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ANTIBACTERIAL ACTIVITIES OF METHANOLIC BARK EXTRACTS OF *BERBERIS ARISTATA* AND *BERBERIS ASIATICA* FROM DIFFERENT ELEVATIONS OF CHAMPADEVI HILL, NEPAL

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ABSTRACT

Berberis is the largest genus of the family Berberidaceae which grows in almost all vegetation type throughout Nepal from 1000-4600 m. Most of the *Berberis* species found in Nepal have medicinal uses. Methanolic extracts of *B. aristata* collected from two elevations (1900 and 2400 m) and *B. asiatica* from three different elevations (1400 m, 1900 m and 2400 m) were analyzed for antibacterial activities against four bacterial strains (ATCC). Agar well diffusion technique was used for antibacterial screening and inhibition was observed for plant extracts of different concentrations ranged from 6.25 mg/ml to 100 mg/ml, Gentamycin (+ve control), and DSMO (-ve control). Methanolic extracts from both species from different elevations showed antibacterial activity against gram positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) as well as gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Antibacterial activity was found decreased with increase of elevation for almost all concentrations of bark extracts from both *Berberis* species. However, comparison between antibacterial activities of bark extracts from two elevations (1900m and 2400m) against all bacterial strains except *E. coli* showed higher ZOI for *B. asiatica.* In case of *E. coli*, the higher ZOI was obtained for *B. aristata*. The results of the present study supported the idea that production of secondary metabolites in plants against microbes depends on possibility of infection which is more or less high in lower elevations in comparison to higher elevations.

Key words: Antibacterial activity, *Berberis*, elevation, nutrient broth, *Staphylococcus*

INTRODUCTION

The planet hosts a vast diversity of plant species distributed across various geographic regions. Approximately 391,000 vascular plant species are currently recognized, including 369,000 angiosperms, as reported by the Royal Botanical Gardens, Kew, UK (Kew, 2016). Of these, about 75,000 species are used in medicinal systems, with over 20,000 higher plant species specifically utilized in traditional treatments by indigenous cultures worldwide (Prakash, 1998). The Nepal Himalaya, known for its exceptional biodiversity, harbors around 10,167 plant species, including 7,000 flowering plants (Shrestha et al., 2000).

Berberis, an angiosperm, is known to use for the cure of different ailments. Every part of the plant such as root, bark, stem and fruits are used in various ayurvedic preparations (Bhattacharjee, 1990). Among the plant parts, stem and root were studied more for their Asian species viz. *B. aristata*, *B. lycium* and *B. asiatica* (Bhardwaj and Kaushik, 2012). Extracts of *Berberis* are used in ophthalmic problems, to treat jaundice, malarial fever, diarrhoea and peptic ulcers (Manandhar, 2002). It also has febrifugal, hypotensive, immuno-stimulating, anti-inflammatory, antimicrobial properties (Musumeci *et al*., 2003). Experimental observation declared that the methanolic as well as ethanolic extracts from stem and root of *B. aristata* respectively, were effective against various bacterial and fungal strains (Lamichhane *et al*., 2014; Mazumder *et al*., 2011; Sharma *et al*., 2008; Shahid *et al*., 2009; Kumar *et al*., 2007; Dar *et al*., 2014). More specifically, berberine compound (alkaloid) isolated from the stem extract of four *Berberis* species (*B. aristata, B. asiatica, B. chitria and B. lycium*) were found effective against different bacterial and fungal strains (Singh *et al*., 2009; Okunade *et al*., 1994).

Antimicrobial activity in plants is primarily attributed to secondary metabolites such as alkaloids, phenolics, and flavonoids (Zaynab et al., 2018). The production of these metabolites varies significantly, often influenced by environmental factors such as elevation and soil properties (Zargoosh et al., 2019). This study hypothesizes that methanolic bark extracts of *Berberis aristata* and *Berberis asiatica*, collected from different elevations, may exhibit distinct antibacterial potentials. Consequently, the primary objective of this research was to evaluate and compare the antibacterial efficacy of these extracts against selected bacterial strains.

