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Research Article

Antidiabetic and Antimicrobial Properties of Some High Altitude Medicinal Plants of Nepal

Rachana Regmi[1], Mitesh Shrestha[2], Megha Raj Banjara[1], and Deegendra Khadka[3*]

[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathamandu, Nepal
[2]Research Institute for Bioscience and Biotechnology, Ekantakuna, Lalitpur, Nepal
[3]Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal

Abstract: Regarding severe side effects caused by synthetic drugs, studies were carried out taking high altitude medicinal plants to evaluate antidiabetic and antimicrobial activities. Antidiabetic property was measured by the inhibition protein tyrosine phosphatase 1B (PTP1B) enzyme with plant extract taking *p*-nitrophenyl phosphate (*p*NPP) as a substrate in the assay. For antimicrobial activity, plant extracts were tested against four pathogenic bacteria; *Staphylococcus aureus, Escherichia coli, Klebisella pneumonia* and *Pseudomonas aeruginosa*. BA17 appeared to be more effective for the inhibition PTP1B enzyme (95-100%) at various concentrations. BU17 showed the maximal zone of inhibition of 16 mm on three microbes in *S. aureus, E. coli*, and *K. pneumonia* at 100 mg/mL.

Keywords: Medicinal plants, Antimicrobial, Protein Tyrosine Phosphatase 1B, High altitude, Type 2 Diabetes Mellitus.

1. INTRODUCTION

Nepal is well known for its enriched biodiversity and of course, herbal plants affluent. Solely on Nepal's alphine zone, more than ten thousand medicinal plants have been covered. In the range of 1,792 to 2, 331 numbers of both aromatic and medicinal plants have been recorded based on their utilization in healing human ailments. Local people have practiced such plants for livelihood, conventional therapies and home remedies since ancient times [1]. Several findings unveiled that the plants are the robust sources of drugs. Hence, medicinal plants are pillar in both traditional and modern medical interventions, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [8]. Compounds having antimicrobial activities are found abundantly in several medicinal plants and used medicinally around the globe as a source of potent drugs [15]. In drug extraction process, various parts of plants are used which include root, stem, flower, fruit, twigs exudates and modified plant organs. Local citizens collect raw drugs material in limited quantities for self-therapeutic purposes while pharmaceutical companies need huge quantity for producing medicine in bulk.

The cause and cure of type 2 diabetes mellitus (T2DM) are still mysterious; however, genetic factors and sedentary living style play crucial role in developing T2DM. Regular monitoring blood glucose level is the appropriate way to get informed before being life threatening. Synthetic drugs for diabetes are scattered in the global market. Among them, drugs like metformin, alpha-glucosidase, thiazolidinediones, and sulfonylurea are currently available in the market. Despite having side effects, people have no other options but rely on these medicines [2]. Mainly the family like Compositae, Berberidacae, Liliaceae and Papaveaccae are renowned for their medicinal value. An alternative of synthetic drug would be natural product which is relatively safer.

The antibiotics typically used in the treatment of human diseases are produced from the bacteria as a mechanism of competition to ensure their

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* Corresponding Author: Deegendra Khadka; deegendra.khadka@nast.gov.np

own survival. Resistivity of microbes in natural environment like soil is developed either by specific mutation or exchanging genetic information (including resistance genes). So, it is easy going to get permission while transmitting the resistance to other microbes [9]. One of the major causes of becoming infectious diseases treatment less effective in the world is the emergence of bacterial strains that exhibit resistance to a variety of antibiotics. A bacterium has also called "superbug" because merely a few antibiotics are available to cure bacterial infected diseases. Using antibiotic widely both for human consumption and animal feed facilitates in development of resistance to a variety of gram-negative and gram-positive pathogenic bacteria [7].

