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Original Research Article

Evaluation of Biological Properties and Isolation of Metabolites of Lichens of Parmeliaceae Family from Himalayan Region of Nepal

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ABSTRACT

The main purpose of this study is to evaluate the bioactive properties and chemical constituents of two lichens *Usnea longissima* and *Parmelia nepalensis*. Both lichens were subjected to sequential extraction with four different organic solvents. Total phenolic content (TPC) was evaluated by Folin Ciocalteu method while total flavonoid content (TFC) was evaluated by aluminum chloride colorimetric method. Antioxidant activities were evaluated by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay and α- amylase inhibition activities was evaluated by starch- iodine method. Antibacterial activity was evaluated by agar well diffusion method and cytotoxicity assay by Brine shrimp lethality assay. Results revealed that methanol and ethyl acetate extract of both lichen species have higher amounts of TPC and TFC. Both lichens showed potent antioxidant activities. The TPC and TFC content showed a positive correlation with the antioxidant activities. Furthermore, ethyl acetate extract of *U. longissima* and chloroform extract of *P. nepalensis* were found to be potent for the *alpha* amylase inhibition. All four fractions of *U. longissima* and *P. nepalensis* have inhibited *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, while *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were found to be resistant to both lichens. Brine Shrimp lethality assays revealed that the hexane extract of *U .longissima* was found to be the most toxic among all tested extracts. Two known secondary metabolite usnic acid and evernic acid were isolated from both lichens. This study revealed that *U. longissima* and *P. nepalensis*possess potent bioactive properties and secondary metabolites as well.

Keywords: *P. nepalensis*, *U. longissima*, Phenolic, Flavonoids, Bioactivities.

Introduction

Lichens are slow growing symbiotic organisms formed by the association of mycobiont (fungi) and photobiont (algae). Photobionts help in the synthesis of nutrients by photosynthesis process while mycobionts help in absorbing minerals. This association makes these organisms resistible to extreme conditions and can grow in any type of substratum like tree, stones, wetland and dry places.[1] Lichens are specially known for their ability to produce unique secondary metabolites and their pharmacological properties[2] and as bio indicators of air pollution.[3] Lichens have been used in folk medicine since long age to treat different diseases.[4] Numerous researches had been carried out for detailed study of biochemical and pharmacological properties of many lichens and their secondary metabolites.[5-8] These days researchers are mainly focused on the isolation and identification of metabolites and their bioactive properties in-vivo and in-vitro. As the world is being more concerned about the natural products and their uses with less or no side effects, lichens may serve as the important source of such natural products with diverse applications.[9,10] What makes researchers more interested in studying lichens is the presence of unique secondary metabolites. Most of the lichen secondary metabolites are only found in lichens so also called as lichen acids. Some of the important lichen acids identified are usnic acid, evernic acid, barbatic acid, diffractaic acid, physodic acid and many more.[11,12] Many researchers had studied about the bioactive properties like antioxidant, anticancer, antimicrobial, anti-viral activities of different lichens and their secondary metabolites.[13-15]

The main purpose of this research was to evaluate different bioactive properties such as antioxidant, enzyme inhibition, antibacterial and cytotoxic activity of two different lichens *U. longissima* and *P. nepalensis* of Parmeliaceae family collected from Himalayan region of Nepal. Although both lichens from different locations have been studied for their chemical constituents[16,17] and bioactive properties.[18-20] Till now, research works on these lichens from Sagarmatha National Park area of Solukhumbu District of Nepal were not reported.

Materials and Methods

Collection of Lichens

Both lichens *U. longissima* and *P. nepalensis* were collected from Sagarmatha National Park of Solukhumbu District of Nepal during the month of June-July, 2016 and identified scientifically by Prof. Dr. Chitra Bahadur Baniya at Tribhuvan University Central Herbarium (TUCH), Central Department of Botany, Kirtipur, Kathmandu, Nepal. The plant voucher specimens (U01 for *U. longissima* and P02 for *P. nepalensis*) were deposited at The National Herbarium and Research

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Extract Preparation
Air dried lichens were ground to powdered form. Eighty four gram (84 g) of powdered *U. longissima* and thirty five gram (35 g) of *P. nepalensis* were sequentially extracted with four different organic solvents; namely, Hexane, Chloroform, Ethyl acetate and Methanol with increasing order of polarity by percolation method. After 3 days, mixture was filtered and filtrate was concentrated by evaporating solvent in rotary evaporator and dry extract were kept in glass vial at 4ºC until use.