MATERIALS AND METHODS

Collection and preparation of plant material

Two species of *Berberis*, *B. asiatica* and *B. aristata*, were collected from different elevations on Chandragiri Hill, Kathmandu, Nepal. *B. aristata* was obtained from elevations ranging between 1900m and 2400m, while *B. asiatica* was collected from three distinct elevations: 1400m, 1900m, and 2400m. The collected specimens were prepared as herbarium samples and deposited in the Tribhuvan University Central Herbarium (TUCH). The bark of the plants was peeled, dried

in the shade for three weeks, and then ground into a fine powder for subsequent extraction.

Extract preparation and dilution

Ten grams of powdered bark from each sample were mixed with 100 ml of methanol in a vial and subjected to sonication (UC-7240BDT, E-Chrome Tech, Taiwan) for 2 hours. The mixture was then filtered through Whatman No. 1 filter paper. The filtrate was left to evaporate at room temperature, following the sonication method. The resulting crude extract was subsequently diluted, with 100 mg of each sample dissolved in 1 mL of dimethyl sulfoxide (DMSO). This stock solution was stored at 4°C and used for antimicrobial screening.

Antimicrobial Activity

Preparation of Culture media

Nutrient Broth media (HI-media laboratories Pvt. Ltd., Mumbai India) was prepared by dissolving 12.5 gram of NB powder in 500 mL distilled water. The media was transferred to the screw capped bottles and sterilized by autoclaving at 15 lbs and 121ºC for 15 minutes. Finally, the suspension media was cooled and used for bacterial culture. Similarly, Mueller Hinton Agar (MHA) media was prepared by using 19 gram of MHA powder (HiMedia Laboratories Pvt. Ltd, Mumbai, India) suspended in 500 mL distilled water. The content was heated to boiling to make media dissolve completely. The media was sterilized by autoclaving at 15 lbs and 121ºC for 15 minutes. The media was poured on sterile petridishes under aseptic conditions for further purposes.

Microorganisms

Two gram positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228) and two gram negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were used for antibacterial assay. Three bacterial strains namely *S. aureus, E. coli and P. aeruginosa* were acquired from Sukra Raj Tropical & Infectious Disease Hospital, Teku, Kathmandu while *S. epidermidis* was acquired from City Hospital, Kalanki, Kathmandu.

Preparation of the standard culture inoculums

The individual pure ATCC cultures of bacteria were streaked on the different nutrient agar plates. Those plates were incubated at 37º C for about 24 hours to obtain pure and isolated colonies. Each distant colony was aseptically transferred to the Nutrient broth for the suspension culture with the help of sterilized inoculating loop. The inoculated bottles were kept on the shaking incubator at 37º C and 150 ppm for overnight. The turbidity of the bacterial suspension was

adjusted at the 0.5 McFarland standards for the antibacterial test. These inoculums were used for the swapping of the plates to test the antimicrobial effects of the plant extracts.

Transfer of bacteria on petriplates

The agar plates for the assay were prepared by labeling them with the date, name of bacteria, name code of plant samples and the concentration of plant samples. The inoculums of bacteria were transferred into Petri dish containing solid nutrient media of MHA using sterile cotton swab. The sterile cotton swab was dipped into well mixed saline test cultures and removed excess inoculums by passing the saturated swab against the inner wall of the culture tube. The swab was used to spread the bacteria on the media in a confluent lawn. One swab was used for one species of bacteria. The culture plates were allowed to dry for few minutes.

Antibacterial screening via agar well diffusion technique

The antibacterial test was performed by modified agar well diffusion method as suggested by Perez *et al*., (1990) with slight modification. Six wells were prepared on the solid MHA media with the help of sterile corkborer (5 mm diameter) and labeled properly with sterile marker pen. Five different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) of the plant samples were prepared in the DMSO. With the help of sterile micropipette 25 µL of each individual plant extract was poured in the above prepared well. The DMSO was taken as the negative control while the gentamycin disc at concentration of 10 µg was taken as the positive control. The plates were incubated overnight at 37º C and zone of inhibition was observed and noted for individual plant extract of individual bacteria for different concentrations. A triplicate of each sample for each bacterial strain was taken for assurance of unbiased data.