To beat the risk factors generated by the consumption of synthetic drugs in the treatment of diabetes and bacterial infections, natural products would be the effective way for the health management. Our team selected high altitudinal medicinal plants for this study. It is believed that the plants grown up in harsh climatic conditions can produce relatively different bioactive compounds which may be the target molecule for the drug development. The main aim of the study is searching a novel natural compound that possesses antidiabetic property and also finding potent natural products having antibacterial characters.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted at Molecular Biotechnology Laboratory, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal. Details of ten selected high altitude medicinal plants with different parts like stem, leaves, roots and their collection are described in **Table 1**. Chemicals were purchased from Sigma-Aldrich (Merck).

2.2. Plant Extract Preparation

Plants including leaves, stem and roots were air dried in shade place for a month. The dried plant materials were ground into the fine powder using grinding machine. For the preparation of plant extract, 21 gram of fine powdered plant matter was dissolved in 150 mL of absolute methanol at room temperature for two successive days. Each day, the dissolved parts were filtered using the Whatman no. 1 filter paper and stored in glass bottle. The final collection of dissolved parts was then evaporated at reduced pressure at 50°C using a rota-evaporator. These obtained solid mass was weighed carefully to express the gram of extract per 100 g of the plant powder. For each sample, extract was prepared individually. Similarly, the extracts were obtained

Table 1. Description of high altitude medicinal plants used in the experiment

S.No.	Scientific name	Vernacular name	Code number	% Yield (MeOH)	Collection sites	Used plants part
1.	*Berberis asiatica*	Chutro	BA17	35	Low camp, Mardi Himal	Leaves and stem
2.	*Betula utilis*	Bhojpatra	BU17	45	Low camp, Mardi Himal	Leaves and stem
3.	*Cassia fistula*	Rajbriksha	CF17	38	Dhangadi, Kailali	Leaves and stem
4.	*Cassia spp.*	-	CS17	30	Dhangadi, Kailali	Leaves
5.	*Murraya koenigii*	Curypatta	MK17	36	Dhangadi, Kailali	Leaves
6.	*Nardostachys grandiflora*	Jatamashi	NG17	30	Low camp, Mardi Himal	Leaves
7	*Neopicrorhiza scrophulariiflora*	Kutki	NS17	43	Mai Pokhari, Ilam	Leaves
8	*Rheum austral*	Padamchal	RA17	35	Dhangadi, Kailali	Leaves and stem
9	*Rhododendron anthopogen*	Laliguras	RhA17	55	Low camp, Mardi Himal	Leaves and stem
10	*Macropanax undulates*	Chenday	MU17	54	Low camp, Mardi Himal	Leaves and stem

from the solvent hexane and ethyl acetate. The extracts were kept at 4°C for further analysis [16].

Percentage Yield (%) of extract = (Dry weight of extract/ Dry weight of plant material) × 100

2.3. *In vitro* PTP1B Enzyme Assay

In the enzyme assay, the PTPase activities were calculated by *p*NPP assay. In this assay, buffer A is composed of the mixture of HEPES (100 mM) and EDTA (5 mM) maintaining pH 7.0. The reaction is carried out at 37 °C using *p*NPP of 1M concentration. The enzyme is diluted with enzyme dilution buffer (25 mMHepes, 5 mM EDTA, 1 mM DTT, 1 mg/ mL bovine serum albumin, pH 7.3). Inibihitors that is plant extract of concentration 250, 500, 750 and 1000 µg/ mL were dissolved in Dimethyl sulfoxide (DMSO). The absorbance at 405 nm was measured to calculate the quantity of *p*-nitro phenol expelled out.