Isolation of Secondary Metabolites
All the four fractions of *U. longissima* and *P. nepalensis* were first analyzed by Thin Layer Chromatography (TLC) which suggested the presence of Evernic acid and Usnic acid in the chloroform and ethyl acetate extracts on comparision with standard of both metabolites. About 6g of the choloroform extract was subjected to Silica gel Column Chromatography. Column was eluted with hexane. Hexane/ Chloroform, (10-90%, 20-80%, 30-70%, 40-60%, 50-50%, 60-40%, 70-30%, 80-20%, 90-10%), 100% Chloroform, 1-10% Chloroform/ Methanol and finally column was washed with 100% methanol to obtained several fractions. Both compounds were purified from hexane/chloroform fraction by several column chromatographies. The pure compounds obtained were identified by doing co-TLC with respective standard compounds.

Determination of total phenolic content (TPC)
Total phenolic content was determined by Folin Ciocalteu (FC) assay according to the method described by previous investigator.[21] Briefly, 0.5 mL of all fractions of both lichens (1 mg/mL concentration) was prepared in methanol. Then 0.8 mL of distilled water and 0.1 mL of FC-reagent were added and allowed to stand for 3 minutes. Then 0.3 mL of Na_2CO_3 (75mg/mL) was added and incubated for 30 minutes and absorbance was measured at 765 nm. Gallic acid (1 mg/mL) was used as standard, all the experiments were performed in triplicates and results were expressed as milligram of Gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Determination of total flavonoid content (TFC)
Total flavonoid content was estimated by Aluminum Chloride colorimetric assay as previously reported with slight modification.[22] Briefly, 1.5 mL of all fractions of both lichens (concentration of 1 mg/mL) was prepared in methanol. Then 75 µL of 5% $NaNO_2$ was added to the samples and incubated for 6 minutes. After incubation, 150 µL of $AlCl_3$ (20mg/mL) was added to the reaction mixture and again incubated for 6 minutes. Further, 0.5 mL of 1M NaOH was added; volume was made up to 3 mL by adding distilled water. The reaction mixture was shaken properly and absorbance was measured at 510 nm wavelength. Quercetin (1 mg/mL) was used as standard, all the experiments were performed in triplicates and results were expressed as milligram of Quercetin equivalent per gram of dry extract (mg QE/g DW).

Antioxidant Activity
DPPH assay
For DPPH assay, procedure explained by previous investigator[23] was followed with slight modifications. Different concentrations of all four fractions of both lichens (50-800µg/mL) were prepared in methanol. Gallic acid (1-5 µg/mL) as standard antioxidant was prepared in distilled water. DPPH solution (0.3mM) was freshly prepared prior to the test and dissolved properly in methanol. Then 1 mL of each concentration of all samples were mixed with 1mL of 0.3 mM DPPH, shaken properly and incubated in dark for 30 minutes and absorbance was measured at 517 nm. Control was prepared by using solvent instead of samples. The experiments were performed in triplicate and percentage Radical Scavenging Activity (RSA) was calculated by using the following formula.

$$\%RSA = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The inhibition concentration at which absorbance is 50% (IC_{50}) was calculated by using average RSA.

ABTS assay
ABTS assay was also performed for evaluating antioxidant activity of both lichens. This assay was performed according to Thaipong *et al*.[24] Briefly, 7 mM of ABTS and 2.45 mM of potassium per sulfate ($K_2S_2O_8$) were prepared in distilled water, mixed in equal volume and incubated in the dark for 12-16 hours at room temperature to generate free radical. ABTS radical was diluted with Phosphate Buffer Saline (PBS) to obtain the absorbance of about 1-1.5. One milliliter of working solution of different concentrations of extracts was prepared in methanol and Gallic acid (10-100 µg/mL) used as standard in distilled water. Then 2 mL of already prepared ABTS radical was added to it, mixed properly and allowed to stand for 15 minutes. After 15 minutes, absorbance was measured at 734 nm. The experiments were performed in triplicate and percentage Radical Scavenging Activity (RSA) was calculated by using the following formula.

$$\%RSA = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The inhibition concentration at which absorbance is 50% (IC_{50}) was calculated by using average RSA.