Data analysis

All the experiments were performed in triplicates for each sample and values were reported as mean ± S.D. All the statistical analysis was done using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Antibacterial activities of the methanolic bark extracts of *Berberis* aristata and *B. asiatica* from different elevations were tested against four ATCC pathogenic bacteria namely *E. coli, P. aeruginosa, S. aureus,* and *S. epidermidis*. Dimethyl

sulphoxide (DMSO) was used as negative control and Gentamycin (10µg) as positive control. The bark extract of both species of *Berberis* from different elevations against four bacterial strains showed quite a good inhibition zone. Measured zone of inhibition for both gram positive and gram negative bacteria were expressed in terms of mm including 5mm diameter of well (Fig. 1-4). Increased zone of inhibition was observed relatively with increase in concentration of the extract. Five concentrations of 100µg/mL, 50µg/mL, 25µg/mL, 12.5µg/mL, 6.25µg/mL were used during the test. Positive control (Gentamycin) showed highest inhibition zone (22mm) against *E. coli* while lowest inhibition zone (19mm) against *S. aureus*. DMSO doesn't showed inhibition zone against all the bacteria confirming it has no antibacterial activity against those bacteria. Thus, the inhibition zone showed by different concentration of extract was taken as due to the virtue of extract alone (Supple. Table 1 and 2).

More specifically, methanolic bark extracts of *B. aristata* and *B. asiatica* showed the zone of inhibition (ZOI) against *E. coli* in all concentration ranged from 6.25mg/mL to 100 mg/mL. Antibacterial activity was found decreased with increase of elevation for almost all concentrations of both bark extracts. However, a comparison between the antibacterial activities of both species at two elevations (1900 m and 2400 m) showed higher values of ZOI for *B. asiatica* (Figure 1).

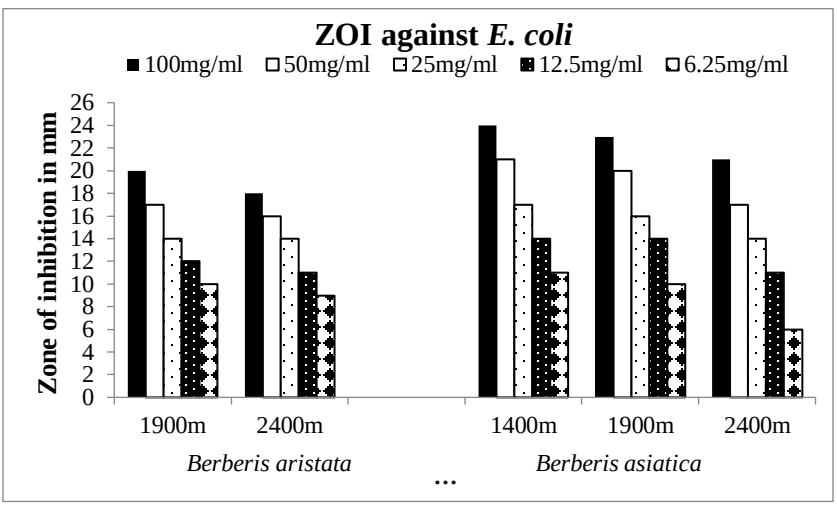

Figure 1. Zone of inhibition of *Berberis aristata* and *B. asiatica* methanolic bark extracts from different elevations against the bacterial strain *E. coli*

Similarly, both extracts showed the zone of inhibition against *P. aeruginosa* in all concentration types except 6.25mg/mL. Antibacterial activity was found decreased with increase of elevation for both bark extracts. In contrast to *E. coli*,

higher antibacterial activity was recorded for *Berberis aristata* at three concentrations (25 mg/mL to 100 mg/mL) in two similar elevations (1900m and 2400m) (Figure 2).

