

Antimicrobial potential of Daruharidra (*Berberis aristata* DC) against the pathogens causing eye infection

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Aim: The present work was carried out to study the antimicrobial potential of the methanolic stem extracts of authentic Daruharidra and four of its substitute plant materials along with eight of those collected from different raw drug markets in South India against six eye-infecting bacteria viz., *Nocardia sp.*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus viridians* and *Escherichia coli*. **Materials and Methods:** The entire testing was carried out by agar well diffusion method after obtaining the samples from clinical laboratory in Coimbatore. Care was taken for complete maintenance of sterile conditions. **Statistical Analysis:** Statistical evaluations were conducted by ANOVA and $P < 0.01$ were considered significant. **Results:** Of the six organisms tested, the extracts showed activity against only three eye infecting pathogens. The highest antimicrobial activity was found against *E. coli* (24.00 ± 0) with an MIC of 23.20 mg/ml and the least against *Nocardia sp* (6.2 ± 0) with an MIC of 6.00 mg/ml. The inhibitory effects of the methanolic extracts on the growth of three organisms viz., *Nocardia sp*, *S. pneumoniae* and *E. coli* are close and are comparable with the standard antibiotic discs used. **Conclusion:** Thus this study suggests that Daruharidra and its traded substitutes may be used in treating eye infection caused by *Nocardia sp*, *S. pneumoniae* and *E. coli*.

Key words: Antimicrobial activity, Daruharidra, eye infection, methanolic stem extract

INTRODUCTION

Daruharidra is one of the herbs mentioned in ancient scriptures of Ayurveda. Ayurvedic Pharmacopeia of India correlates Daruharidra to *Berberis aristata* DC of family Berberidaceae.^[1,2] The plant is native to the whole range of the Himalayas and also occurs in Nilgiri range in southern India.^[3] The root and wood are rich in the yellow alkaloid, Berberine, a bitter substance, which dissolves in acids and forms salts of the alkaloid.^[4]

Internally Daruharidra is useful against a vast range of diseases. Studies indicate that it is commonly used to treat eye infections, ENT infections, skin disease, menorrhagia, cholera, jaundice, wound healing and urinary tract infections, indigestion and vaginal disorders.^[5-10]

Owing to its high medicinal value, Daruharidra is of trade importance and an endemic species of conservation

concern, which has quite high demand in herbal drug market. So to meet the need, herbal drug providers supply mixture of different *Berberis* species.^[11] Market survey in India indicates that *Berberis asiatica*, *Berberis lycium*, *Cosinium fenestratum* and *Morinda umbellata* are traded as substitutes of *B. aristata*.^[12]

Different species of the same genus may have totally different or weaker pharmacological action when compared with the authentic counterpart.^[13] *B. aristata*, reported to possess antimicrobial,^[14,15] antioxidant, anti-hyperglycemic,^[16] anti-amoebic^[17] and wound-healing properties.^[18] *B. asiatica* possesses antimicrobial and anti-tumour effect.^[14,19] *B. lycium* has reports on anti-bacterial, anti-diabetic effect and used to treat bleeding piles.^[14,20] *C. fenestratum* is known for its antibacterial property^[21] and also used for treating eye diseases and influenza.^[22] *M. umbellata* is used to treat dysentery and diarrhea^[23], also possess antileukaemic and antioxidant properties.

Eye infection is the common disease in the world, which plays a major role in medicinal practice. Microbial infections such as microbial keratitis, scleritis, orbital cellulitis, endophthalmitis were caused by eye infecting bacteria such as *Escherichia coli*, *Nocardia sp*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*.^[24,25] Although

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Daruharidra is traditionally used to cure ophthalmia and other eye diseases^[10], there was no valid scientific data available to prove antimicrobial activity against eye-infecting microorganisms. In view of the fragmentary nature of this work, it was planned to examine the *in vitro* antibacterial activity of the methanolic stem extracts of *B. aristata* and its substitutes (*B. asiatica*, *B. lycium*, *C. fenestratum* and *M. umbellate*) with the stem samples procured as 'Daruharidra' from eight different markets in south India against clinically isolated eye-infecting bacteria.