Alpha-amylase inhibition assay
α-amylase inhibition assay was performed by starch iodine method[25] with slight modifications. Stock solutions of 1mg/mL of lichen extracts were prepared in 99% DMSO and then diluted to different concentrations (20-100 µg/mL) with phosphate buffer up to final volume of 500 µL. 100 µg/mL fungal alpha amylase (diastase) was prepared in Phosphate Buffer Saline (PBS) and 100µl was added to prepared samples and incubated for 10 minutes at 37˚C. Further 200µl of 1% starch was added and further incubated for 30 minutes at 37˚C. α-amylase blank and substrate blank were also prepared simultaneously where distilled water was used instead of enzyme and starch respectively. After 30 minutes of incubation, 50 µl of iodine solution was added in each reaction mixture and diluted with 3 mL distilled water. Absorbance was measured at 565 nm and each experiment was performed in triplicate. Percentage inhibition of α-amylase was calculated by the following equation.

$$\% \text{ Inhibition} = \frac{\text{A-C}}{\text{B-C}} \times 100$$

Where, A is the absorbance of sample, B is the absorbance of α-amylase blank and C is the absorbance of substrate blank. IC_{50} value was calculated by plotting percentage inhibition against sample concentration.

Antibacterial assay
Antibacterial activity was evaluated by agar well diffusion method adopted by previous investigator.[26] Single colony of five pathogenic bacteria, *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), were inoculated in nutrient broth and incubated for 12-16 hours and compared with 0.5 McFarland standards. Muller Hinton Agar (MHA) agar plates were then swabbed with the respective bacteria and wells were made with 5 mm borer. All fractions of both lichens were dissolved in 100% DMSO to make concentration of 20mg/mL and Streptomycin was dissolved in distilled water to make concentration of 100µg/mL. 50 µL of all prepared samples, Streptomycin as a positive control and 100% DMSO as negative control were loaded in different wells, allowed to diffuse for 2-3 hours and incubated for 16-18 hours at 37˚C. After 18 hours, zone of inhibition was measured and the results were expressed as mean ± standard deviation.

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Cytotoxicity assay

Cytotoxicity of both lichen extracts was evaluated by Brine Shrimp Lethality Assay as previously described by Olowa and Olaga.[27] Artificial sea water was prepared by dissolving 40g sea salt in one liter distilled water and shrimp eggs were added in it to hatch for 48 hours. Three different concentrations of extracts (10, 100 and 1000) µg/mL were prepared in suitable solvents (Chloroform, Ethyl acetate and Methanol) and required amounts were transferred to the respective vials labeled as 10, 100 and 1000 µg/mL allowing solvents to dry completely. After 48 hours, 10 mature shrimps were transferred into each vials containing 5mL sea water and allowed to stand for 24 hours. After 24 hours, toxicity of the lichen extracts was analyzed by observing dead shrimps, obtaining mortality percentage and LC_{50} values.

Statistical analysis

Origin Pro 6.1, SPSS version 20 and MS-Excel 2010 software were used for analysis of data. ANOVA were carried out for determining significance level and Spearmen's correlation was performed for obtaining correlation coefficients. Probit (probability unit) analysis was performed for determining the LC_{50} value for brine shrimp lethality assay.

Results and Discussion

Extraction

Air dried plant were extracted with four different solvents; hexane, chloroform, ethyl acetate and methanol in the increasing order of polarity. The percentage yield of the extracts was listed in the Table 1. The highest yield, of *U. longissima*, was obtained with chloroform (8.05%) followed by methanol (3.15%). While the highest yield of *P nepalensis*, was obtained with methanol (4.15%), followed by chloroform (3.07%).

Isolation of secondary metabolites

Secondary metabolites of lichens were isolated by performing column chromatography of chloroform extracts of both lichens. Usnic acid was isolated from extract of both lichens while Evernic acid was isolated from *U. longissima* extract. Both metabolites Usnic acid and Evernic acid (Figure 1a and 1b) were identified by comparing with the standard compounds.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC and TFC of all four fractions of *U. longissima* and *P. nepalensis* are summarized in Table 2. According to the result obtained, Ethyl acetate fraction of *U. longissima* contains the highest amount of TPC (43.88 ± 0.42mgGAE/g DW) and TFC (171.13 ± 9.04mgQE/g DW). Whereas, in *P. nepalensis*, methanolic fraction contained highest amount of TPC (39.68 ± 0.76 mgGAE/g DW) and ethyl acetate fraction contained the highest TFC (151.53 ± 5.49 mgQE/g DW).