In case of gram positive bacteria *S. aureus*, bark extract of *B. aristata* showed zone of inhibition in all concentrations except 6.25 mg/mL which was found decreased in higher elevation at different concentrations. However, the response of bark extract of *B. asiatica* showed almost similar range of ZOI in different elevations except for 100 mg/mL where it showed decrease in ZOI from lower to higher elevations (Figure 3). In case of lowest extract concentration (6.25 mg/mL) ZOI was only recorded from lower elevation (1400m) for *B. asiatica* (Figure 3). The bark extracts of *B. aristata* and *B. asiatica* collected from different elevations showed ZOI against *S. epidermidis* in all concentration ranged except 6.25mg/mL. Bark extract of *B. asiatica* from middle elevation (1900m) showed antibacterial activity even at lowest concentration (6.25mg/mL). Both extracts from lower elevation were found to show higher antibacterial activity, although, *B. asiatica* seemed to have greater antibacterial potential than that of *B. aristata* (Figure 4).

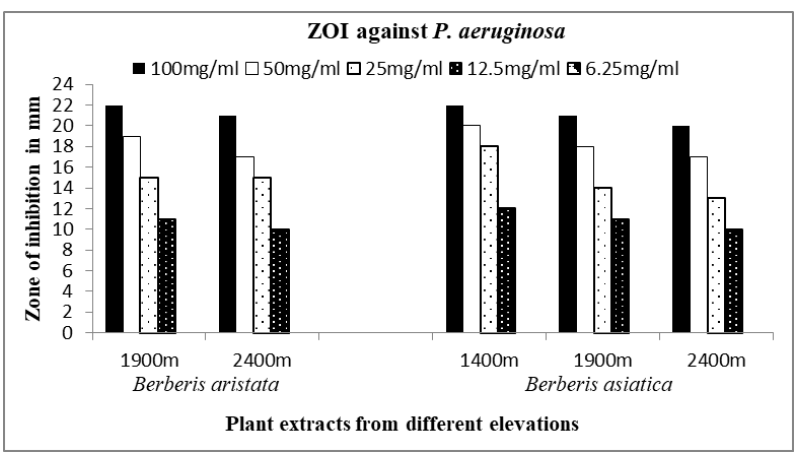

Figure 2. Zone of inhibition of *Berberis aristata* and *B. asiatica* methanolic bark extracts from different elevations against the bacterial strain *P. aeruginosa*

Angiospermic plants produce secondary metabolites, especially antimicrobial compounds to act like a defense mechanism against microorganisms. On the contrary, microorganisms have the genetic ability to mutate and acquire resistance to antibiotics and have become a major global health problem. This compelled the scientists to search out new drugs from plant origin (Khoobchandani *et al.*, 2010). Flavonoid compounds exhibit inhibitory effects against Bacteria. Flavonoids, hydroxyl group on their ß- rings are more active against microorganisms and have

also been found that the more hydroxylation, more the antimicrobial activity (Sato *et al*., 1996). The hydroalcoholic extract of *Berberis* and alkaloid (Berberine) has stronger and broader spectrum as compared to fungal strain (Singh *et al*., 2007).

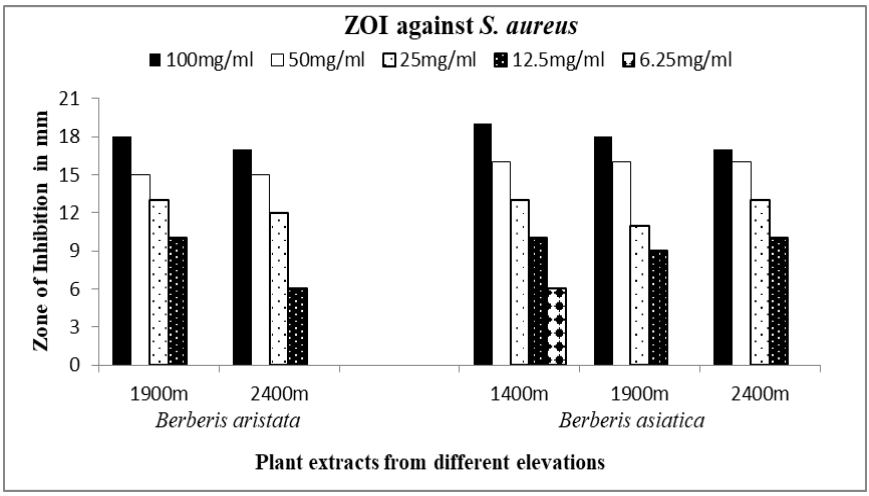

Figure 3. Zone of inhibition of *Berberis aristata* and *B. asiatica* methanolic bark extracts from different elevations against the bacterial strain *S. aureus*