2.4. Inhibition Studies

For inhibition studies, enzyme was diluted in the enzyme dilution buffer (2.2µL of enzyme and 107.8µL of enzyme dilution buffer). Reaction mixture i.e. bulk was prepared which contain 250µLof water, 100µL of 5x reaction buffer with DTT and 50µLof diluted enzyme. PTP1B activity was measured by the addition of 5 µL of 1M *p*NPP (as substrate) in the reaction mixture along with or without different concentrations i.e. 250, 500, 750 and 1000 µg/mL of inhibitors. After incubation for 10 min at 37°C, the reactions were halted with 950 µL of 0.5M Sodium Hydroxide (NaOH). The amount of *p*-nitro-phenol formed was measured by UV absorbance at 405 nm [3]. The percentage inhibition of PTP1B was calculated as follows:

$$\% \text{ inhibition} = [(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$$

Where, Abs_{blank} is absorbance of the blank and Abs_{sample} is absorbance of the sample [17].

2.4.1 Determination of Antibacterial Activity

2.4.4.1 Preparation of the standard bacterial culture inoculums

Four active bacterial strains namely *Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC700603) and Pseudomonas aeruginosa (ATCC 27853*) were used. Three or four isolated colonies of each strain were inoculated in the 5 mL nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard (0.5 %). The inoculated culture bottles were kept in the incubator at 37 °C for 3-4 hours. The turbidity of the sub-cultured bacterial suspension was adjusted at 0.5 % McFarland standards (freshly prepared a day before the experiment). These bacterial inoculums were used for the swabbing on the Muller Hinton agar (MHA) plates to test the antimicrobial effects of plant extracts.

2.4.4.2 Antibacterial Activity Assay

For antibacterial activity assay, the well diffusion method on MHA media procedure was used to evaluate effectiveness of plant extracts against bacterial activity [3]. While making wells on MHA media, a cork borer having 6 mm in diameter was used. Bacterial inoculums with the concentration of 10^6 CFU/mL were spread on the solid media with a sterile cotton swab. 20 µL of the working solution of plant extract with the concentration of 100, 50, 25 and 12.5 mg/mL and same volume of extraction solvent (methanol and DMSO) was used as negative control, whereas 1mg/mL streptomycinas positive control was filled in the wells with sterile micropipette. Plates were left for some time till the extract diffused into the medium with the lid closed and incubated at 37°C for 24 h. After overnight incubation the plates were observed for the zone of inhibition (ZOI) and the diameter of the inhibition zone were measured using scale.

3. RESULT

BA17 and MK17 have high inhibitory potential against PTP1B. This plant extract has a potential property to be used against the treatment of the disease associated with the over activity of the PTP1B. The BA17 and MK17 can be the possible sources for the treatment of T2DM in traditional medicine. Among ten medicinal plants sample, BA17 showed promising result against the inhibition of PTP1B as shown in **Fig. 1. Table 1** shows summary of plant sample with their code numbers.

Methanolic plant extracts showed the zone of inhibition against microbes ranged the concentration

