Shimadzu, Japan) at Department of Chemistry, University of Yangon. The ^{1}H NMR and^{13}C NMR spectra of isolated compound **(**SL 1**)** were recorded in $CDCl_3$ and DMSO by 400 MHz NMR spectrometer at the Department of Organic and Biomolecular Chemistry, Nagoya University and Division of Natural Products Chemistry, Institute of Natural Medicine, University of Toyama, Japan.

Investigation of Anticancer Activity by MTS Assay with Different Cancer Cell Lines

The cytotoxicity of ursolic acid was investigated by using MTS assay against H1299(lung cancer cell line), Clone no.9 (lung cancer cell line), A549 (lung cancer cell line) and MCF-7 (breast cancer cell line) at Laboratory of Biological Chemistry, Department of Chemistry, Faculty of Science, Hokkaido University, Japan. The cell number in the mixture solution was counted by Hemocytometer under microscope. Each cell lines was seeded in 96-well plates and incubated in incubator (37 ºC, 5%CO_2) for one day. And then stimulated with ursolic acid on various cancer cell lines3.0 µL of DMSO was mixed with 697 µL of Opti-MEM. This mixture was assigned as Solution A. 2.8 µL of compound stock solution was mixed with 657 µL Opti-MEM to become 17 µg/mL concentration. From the stock solution concentration of 17

µg/mL of the compound, 440 µL was diluted with 110 µL of Solution A to become 13.6 µg/mLconcentration. This stock solution was continuously diluted with Solution A to get the compound solution with the concentrations of 10.2, 6.8 and 3.4 µg/mL in conical tubes respectively. Each of these solutions 200 µL was mixed with 800 µL of DMEM in conical tube respectively. Finally, 100 µL of each diluted solution was retaken into 96 well plate and incubated (37 ºC, 5% CO_2) in incubator for three days.

Finally, old medium, that containing cancer cell was removed by aspirator and washed with 1000 µL of PBS (phosphate buffered saline) by mutichannels pipette. That PBS (phosphate buffered saline) was removed by aspirator again. 50 µL of DMEM medium and 10 µL of Cell Titter 96 ® Aqueous One Solution Cell Proliferation Assay solution were added into 96 well plate. It was incubated (37ºC, 5% CO_2) in incubator for 30 min. Finally, the absorbance was measured at 490 nm in plate reader.

Results and Discussion

Antitumor Activity of Sular-na-phar

The inhibition of *A.tumefaciens-induced*tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity that can detect a broad range of known and novel antitumor effects. The validity of this bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals. It was demonstrated that inhibition of crown gall tumor initiation on potato disc showed an apparent correlation with compounds and plant extracts. The antitumor activity of the H_2O and EtOH extracts of whole plant of Sular-na-pharwas investigated using PCG test with the bacterium *A. tumefaciens* obtained from Fermentation Department. The tested samples were dissolved in DMSO, diluted and mixed with the bacterial culture for inoculation. After preparing the inoculums, the bacterial suspension was inoculated on the cleaned and incubated for 3 days, at room temperature. After that, the tumors were appeared on potato discs and checked by staining the knob with Lugol's (I_2+KI) solution. In the control, the formation of white knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The active test samples did not form any tumors on the potato discs and its surface remained blue.

From this experiment, it was found that the H_2O extract of whole plant of Sular-na-pharwas good in preventing the tumor formation with the dose 0.05 g/mL, 0.10 g/mL and 0.20 g/mL in *vitro* potato disc assays. The EtOH extract of whole plant of Sular-na-phar did not exhibit the growth of tumor even at the concentration of 0.20 g/mL. The results are shown in Table 1.

Table 1: Tumour Inhibitory Property of Different Concentrations of H_2O and EtOH Extracts of Whole Plant of *O. corymbosa* (Sular-na-phar)

Extracts	Concentration of Samples (g/mL)	Tumor*
Control	0.00	+
H_2O	0.05	-
	0.10	-
	0.20	-
EtOH	0.05	+
	0.10	+
	0.20	+

*(+) Tumor appeared, (-) No tumor appeared

Antimicrobial Activity of Crude Extracts of Sular-na-phar

Four crude extracts of such as PE, EtOAc, EtOH, and H_2O extracts of *O. corymbosa* were screened for antimicrobial activity against six different pathogenic microbes using agar well diffusion method. This method is based on zone diameter including the well diameter, in millimeter (mm).