Antioxidant assay

DPPH and ABTS free radical scavenging activity of all four extracts of *U. longissima* and *P. nepalensis* were investigated by determining the percentage inhibition of free radicals and IC_{50} values (Table 4). Figure 2 and Figure 3 showed concentration dependent inhibition of free radicals. Among four extracts of U. longissima, the lowest IC_{50} value was obtained for ethyl acetate extract (219.49 ± 3.99 µg/mL) in DPPH assay and for chloroform extract (9.45 ± 0.20 µg/mL) in ABTS assay. Similarly, among the four extracts of *P. nepalensis*, the lowest IC_{50} value was obtained for methanol extract (270.75 ± 9.3 µg/mL) in DPPH assay and for ethyl acetate extract (2.39 ± 0.18 µg/mL) in ABTS assay. Hexane extract of both lichens was found to be least potent. Lower the IC_{50} values, higher the antioxidant activities. Thus chloroform, ethyl acetate and methanolic extracts of both lichens were found to be potent free radical inhibitors.

The correlation between DPPH and ABTS assays with TPC and TFC of all four extracts of both lichens were analyzed using the correlation coefficient. Result revealed that DPPH assay is significantly correlated with TPC and TFC in both lichens species while ABTS assay is only significantly correlated only with TFC (Table 3). This is in support of previous reports that phenolics and flavonoids are the major constituents of lichens which are responsible for the different bioactive properties but not obligatory in every case.[28,29]

Natural products are gaining attention of researchers due to their potent bioactivity with less or no side effects.[30] Many research have been carried out to study natural antioxidants and antioxidant activities of different lichen metabolites. Our study also focused on free radical scavenging ability of different extracts of two lichens. In our study, Ethyl acetate and methanol extract of both lichens showed relatively high DPPH free radical scavenging activity compared to other two solvent extract, which might be due to the presence of higher amount of TPC and TFC. Varying IC_{50} values for the DPPH and ABTS assay by same sample are reported previously as well and reason might be the different affinity of hydrogen donors (phenols and flavonoids) to the different free radicals.[31]

Alpha-amylase inhibition assay

α- amylase inhibition activity of all fractions of both lichens was determined by starch iodine method. The general principle behind this method is when lichen extract inhibits the enzyme α-amylase; starch does not degrade into glucose and gives blue color after addition of iodine. This activity was evaluated by determining percentage inhibition of enzyme by the samples and IC_{50} values of each fraction were then calculated. Figure 4 showed concentration dependent inhibition of enzyme by samples. Our findings revealed that chloroform and ethyl acetate fractions of *P. nepalensis* are more potent enzyme inhibitors with IC_{50} values of 138.60 ± 6.66µg/mL and 183.67±3.75µg/mL respectively. Among the two lichens; *P. nepalensis* was found to be more promising to inhibit α-amylase.

Since very few studies had been carried out on α-amylase inhibitory activity of lichens.[32,33] This study might be the addition of one more step in the study of enzyme inhibition activity of lichens. Inhibitory activity might be due to the blocking of active site of the enzyme by the inhibitors such as phenolics, flavonoid and other compounds present in lichens as reported in previous works.[34]

Antibacterial activity

Agar well diffusion method was followed for determination of antibacterial activity of lichen extracts. Overall results are presented in Table 5. Five pathogenic bacteria were used for susceptibility test, among which *B. subtilis*, *E.coli* and *S. aureus* were found to be susceptible to both lichen extracts. Most promising antibacterial activity was shown by chloroform extract of *P. nepalensis* with effective zone of inhibition against *E. coli* (34 ± 1.41 mm), *S. aureus* (33.5 ± 2.12 mm) and *B. subtilis* (23.5 ± 2.12 mm). *K. pneumoniae* and *P. aeruginosa* were resistant to all extracts. Streptomycin used as positive control was sensitive to all tested bacteria while negative control DMSO had no effect.

Numerous researches had been carried out for the study of antimicrobial activity of different types of lichens all over the world. Pure lichen metabolites have also been screened for their antimicrobial sensitivity.[35] Antibacterial activity of chloroform extract of *P. nepalensis* against E. coli and S. aureus had drawn special attention and need to be studied further due to exceptionally highest zone of inhibition obtained. However, all fractions of both lichens were insensitive towards the *K. pneumonia* and *P. aeruginosa*.