MATERIALS AND METHODS

Plant authentication and sample collection

The raw drugs (*B. aristata*, *B. asiatica*, *B. lycium*, *C. fenestratum* and *M. umbellate*) were obtained as voucher samples from the Repository of Medicinal Resources, Foundation for Revitalisation of Rural and Local Health Traditions (FRLHT), Bangalore, Karnataka, India. Stem samples sold as 'Daruharidra' were procured from eight different raw drug markets in South India [Table 1].

Extraction of plant material

The extracts of both the authentic and the market samples were carried out using methanol.^[26]

Test micro-organisms

Clinically isolated six eyes infecting microorganisms namely *Nocardia sp.*, *S. aureus*, *S. pneumonia*, *P. aeruginosa*, *S. viridians* and *E. coli* were collected from the Microbiology Department, Aravind Eye Hospital, Coimbatore, Tamil Nadu, India. The micro-organisms were subcultured on nutrient agar and maintained at 4°C for further use.

Screening for antimicrobial activity

The methanolic stem extracts of Daruharidra plant samples were used for evaluation of their antimicrobial activity by agar well diffusion method and Minimum Inhibitory

Concentration (MIC) for each test organism was determined by following the modified agar well diffusion method.^[27] All the tests were conducted using Mueller Hinton Agar at a bacterial concentration of 1×10^5 CFU/ml. The concentration of the extracts ranged between 6.00 mg/ml and 14.7 mg/ml.

Commonly prescribed eye drops by ophthalmologist such as Gentamycin (allopathic eye drop; 5 µl/well) and Ilaneer kuzhambu (ayurvedic eye drop; 5 µl/well) procured from the local market of Coimbatore, Tamil Nadu, India along with Berberine (1 µg/µl) and Ampicillin (1 µg/µl) were used as positive controls while distilled water was used as negative control.

Statistical analysis

The experimental results were repeated four times, in triplicates each time, and expressed as Mean ± Standard deviation. The results were statistically evaluated by ANOVA and $P < 0.01$ were considered as significant.

RESULTS

Methanolic stem extracts of Daruharidra (*B. aristata*), its substitutes and market samples showed antimicrobial activity against three microorganisms out of the six tested organisms. Positive controls produced significantly sized inhibition zones against the tested bacteria (ranging between 17.75 mm and 7 mm) and the negative control produced no observable inhibitory effect against any of the test organism [Tables 2 and 3].

From Tables 2 and 4 it is clear that all the five authentic Daruharidra samples showed sensitivity to three microbes (*Nocardia sp.*, *S. pneumonia* and *E. coli*) of these, *Nocardia sp.* showed highest antimicrobial activity for *B. lycium* (22.30 mm) with an MIC of 14.98 mg/ml, followed by *M. umbellate*, *C. fenestratum*, *B. asiatica* and *B. aristata*. Similarly *S. pneumonia* showed highest activity for *M. umbellate* with a zone of 12 mm and MIC of 15.46 mg/ml, followed by *B. asiatica*, *B. aristata*, *B. lycium* and *C. fenestratum* against *E. coli*. *B. lycium* showed zone of inhibition with a diameter of 23 mm and an MIC of 14.98 mg/ml higher than the other authentic samples.

B. aristata showed highest zone of inhibition for *E. coli* followed by *S. pneumonia* and *Nocardia sp.* whereas *B. asiatica* showed the highest zone of inhibition for *S. pneumonia* followed by *Nocardia sp.* and *E. coli*. *B. lycium* and *C. fenestratum* showed highest zone of inhibition for *E. coli* then for *Nocardia sp.* and *S. pneumonia*. *M. umbellate* showed better zone of inhibition for *Nocardia sp.* followed by *S. pneumonia* and *E. coli* [Table 2].