According to the results presented in Table 2, PE extract of *O. corymbosa* (Sular-na-phar) did not show any antimicrobial activity against all of the microorganisms tested. Moderately significant antibacterial activities were possessed by EtOH extract of selected medicinal plant (13mm-16mm). H_2O extractof the selected medicinal plant inhibited the all strains of microorganisms and significant zone of inhibition ranges between 18mm-22mm. Moreover, EtOAc extract of Sular-na-phar significantly inhibited all strains of microorganisms and significant zone of inhibition ranges between 20mm-32mm. It's zone diameter is wider than the other extracts.

Table 2: Inhibition Zone Diameters of Various Extracts of Sular-na-phar against Six Microorganisms by Agar Well Diffusion Method

No.	Microorganisms	Inhibition Zone Diameters (mm) of Different Extracts			
		PE	EtOAc	EtOH	H_2O
1	*Bacillus subtilis*	-	25 (+++)	13(+)	18(++)
2	*Staphylococcus aureus*	-	32(+++)	15(++)	20(+++)
3	*Pseudomonas aeruginosa*	-	20(+++)	-	20(+++)
4	*Bacillus pumilus*	-	28(+++)	16 (++)	20(+++)
5	*Candida albicans*	-	27(+++)	16 (++)	18(++)
6	*Escherichia coli*	-	30(+++)	14 (+)	22(+++)

Agar well – 10mm
10mm ~ 14mm (+)
15mm ~ 19mm (++)
20mm above (+++)

Antioxidant Activity of Sular-na-phar

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The reduction of the radical is followed by a decrease in the absorbance at 517nm. The results are shown in Table 3. From these observations, the radical scavenging activity of EtOH extract was greater than that of H_2O extract. EtOH extract of Sular-na-phar inhibited 50 % of free radical at the concentrations of 112.55 μg/mL (IC_{50}) and also H_2O extract inhibited 50 % of free radical at the concentration of 198.53μg/ mL (IC_{50}). So, antioxidant activity of EtOH extract is more stronger than H_2Othat of extract.

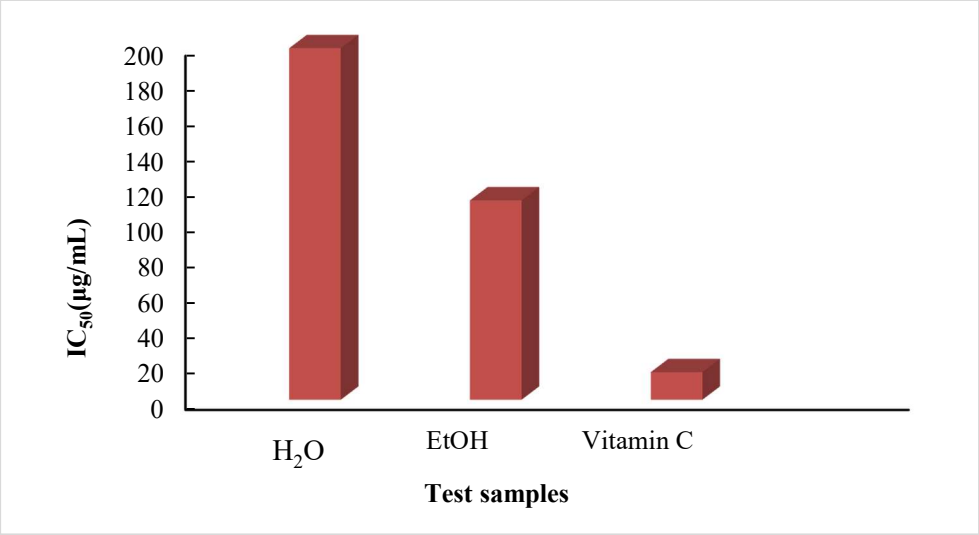

Figure 1: A bar graph of IC_{50}(µg / mL) of H_2O and EtOH crude extracts of (Sular-na-phar)