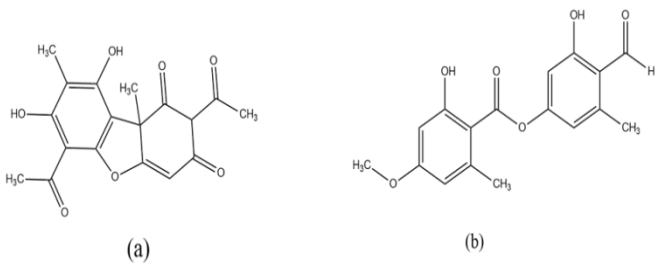

Figure 1: Chemical Structure of (a) Usnic acid (b) Evernic Acid.

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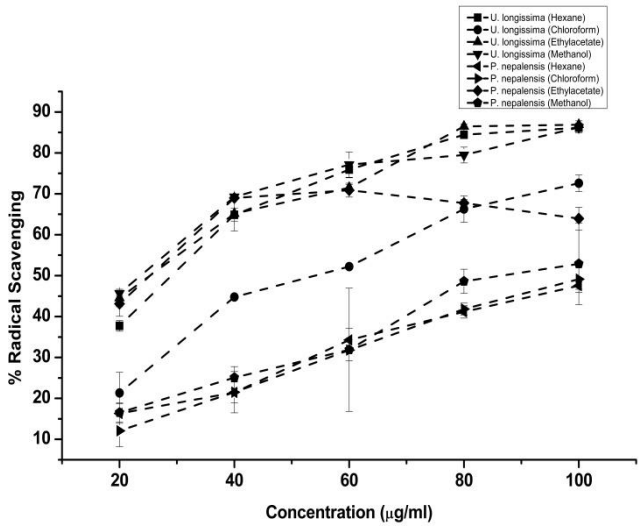

Figure 2: Percentage Inhibition of DPPH free radicals by different extracts of *U. longissima* and *P. nepalensis.*

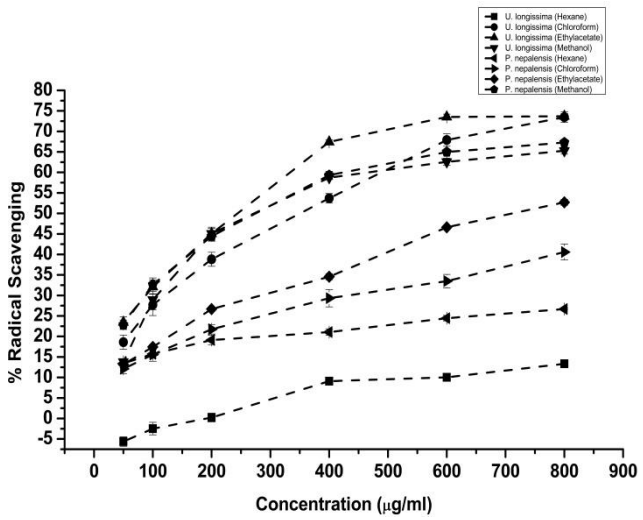

Figure 3: Percentage Inhibition of ABTS free radical by different extracts of *U. longissima* and *P. nepalensis*.

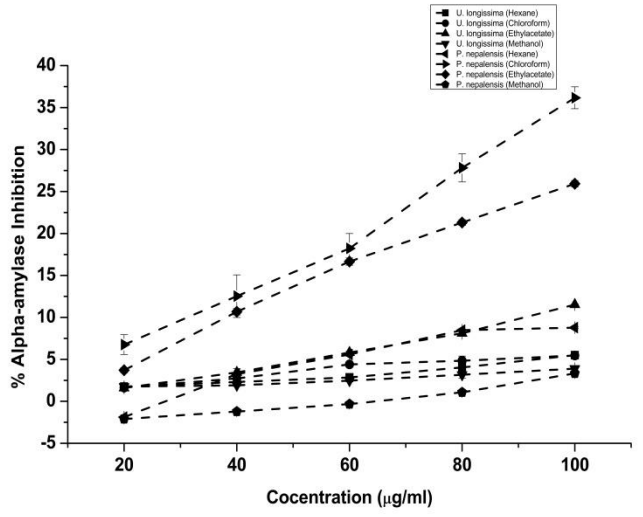

Figure 5: Percentage Inhibition of *α*-Amylase enzyme by different extracts of *U. longissima* and *P. nepalensis.*