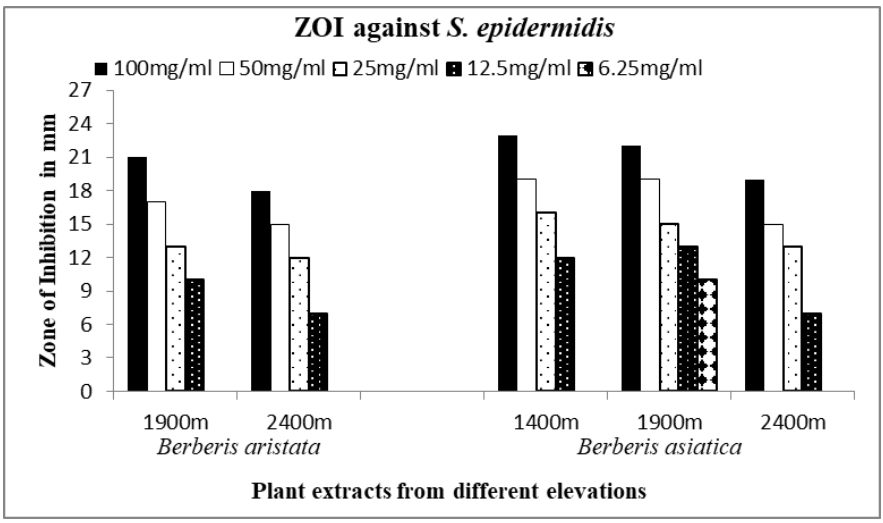

Figure 4. Zone of inhibition of *Berberis aristata* and *B. asiatica* methanolic bark extracts from different elevations against the bacterial strain *S. epidermidis*

Stem bark extract of selected species *Berberis* under study showed potential antibacterial activity against the gram positive (*Staphylococcus aureus* and

Staphylococcus epidermidis) as well as gram negative bacteria (*Esterichia coli* and *Pseudomonas aeruginosa*). Thus, present outcome shows similarity with the outcome of Dar *et al*. (2014) that the methanolic extract of *Berberis aristata* stem is highly active against both Gram positive and Gram negative bacteria. Experimental observation declared that the *Berberis aristata* stem extract was sensitive against *Candida albicans, Salmonella typhii, Pseudomonas aeruginosa* and *Escherich coli*, while it didn't show any activity against *Klebsiella pneumoniae, S. aureus* (Lamichhane *et al*., 2014) which is quite similar to the present result however inhibition zone was also obtained against *S. aureus* in present study. The high inhibition zone was obtained against *Esterichia coli* while low inhibition zone was obtained against *Staphylococcus aureus*. Difference in the area of collection of plant along with maturity of plant, collection time, extraction set up, extraction time might have contributed to this varied result.

Stem extract of *Berberis asiatica* tested against the Gram-negative bacteria, *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Proteus mirabilis, P. vulgaris, Salmonella paratyphi-A, Shigella dysenteriae-1* and *Pseudomonas aeruginosa* showed zone of inhibition. *Pseudomonas aeruginosa*, however, had a considerably large zone of inhibition (Bhandari *et al*., 2000) which is comparable to result from this study. Alcoholic root extract of *Berberis aristata* was effective against *Staphylococcus aureus, Staphylococcus epidermidis* and *Esterichia coli* while ineffective against *Pseudomonas aeruginosa* (Shahid *et al*., 2009) which is similar to result of this study however bark extract used in present study also shows effectiveness against *Peudomonas aeruginosa*. This variation may arise due to use of different plant parts and moreover other factors mentioned earlier may also affect the result.