Table 3: (IC_{50})of H_2OandEtOH Crude Extracts of Sular-na-phar

Samples	IC_{50} (µg/mL)
H_2O extract	198.53
EtOH extract	112.55
Standard Vitamin C	15.55

Cytotoxicity of Sular-na-phar

The cytotoxicity on EtOH and H_2O extracts of whole plant of Sular-na-phar was evaluated by Brine Shrimp cytotoxicity bioassay. This bioassay is general toxicity screening for bioactive plants and their derivatives. A model animal that has been used for this purpose is the Brine Shrimp, *Artemia salina.*

The cytotoxicity of EtOH and H_2Oextracts of the whole plant of Sular-na-phar was expressed in terms of mean SEM (standard error mean) and LD_{50} (50% Lethality Dose) and the results are shown in Table 4. In this experiments, standard potassium dichromate ($K_2Cr_2O_7$) and caffeine were chosen because $K_2Cr_2O_7$ is well-known toxicity in this assay and caffeine is a

natural product. The effect of cytotoxicity was not found on the Brine Shrimp up to maximum dose of 1000 μg/mL.

Table 4: Cytotoxicity of Whole Plant of Sular-na-phar (Sular) against *Artemia salina* (Brine Shrimp)

Tested samples	No. of Death of Brine Shrimp (Mean ± SEM)				LD_{50} (μg/mL)
	1000	100	10	1	
EtOH extract	3.33±0.58	1.33±0.58	0.00±0.58	0.00±0.00	>1000
H_2O extract	1.00±0.58	0.00±0.00	0.00±0.00	0.00±0.00	>1000
$K_2Cr_2O_7$	10.00±0.00	10.00±0.00	2.00±0.00	0.00±0.00	43.74
Caffeine	5.00±1.16	3.00±0.58	2.33±1.20	0.00	1000

Structural Elucidation of Compound SL 1

SL 1 : Ursolic acid: white colour crystal, 0.0285 %, 77.6 mg (R_f= 0.3, PE : EtOAc = 3:1, melting point 286˚C); FT IR of 3400 (OH), 2925, 2687 (CH), 1688 (C=O), 1455 (CH), 1029 (C-O); ^{1}HNMR (400MHz, $CDCl_3$) : δ 0.74 - 2.10 (44H, m), 3.22 (1H, m), 5.22 (1H, m): ^{13}CNMR (400 MHz, $CDCl_3$) δ 15.1, 15.3, 16.6, 16.7, 18, 23.0, 23.2, 23.9, 26, 27.7, 28.0, 29.0, 30.4, 32.8, 36.6, 38.8, 39.2, 39.2 ,41.8, 47.3, 47.5, 53.61, 55.03, 78.57, 125.27, 137.97, 180.41 agreed with the data reported by Guvenalp (2005).

Anticancer Activity of Ursolic acid by MTS Assay with Different Cancer Cell Lines

The compound isolated, ursolic acid was determined the anticancer activities. It indicated the anticancer activities, such as lung cancer cell line and breast cancer cell line. The cytotoxicity of ursolic acid was investigated using MTS assay against H1299, Clone no.9, A549 and MCF-7 cancer cell lines. If percentage of cell viability will be high, the living cell will be

accumulated. If so, anticancer activity will not be effective. According to the result data, cell viability cannot be inhibited by ursolic acid for H1299 and Clone no. 9 cell lines significantly. However, the cell viability of A549 and MCF-7 cell can be inhibited by ursolic acid. That can be evident by comparing with etoposide (positive control). Although inhibition of cell in ursolic acid for A549 cell were 100, 101, 84, 16, 12 and 13 in 0µg/mL, 3.4µg/mL, 6.8µg/mL, 10.2µg/mL, 13.6µg/mL, and 17µg/mL, the etoposide was 67 for concentration of 17µg/mL. For MCF-7, the cell inhibition values of ursolic acid were 96, 46, 22, 20 and 20 in different concentrations, but the cell inhibition of etoposide was 60 for concentration of 17µg/mL. The results are shown in Figure 2 and Table 5. So, the maximum concentration inhibited the cell viability was 13.6µg/mL form A549 and MCF 7 cell lines. On the other hand, H1299 (lung cancer cell line) and Clone no.9 (lung cancer cell line) cannot be inhibited by ursolic acid**,** but A549 (lung cancer cell line) and MCF-7 (breast cancer cell line) can be inhibited by ursolic acid.