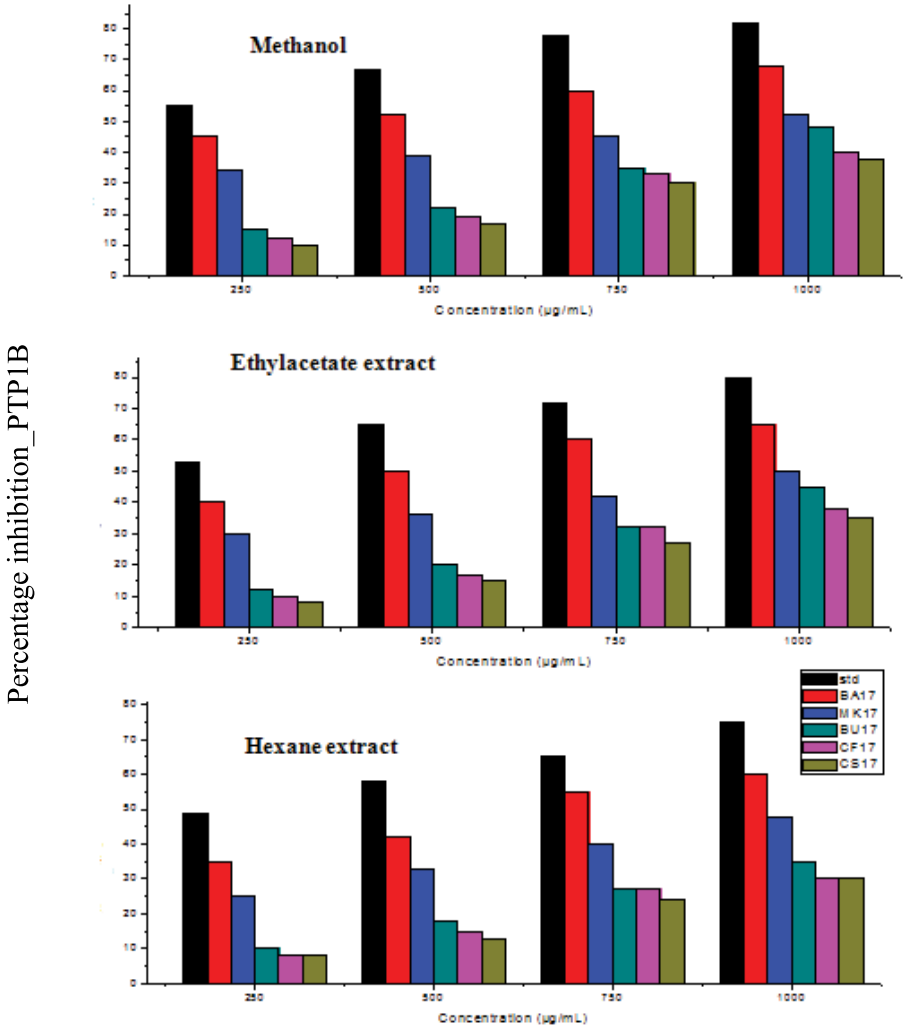

Fig. 1. Inhibitory effects of extracts of the selected plants on PTP1B activities

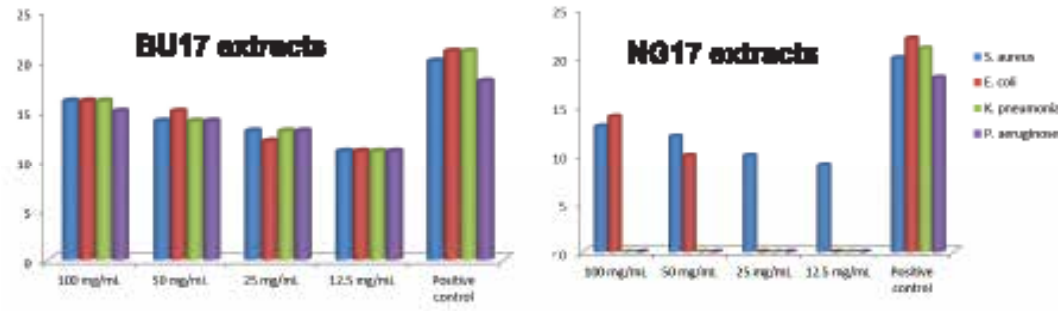

Fig. 2. Zone of Inhibition (ZOI) of BU17 and NG17 plant extracts against four different bacterial strains

