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A COMPARATIVE ANALYSIS OF THE ANTIBACTERIAL ACTIVITY OF *BERBERIS ARISTATA* AND *BERBERIS ASIATICA* ON GASTROINTESTINAL PATHOGENS

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ABSTRACT

Objective: The study had been envisioned to assess the antibacterial activity of the different parts viz. root, bark, stem, leaf and fruit of *Berberis aristata* and *Berberis asiatica* with a view to compare the potentiality of these respective plants as an antimicrobial agent. **Methods:** The study involved the identification, collection, extraction and antibacterial analysis of the respective extract. The crude extract was prepared by Solvent Extraction method to make a final concentration of 100mg/ml and analysed using Agar well diffusion method against 6 gastrointestinal pathogens: *Bacillus subtilis* (ATCC 23857), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella* Typhi (ATCC 27870). **Results:** The extract was overall more effective against gram positive organisms. The root and bark extract of both plants presented the maximum inhibitory action against all test organisms in a comparable range. The root extract was most effective against *B. subtilis* with respective inhibition size of 24mm and 22mm for *B. aristata* and *B. asiatica*. The bark extract showed the highest activity against *S. aureus* for both plants. Stem extract of *B. aristata* and *B. asiatica* inhibited 5 and 3 bacterial strains repectively. The leaf and fruit extract of both the plants exhibited the least activity. **Conclusion:** The present study reflected the potentiality of *B. asiatica* as a substitute to *B. aristata* as an antimicrobial agent and emphasizes the need to screen for the overall study of the plant so as to utilize them for therapeutic purposes.

KEYWORDS: *Berberis aristata*; *Berberis asiatica*; Agar well diffusion; Gastrointestinal pathogens; Antibacterial activity; Zone of Inhibition.

INTRODUCTION

Plants are a valuable source of medicinal agents with proven potential against infectious agents. The increasing prevalence of Multi Drug Resistant strains and those with reduced susceptibility to antibiotics has led to the quest for new infection fighting strategies (Bajracharya et al 2008; Khakurel et al. 2014). Many plant extracts and phytochemicals are known to have antimicrobial properties and can be of great significance in therapeutics (Subba and Basnet 2014; Mitra and Obrell 2009).

Berberidaceae, a family of flowering plants, commonly called the barberry family has been recognized for their efficacious medicinal properties. It consists of spiny deciduous evergreen shrubs characterized by yellow wood and flowers (Srivastava et al 2005). Berberine, a type of isoquinolone alkaloid, is the major bioactive constituent of barberry. Phytochemical analysis also revealed the presence of alkaloids, tannins, phenolic compounds, sterols and triterpenes (Najmeh et al 2014).

Berberis aristata (Daruharidra) is the most widely used *Berberis* species for therapeutical purposes. The root is mostly used as medicinal part either as a single plant remedy or in polyherbal formulations (Rathi et al 2013). Pharmacological studies have reviewed its proven activity as hypoglycemic, antibacterial, antifungal, antipyretic, analgesis, anti-inflammatory, hepatoprotective, strong wound healer, anticancer and antioxidant. Attempts have been made to explore the possibilities of utilizing other parts rather than the roots of these species and searching for an alternative with an aim to conserve these species which has been over exploited due to the diverse use of its roots (Sharma et al 2011; Singh et al 2009).

Berberis asiatica is a common substitute to *Berberis aristata* and has been used as anti-inflammatory, antimicrobial, antioxidant, anticancer, antidiabetic, spasmolytic, antipyretic and antiseptic (Patni et al 2012). Its inner bark is yellow and resembles turmeric, hence known as tree turmeric or Daruhaldi in Sanskrit. *B. aristata* and *B. asiatica* differ morphologically in context

of leaves, inflorescence and stem (Upadhyay et al 2012; Srivastava and Mishra 2004).

This study was conducted to examine the in-vitro antimicrobial activity of the crude preparation of different parts of these plants with an objective of substantiating the validity of their use and to analyze the potentiality of *B. asiatica* as a substitute to *B. aristata* as an antimicrobial agent.