Table 5: Average Cell Viability of Various Cell Lines at Different Concentrations of Ursolic Acid of theSular-na-phar

Cell lines	**Average cell viability of various cell lines at different concentrations of ursolic acid(µg/mL)**						**Control**
	0	**3.4**	**6.8**	**10.2**	**13.6**	**17**	**17**
H1299	0.506	0.518	0.530	0.561	0.534	0.401	0.256
	100	102	105	111	105	79	51
Clone no.9	0.453	0.553	0.583	0.562	0.541	0.571	0.251
	100	122	129	124	119	126	55
A549	0.754	0.760	0.631	0.120	0.019	0.096	0.504
	100	101	84	16	12	13	67
MCF –7	0.461	0.443	0.21	0.103	0.009	0.091	0.279
	100	96	46	22	20	20	60

*Absorbance values were measured at 490nm.

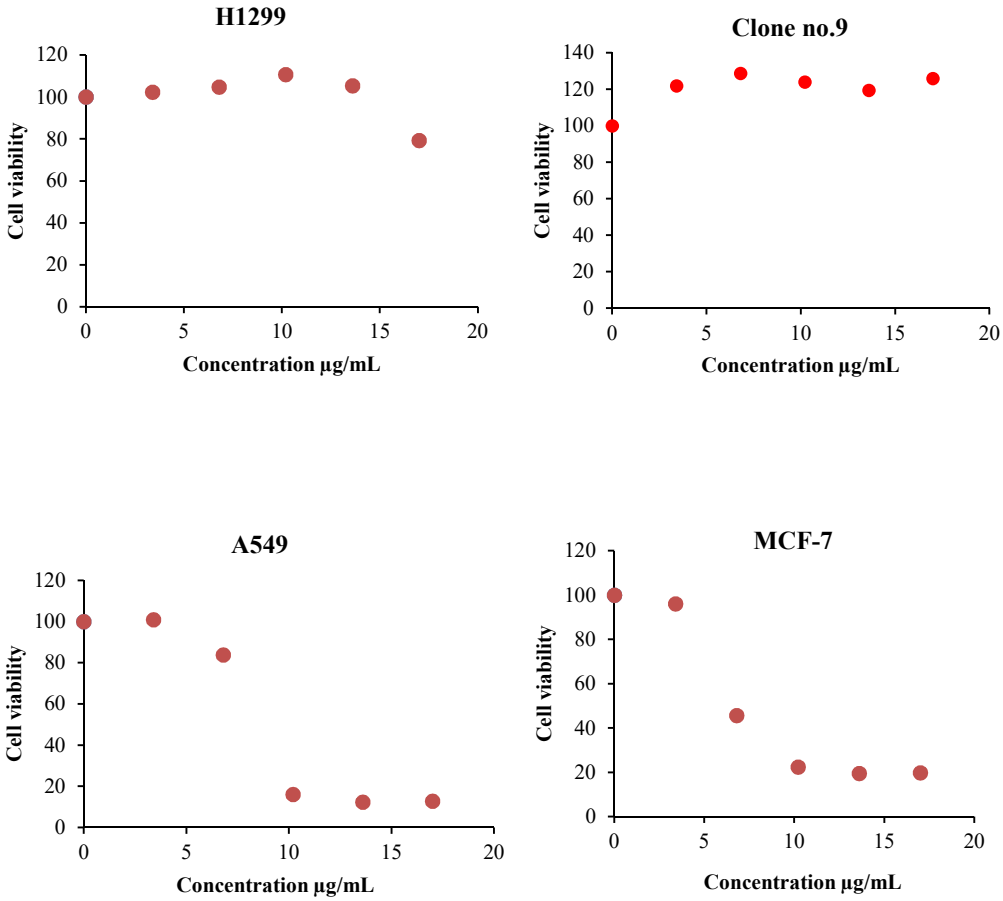

Figure 2: Cell viability of Various Cell Lines at Different Concentrations of Ursolic acid

Conclusion

Antitumor activity investigated by potato crown gall (PCG) assay revealed that H_2O extract of *O. corymbosa* (Sular-na-phar) inhibited tumour formation but EtOH extract could not inhibit.