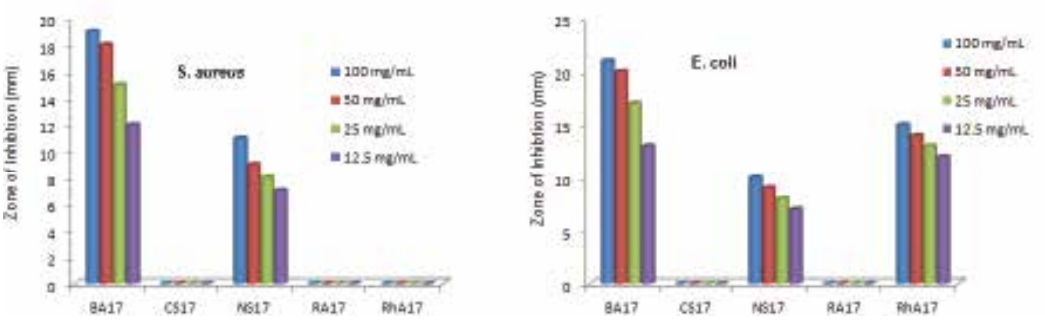

Fig. 3. Zone of Inhibition (ZOI) of five plants extracts against S. aureus and E. coli bacterial strains

6.25 mg/mL to 100 mg/mL. BU17 plant extract showed the highest zone of inhibition of 16 mm, 14 mm, 13 mm and 11mm on *S. aureus* at 100, 50, 25 and 12.5 mg/mL concentration respectively and the detailed on other bacterial strains are depicted in the **Fig.2** and **Fig. 3.**

4. DISCUSSION

In this study, plants having medicinal values were selected from higher altitudes of Nepal. Higher altitude plants which are locally used as medicine are selected because they are rich sources of the bioactive compounds in the view of their growth in stressful conditions [11]. Climatic conditions, parts of the plant used, extraction time, and temperature and extraction procedure (including the solvent selection) plays a vital role in isolating bioactive compounds that have pharmacological activity [10]. In this study, selected plants were freshly collected and shade dried to avoid the loss of the bioactive compound. Drying in the artificial environments at low temperature reduces the loss of large moisture content and prolonged storage time [6].The dried sample were ground mechanically and subjected to solvent for extraction. Methanol is the solvent used for the extraction because alcohol solvent presumably ruptures the cell membrane and extracts greater amount of endo-cellular materials [12].

Overexpression of PTP1B is associated with the resisting insulin molecule to bind on insulin receptor and eventually glucose molecule are unable to get inside the cells which could lead elevating blood glucose level, called T2DM. PTP1B enzyme has been identified as one of the primary drug targets for treatment of T2DM [13]. It has been reported that natural inhibitors like berberine, and iso-quinoline alkaloid possess potent antidiabetic properties to inhibit PTP1B enzyme [5]. Papaverine, a structural analog of berberine, which belongs to member of iso-quinoline alkaloids have also exhibit potent PTP1B inhibitory activity thereby lowering fasting blood glucose level *in vivo* [4]. BU17 plant contains alkaloids like berbamine and berberine which may be responsible for the highest inhibitory activity. Although hypoglycemic effects of some of the plants have been reported, the mechanism of action has not been fully elucidated [14]. BU17*,* MK17 and NS17 have greater inhibitory effect on PTP1B. This study showed that BU17and MK17 can be better inhibitors of PTB1B in the future for the treatment of T2DM.

BA17, NS17 and RhA17plant extracts showed higher zone of inhibition for *S. aureus* and *E. coli.* But they did not display any inhibition zone on *K. pneumoniae* and *P. aeruginosa*. The CS17 plant extract showed nearby zero inhibition zones on *S. aureus, E. coli*, *K. pneumonia* and *P. aeruginosa*. Gram-negative bacteria (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) had been found to be less susceptible to plant extracts than Gram positive (*S. aureus*). Gram-positive bacteria are highly sensitive in comparison to gram-negative bacteria because of possessing less effective permeability barrier of the outer peptidoglycan layer.

5. CONCLUSION

Methanolic extract of different higher altitudinal plants possess inhibitory potential against the PTP1B, especially the extract of BA17 and MK17 possess significant inhibition which indicates possible option for the treatment of the diseases associated with the over activity of the PTP1B

for example T2DM. BU17 showed the promising antibacterial activity against *S. aureus*, *E. coli, K. pneumoniae* and *P. aeruginosa* revealing as possible alternative to antibiotics.

6. ACKNOWLEDGEMENT

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