MATERIALS AND METHODS

Plant Materials

This study was conducted in the Microbiology laboratory of St. Xavier's College during June 2017. The fresh parts of *Berberis* plant- root, stem, leaves, bark and fruit were collected from Godawari as has been confirmed by The National Herbarium and Plant Laboratories, Godawari, Nepal.

Preparation of Crude Extract

The plant parts collected were washed, shade dried at room temperature and later pulverized by mechanical grinder. The powder was passed through a 40-mesh sieve and stored in a closed vessel until use. The materials (100 gm each) were cold perlocated with 50% ethanol at room temperature for 48 hours. The extracts were decanted, filtered through Whatman No. 1 filter paper and sterilized using membrane filter. It was then concentrated in reduced pressure below 40°C on rotary evaporator and finally dried in water bath. 1gm crude ethanol extract was mixed with 10ml of 1% Dimethyl Sulfoxide (DMSO) solution, vortexed to make homogenous suspension of 1gm/10ml, i.e. 100mg/ml. This suspension was stored at 4°C and used for bacterial screening (Das et al 2010).

Microbial Strains and Growth Conditions

The extracts were assessed for their antimicrobial activity against 6 gastrointestinal bacteria. 3 species were gram positives: *Bacillus subtilis* (ATCC 23857), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and 3 were gram negatives: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhi (ATCC 27870). These organisms were procured from National Public Health Laboratory, Kathmandu. Cultures were grown on Nutrient Broth (NB) at 37°C for 18-24 hours and were maintained on respective agar slant at 4°C.

Antimicrobial Essay

The antimicrobial activity was evaluated by Agar Diffusion Method. Test cultures were incubated at 37°C on NB for few hours to obtain turbidity equivalent to 0.5 McFarland standards. This was swab inoculated in sterilized Mueller Hinton Agar. After 10 minutes, wells of 6mm diameter were made with a sterile cork borer and the extract was dispensed aseptically into the wells. The plates were allowed to stand for about 30 minutes. The diameter of Zone of Inhibition (ZOI) was measured after overnight incubation at 37°C. All test systems included respective controls (Das et al 2010; Bhardwaj and Kartik 2013).

RESULTS AND DISCUSSION

The study was undertaken to assess the antibacterial activity of different parts of 2 *Berberis* species viz. *Berberis aristata*, which has already been well established for its therapeutic uses long since and *Berberis asiatica*, which possess great medicinal values and can be a significant substitute to *B. aristata* in the long run. The plant parts: root, bark, stem, leaf and fruit of both these species were taken for the evaluation of their activity against 6 gastrointestinal pathogens. Flowers of the plant were not collected since sampling and analysis was done in June which is indeed the fruiting season of the plant. The results pertaining to the inhibitory action of these plant extracts have been presented in Table 1 and Table 2.

Analysis of antibacterial activity presented that overall the gram positive bacteria are inhibited to a greater extent than gram negative organisms as has been indicated by the inhibition zone diameter. This result is consistent with another study by Singh at el 2009 where gram positive organisms were more sensitive to the plant extracts of both *B. aristata* and *B. asiatica* as compared to the gram negative organisms.

Table 1: Antibacterial activity of different parts of *Berberis aristata* plant.

S.N	Bacteria	Zone Of Inhibition (mm)				
		Root	Stem	Bark	Leaf	Fruit
Gram Positive Bacteria						
1.	*Bacillus subtilis*	24	19	15	12	
2.	*Enterococcus faecalis*	18	20	18	9	8
3.	*Staphylococcus aureus*	20	17	20		
Gram Negative Bacteria						
4.	*Escherichia coli*	13	10	17		10
5.	*Pseudomonas aeruginosa*	14		13		
6.	*Salmonella* Typhi	16	11	17		

Table 2: Antibacterial activity of different parts of *Berberis asiatica* plant.