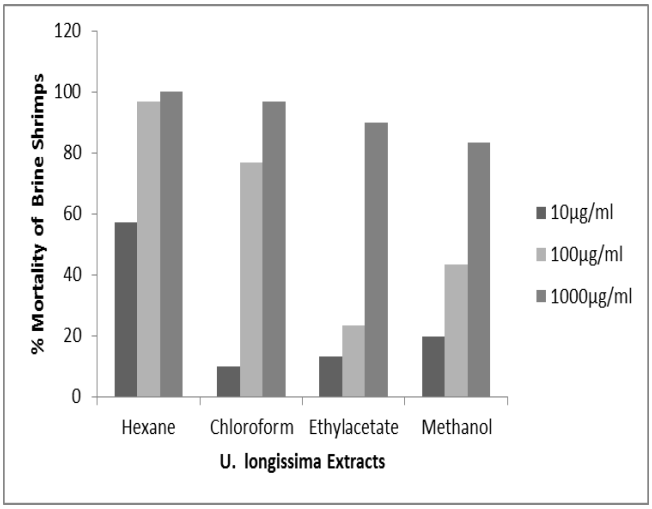

Figure 5: Percentage mortality of brine shrimps for different concentrations of extracts of *U. longissima*

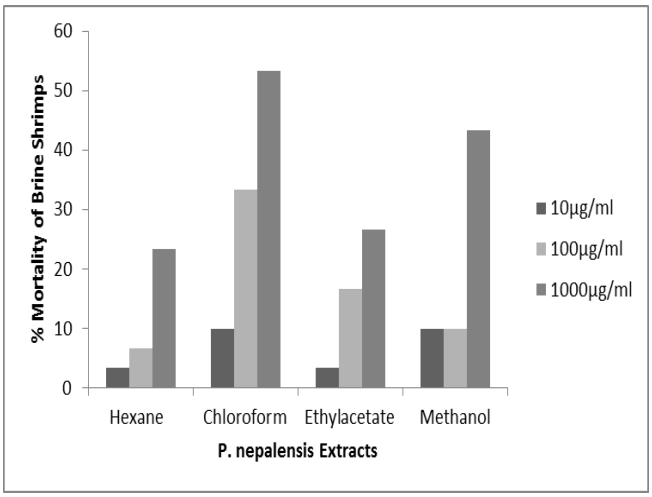

Figure 6: Percentage mortality of brine shrimps by different concentrations of extracts of *P. nepalensis*

The resistance of *K. pneumoniae* and *P. aeruginosa* to all the lichen extracts might be due to their complex cell wall structure and protection mechanism that crude extract could not damage.[36,37] The mechanism behind the antibacterial activity of lichen extract might be due to the halt of RNA and DNA synthesis[38] and inhibition of protein synthesis[39] of susceptible bacteria by the antibiotic compounds present in the lichen.

Cytotoxicity assay

Cytotoxicity of the lichen extracts were evaluated by performing Brine Shrimp Lethality assay. The 48 hours old shrimps were treated with three different concentrations of the samples in triplicate. Cytotoxicity level was determined by mortality rate of shrimps and LC_{50} values were calculated. Figure 5 and 6 show the concentration dependent cytotoxic activity of lichen extracts. Highest cytotoxicity was determined for hexane extract of *U. longissima* with LC_{50} value 8.974 µg/mL followed by Chloroform extract 47.794µg/mL. Methanolic and ethyl acetate extracts were found to be comparatively less toxic with LC_{50} values 107.94 µg/mL and 171.65µg/mL respectively. *P. nepalensis* extracts were found to be less cytotoxic compared to *U. longissima*.

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Earlier studies of cytotoxic activity reported that lichen extracts and their metabolites possess effective cytotoxic effects.[40] This study also strongly supported that lichen *U. longissima* possess strong cytotoxic effects due to the presence of active secondary metabolite Usnic acid, also supported by Ding *et al*.[41]

Table 1: Total percentage yield of extracts of *U. longissima* and *P. nepalensis*

Solvents used	*U. longissima*		*P. nepalensis*	
	Weight of extract (g)	Yield (%)	Weight of extract (g)	Yield (%)
Hexane	0.823	0.97	0.27	0.77
Chloroform	6.759	8.05	1.075	3.07
Ethyl acetate	0.436	0.51	0.676	1.93
Methanol	2.646	3.15	1.455	4.15

Table 2: Total phenolic content (TPC) and flavonoid content (TFC) of *U. longissima* and *P. nepalensis* crude extract.