CONCLUSION

The study highlights the antibacterial potential of methanolic bark extracts of *Berberis aristata* and *Berberis asiatica* collected from varying elevations of Champadevi Hill, Nepal. The findings suggest a negative correlation between antibacterial activity and elevations, as extracts from lower elevations exhibited stronger antibacterial effects. This trend may be attributed to the higher production of secondary metabolites, such as flavonoids and alkaloids, in plants growing at lower altitudes, where warmer conditions and higher microbial activity might drive the need for enhanced chemical defenses. Among the two species, *B. asiatica* generally outperformed *B. aristata* in antibacterial efficacy, except against *E. coli*, where *B. aristata* showed better performance. These results underscore the influence of environmental factors on the bioactive properties of medicinal plants and their potential as sources of natural antibacterial agents.

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Supplementary Tables

Supplementary Table 1. Antibacterial activity of *Berberis asiatica* and *B. aristata* from different elevations against two gram negative bacterial strains

Samples*	Tested organism	Zone of inhibition in mm (with diameter of well 5 mm)						
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	+Ve control	-Ve control
BAS-1400m	*E. coli*	24.3±1.1	20.6±0.5	15.0±3.0	12.6±2.0	10.0±1.0	22	0
	P. aeruginosa	22±2.6	19.6±2.5	18.3±3.0	13.6±1.1	11.3±0.5	21	0
BAS-1900m	*E. coli*	22.6±1.1	20.0±2.0	16.0±1.7	13.6±2.0	10.3±2.5	22	0
	P. aeruginosa	21.0±1.7	17.6±1.5	13.6±2.5	10.0±0.0	0.0±0.0	21	0
BAS-2400m	*E. coli*	19.6±1.5	17.3±1.1	14.3±1.1	11.0±1.0	6.0±5.2	22	0
	P. aeruginosa	20.3±1.1	17.3±0.5	13.0±0.0	11.0±1.0	0.0±0.0	21	0
BAR-1900m	*E. coli*	19.6±1.5	17.3±1.5	14.3±1.5	11.6±1.1	10.0±1.0	22	0
	P.aeruginosa	22.0±2.6	19.3±3.0	15.3±3.5	11.0±2.0	2.6±4.6	21	0
	E. coli	17.6±0.5	16.0±1.0	14.3±1.5	11.6±1.1	10.0±1.0	21	0

BAR-2400m)	*P. aeruginosa*	21.0±2.6	17.0±3.2	15.3±3.2	9.6±1.5	0.0±0.0	21	0

* BAS: *Berberis asiatica*, BAR: *Berberis aristata*

Supplementary Table 2. Antibacterial activity of *Berberis asiatica* and *B. aristata* from different elevations against two gram positive bacterial strains

Samples	Tested organism	Zone of inhibition in mm (with diameter of well 5 mm)						
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	+Ve control	-Ve control
	SAU	19.3±0.5	15.3±1.1	12.3±1.5	10.3±1.5	0±0.0	22	0
BAS-1400m	*SEP*	23.3±0.5	19.3±0.5	16.0±0.0	12.6±0.5	8±0.0	21	0
	SAU	18.6±1.1	15.0±1.0	11.0±0.0	9.0±1.7	0±0.0	22	0
BAS-1900m	*SEP*	22.0±1.7	19.3±1.5	15.3±1.5	12.3±1.1	9.3±1.0	21	0
	SAU	17.3±0.5	15.3±1.1	12.6±1.5	10.0±0.0	0±0.00	21	0
BAS-2400m	*SEP*	18.0±0.0	15.0±2.0	12.6±2.0	9.3±1.5	0±0.00	22	0
	SAU	18.3±1.1	15.6±1.1	12.3±1.1	9.3±1.1	0±0.00	21	0
BAR-1900m	*SEP*	20.6±0.5	16.6±1.1	13.3±0.5	10.3±0.5	2.6±4.6	21	0
	SAU	17.0±1.0	15.3±0.5	12.0±2.0	9.0±2.0	0±0.0	21	0
BAR-2400m	*SEP*	18.0±0.0	15.0±2.0	11.6±0.5	9.3±1.5	0±0.0	21	0

* BAS: *Berberis asiatica*, BAR: *Berberis aristata*