S.N	Bacteria	Zone Of Inhibition (mm)				
		Root	Stem	Bark	Leaf	Fruit
Gram Positive Bacteria						
1.	*Bacillus subtilis*	22	17	16	14	
2.	*Enterococcus faecalis*	20		18	11	
3.	*Staphylococcus aureus*	19	15	19		7
Gram Negative Bacteria						
4.	*Escherichia coli*	11	8	14	10	10
5.	*Pseudomonas aeruginosa*	13		12		
6.	*Salmonella* Typhi	14		17		

The root and bark seemed to be the most effective against the test organisms as these extracts inhibited all test organisms to a higher extent. This is followed by the stem extract which reflected quite good activity against the organisms. The leaf and the fruit extract inhibited only few test organisms with comparatively small ZOI. Moreover, the inhibition zone for extracts of root, bark and stem for *B. aristata* and *B. asiatica* is in comparable range; however, *B. aristata* showed slightly higher activity in most cases.

The largest inhibitory activity was presented by the root extracts of these plants. The root extract seemed to be most effective against *B. subtilis*; the ZOI was 24mm for *B. aristata* root extract and 22 mm for *B. asiatica* root extract. The root extract was least effective against *E. coli* with an inhibition zone of 13mm and 11mm for *B. aristata* and *B. asiatica* respectively. Overall, the root extract of *B. asiatica* showed slightly lower activity against the test organisms except for *E. faecalis*; the ZOI was 20mm for *B. asiatica* and 12mm for *B. aristata*.

The bark extract of these plants also presented a great potential for inhibiting these organisms. The extract of both plants showed a quite consistency in their activity against all these test organisms. *B. aristata* extract showed the highest activity against *S. aureus* and the least against *P. aeruginosa* with ZOI of 20mm and 13mm respectively. This result was contrary to a similar study by Lamichhane et al 2014 where the bark of *B. aristata* was sensitive against *S.* Typhi and *P. aeruginosa* while it showed no activity against *S. aureus*. Similarly, the highest and the least inhibition size for *B. asiatica* bark extract was 19mm and 12 mm for same strains of bacteria.

The stem extract of *B. aristata* inhibited 5 test organisms with the highest activity against *E. faecalis*, a ZOI of 20mm followed by 19mm inhibition zone for *B. subtilis* and 17mm for *S. aureus*. It seemed to have a greater action against the gram positive organisms. It did not show any action against *P. aeruginosa*. On the other hand, the stem extract of *B. asiatica* was effective only against 3 test organisms; a 17mm ZOI for *B. subtilis*, 15mm for *S. aureus* and 8mm for *E. coli*. It did not inhibit *E. faecalis*, *P. aeruginosa* and *S.* Typhi. Comparatively, the stem extract of *B. aristata* was found to be more effective with inhibition against higher number of species and greater inhibition size.

The leaf extract of both plants showed highest activity against *B. subtilis* with ZOI of 14mm for *B. asiatica* followed by 12mm for *B. aristata* extract. The inhibitory action of leaf extract was larger for *B. asiatica* altogether. *B. aristata* showed activity against only 2 gram positive species, *B. subtilis* and *E. faecalis* while *B. asiatica* showed activity against *B. subtilis*, *E. faecalis* and *E. coli*.

The fruit extract showed the least inhibitory action without any consistency in the action between the two species. *B. aristata* inhibited *E. faecalis* and *E. coli* with highest activity of 10mm against *E. coli*. *B. asiatica* also inhibited 2 species; *S. aureus* and *E. coli* with highest inhibition of 10mm for *E. coli*. A comparable inhibition was obtained in a study by Saklani et al 2011; the 100 mg/ml ethanolic fruit extract of *B. asiatica* showed 10mm ZOI for *S. aureus* and 11 mm for *E. coli*.

CONCLUSION

The study has presented the screening of the crude extract of different parts of *Berberis aristata* and *Berberis asiatica* against common gastrointestinal bacteria. All parts of the plant possessed antibacterial activity in varying ranges. The root and bark tended to have the highest inhibitory action followed by the stem, leaf and fruit as has been indicated by the measurement of ZOI. Moreover, the comparative evaluation of the antibacterial activity of these different parts validates the potentiality of *Berberis asiatica* as a substitute to *Berberis aristata*. This so indicates the need for the further researches that needs to be carried out to identify, quantify and verify its active components, suitable dose, yielding variety, etc for their efficacy in medicinal purposes.

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