	U. longissima		*P. nepalensis*	
	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Hexane	25.86 ± 0.40*	26.27 ± 2.20	10.36 ± 0.22	82.17 ± 4.28**
Chloroform	29.09 ± 1.11*	74.11 ± 4.50	14.71 ± 0.088	72.77 ± 9.06**
Ethyl acetate	43.88 ± 0.42	171.13 ± 9.04	32.18 ± 0.97	151.53 ± 5.49***
Methanol	32.59 ± 0.51	107.85 ± 2.60	39.68 ± 0.76	149.31 ± 8.86***

[Note: All the values are significantly different at $p < 0.05$ except the values marked with equal number of sign*]

Table 3: Spearman's rho Correlation coefficient of antioxidant activity with TPC and TFC of *U. longissima* and *P. nepalensis*

Assay	Correlation (R^2)			
	U. longissima		*P. nepalensis*	
	TPC	TFC	TPC	TFC
DPPH Assay	0.900**	0.733*	0.940**	0.577*
ABTS Assay	0.333	0.683*	0.374	0.681*

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Table 4: DPPH, ABTS, α-amylase inhibition and Brine Shrimp lethality assay of *U. longissima* and *P. nepalensis*

Test Samples	Extract	DPPH Assay IC_{50} (µg/mL)	ABTS Assay IC_{50} (µg/mL)	α-Amylase Inhibition Assay IC_{50} (µg/mL)	Brine Shrimp Lethality Assay LC_{50} (µg/mL)
U. longissima	Hexane	2035.46 ± 3.81	28.86 ± 0.25	1001.79 ± 13.13	8.974
	Chloroform	401.68 ± 5.74	9.45 ± 0.20	940.91 ± 17.55	47.794
	Ethyl acetate	219.49 ± 3.99	11.25 ± 0.59*	412.98 ± 3.59	165.96
	Methanol	261.16 ± 2.08	10.26 ± 0.19*	742.54 ± 71.7	107.655
P. nepalensis	Hexane	2045.52 ± 42.20	23.75 ± 0.11**	371.55 ± 5.89	19245.97
	Chloroform	1006.16 ± 15.20	24.91 ± 1.42**	138.60 ± 6.66	656.71
	Ethyl acetate	696.56 ± 7.93	2.39 ± 0.18	183.67 ± 3.75	4531
	Methanol	270.75 ± 9.30	21.52 ± 1.10	783.51 ± 7.36	1315.82
Standard	Gallic Acid	5.54 ± 0.12	1.74 ± 0.11		

Note: All the means are significantly different at $p < 0.05$ except for those denoted by same number of *

Table 5: Zone of inhibition shown by *U. longissima* and *P. nepalensis* against different pathogenic bacteria

Test Samples		Zone of Inhibition (mm)				
		B. subtilis	***S. aureus***	***E.coli***	***K. pneumonia***	***P. aeruginosa***
U. longissima	Hex	16.00 ± 1.41	15.66 ± 1.15	15.33 ± 2.51	ND	ND
	$CHCl_3$	24.50 ± 0.70	6.33 ± 0.57	5.33 ± 0.57	ND	ND
	EtOAc	16 ± 0.40	10.33 ± 0.57	9.33 ± 1.52	ND	ND
	MeOH	17.00 ± 1.41	10.33 ± 0.57	9.66 ± 1.52	ND	ND
P. nepalensis	Hex	23 ± 0.20	14.33 ± 0.55	13 ± 0.10	ND	ND
	$CHCl_3$	23.50 ± 2.12	33.50 ± 2.12	34.00 ± 1.41	ND	ND
	EtOAc	17.50 ± 0.7	11.00 ± 2.82	7.50 ± 0.70	ND	ND
	MeOH	6.00 ± 1.41	ND	ND	ND	ND
Streptomycin		17.00 ± 1.42	17.00 ± 2.00	16.30 ± 0.58	17.66 ± 0.58	17

[Note: (ND) = Not Detected, Hex- Hexane; $CHCl_3$- Chloroform; EtOAc-Ethyl acetate; MeOH-Methanol Concentrations used; Lichen extracts = 20 mg/mL, Streptomycin = 100 µg/mL]

Conclusion

This study revealed that lichen collected from Sagarmatha National Park of Solukhumbu District of Nepal possess significant biological activities such as antioxidant, alpha-amylase, cytotoxic and antimicrobial activities. Also these lichens are good source of Usnic acid and evernic acid.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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