In antimicrobial activity of four different extracts (PE, EtOH, EtOAc and H_2O) of the whole plant of *O. corymbosa* (Sular-na-phar), PE extract did not show any antimicrobial activity against all of the microorganisms tested. Moderately significant antibacterial activities were possessed by EtOH extract (13mm-16mm) and H_2O extract inhibited the all strains of bacteria with the significant zone of inhibition ranged between 18mm-22mm. Among the four extracts, EtOAc extract significantly inhibited all strains of bacteria with the significant zone of inhibition ranged between 20 mm-32 mm. In screening of free radical scavenging, the IC_{50} values of H_2O and EtOH extracts of Sular-na-pharwere observed to be 198.53 µg/mL and 112.55 µg/mL, respectively. Ethanol extract of sample showed more stronger radical scavenging activity than water extract. The cytotoxicity effect of plant extract was not found on Brine Shrimp up to maximum dose of 1000 µg/mL. The silica gel column chromatographic separation gave : 0.0258%, 77.6mg of ursolic acid (R_f= 0.3, PE : EtOAc = 3:1, melting point 286˚C). The cytotoxicity of ursolic acidas investigated using MTS assay against H1299, Clone no.9, A549 and MCF-7 cancer cell lines. In this experiment, various concentrations such as 3.4, 6.8, 10.2, 13.6, 17 µg/mL were used and etoposide (17 µg/mL) was also used as positive control. Among all these four cell lines, H1299 (lung cancer cell line) and Clone no.9 (lung cancer cell line) could not be inhibited by ursolic acid**,** but A549 (lung cancer cell line) and MCF-7 (breast cancer cell line) can be inhibited by ursolic acid.

Acknowledgements

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research.

References

Agrawal, S. K. (2013). "Evaluation of Antiulcer Activity of *Oldenlandia corymbosa* (L.)". *International Journal of Research and Development in Pharmacy and Life Sciencies,*vol.2 (2), pp.363-367

Chakraborty, A. K., Rambhade, S. and Patil, U. K. (2011). "*Chromolaena odorata* (L.) : An Overview". *Journal of Pharmacy Research,*vol.4(3), pp.573-576

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

Ferrigni N. R., Putnan, J. E., Aderson, B., Jacobsen, L.B., Nicols, D. E., Moore, D. S. and Mclaughlin, J. L. (1982). "Modificationi and Evaluation of the Potato Disc Assay and Antitumor Screening of Euphorviaceae Seeds" *.J. Nat. Prod*, vol. 45(6), pp. 678-686

Guvenalp, Z., Kilic, N., Kazaz, C., Kaya, Y. and Demlrezer, O. (2005). "Chemical Constituents of *Galiumtortumense*". *Turk J Chem,* vol.30, pp.515-523

Marinova, G. and Batchvarov, V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH''. *Bulgarian Journal of Agricultural Science*, vol.17, pp.11-24

Navin, R. and Kim, S. M. (2016).Therapeutic Interventions Using Ursolic Acid for Cancer Treatment". *Med chem (Los Angeles)*, vol.6, pp.339-344

Sahgal, G., Ramanathan, S., Sasidharan, S., Mordi, M.N., Ismail, S. and Mansor, S. M. (2010). "Brine Shrimp Lethality and Acute Oral Toxicity Studies on *Swieteniamahagonic* (L.) Jacq. Seed Methanolic Extract''. *Pharmacognosy Res.,* vol. 2(4), pp.215-220

Thandar Aung. (2006). *Isolation, Characterization and Identification of Some Bioactive Constituents and Investigation of Cholesterol Lowering Effect of Euphorbitahirta* L. *(Kywe - Kyaung – Hmin-Say) and Oldenlandia corymbosa* L. *(Sular-Na- Phar*), PhD dissertation, Department of Chemistry, University of Yangon, 9 -51

The Wealth of India. (1950). *A Dictionary of India Raw Materials and Industrial Products*. New Delhi: vol. 2, pp.1-9