Comparison between authentic Daruharidra (*B. aristata*) and market samples in Tables 3 and 5 revealed market sample 4 to show maximum inhibitory zone of 24 mm against *E. coli*

Table 1: Place of collection of stem samples of Daruharidra (*B. aristata*) its substitutes and market samples

Plant material	Place of collection
<i>B. aristata</i> (Authentic sample 1)	FRLHT, Bangalore, India
<i>B. asiatica</i> (Authentic sample 2)	FRLHT, Bangalore, India
<i>B. lycium</i> (Authentic sample 3)	FRLHT, Bangalore, India
<i>C. fenestratum</i> (Authentic sample 4)	FRLHT, Bangalore, India
<i>M. umbellate</i> (Authentic sample 5)	FRLHT, Bangalore, India
Market sample 1	Theni, Tamilnadu, India
Market sample 2	Coimbatore, Tamilnadu, India
Market sample 3	Thrissur, Kerala, India
Market sample 4	Coonoor, Tamilnadu, India
Market sample 5	Chennai, Tamilnadu, India
Market sample 6	Bangalore, Karnataka, India
Market sample 7	Madurai, Tamilnadu, India
Market sample 8	Thiruvananthapuram, Kerala, India

Table 2: Antimicrobial activity of methanolic stem extracts of Daruharidra (*B. aristata*), and its substitutes on eye pathogens determined by agar well diffusion method

Extracts	Diameter of growth of inhibition zones (mm)					
	N	Sa	Stp	Pa	Sv	Ec
<i>B. aristata</i> (authentic sample 1)	08.40±0.29	-	10.00±0.82	-	-	21.00±0.00
<i>B. asiatica</i> (authentic sample 2)	10.30±0.00	-	11.00±0.00	-	-	07.25±0.29
<i>B. lycium</i> (authentic sample 3)	22.30±0.00	-	09.00±0.00	-	-	23.00±0.00
<i>C. fenestratum</i> (authentic sample 4)	16.00±0.00	-	07.60±0.00	-	-	18.00±0.00
<i>M. umbellate</i> (authentic sample 5)	17.30±0.00	-	12.00±0.00	-	-	10.00±0.00
Ayurvedic eye drop (Ilaneer kuzhambu)	14.00±0.82	-	11.00±0.00	11.00±0.00	10.25±0.96	12.50±0.82
Allopathic eye drop (Gentamycin)	19.20±0.00	11.12±0.75	16.00±0.00	-	10.00±0.82	14.00±0.82
Berberine	07.00±0.00	08.00±0.82	14.00±0.00	-	-	10.00±0.00
Ampicillin	17.75±0.96	08.25±0.50	40.00±0.00	-	-	12.50±0.00
Distilled Water	-	-	-	-	-	-

N – *Nocardia* sp; Sa – *Staphylococcus aureus*; Stp – *Streptococcus pneumoniae*; Pa – *Pseudomonas aeruginosa*; Sv – *Staphylococcus viridians*; Ec – *Escherichia coli*. Values including diameter of the well (6 mm) are means of three replicates±standard deviation. The data were analyzed by ANOVA

Table 3: Antimicrobial activity of methanolic stem extracts of Daruharidra (*B. aristata*) and market samples on eye pathogens determined by agar well diffusion method

Extracts	Diameter of growth of inhibition zones (mm)					
	N	Sa	Stp	Pa	Sv	Ec
<i>B. aristata</i> (authentic sample 1)	08.40±0.29	-	10.00±0.82	-	-	21.00±0.00
Market sample 1	07.50±0.00	-	10.00±0.00	-	-	19.25±0.50
Market sample 2	07.60±0.00	-	11.00±0.00	-	-	20.38±0.25
Market sample 3	08.50±0.00	-	12.00±0.00	-	-	21.00±0.00
Market sample 4	08.60±0.00	-	11.00±0.00	-	-	24.00±0.00
Market sample 5	07.80±0.00	-	10.75±0.50	-	-	21.00±0.00
Market sample 6	06.20±0.00	-	08.75±0.50	-	-	18.00±0.00
Market sample 7	07.80±0.00	-	09.75±0.50	-	-	20.00±0.00
Market sample 8	09.50±0.41	-	08.00±0.00	-	-	18.00±0.00
Ayurvedic eye drop (Ilaneer kuzhambu)	14.00±0.82	11.00±0.82	11.00±0.00	11.00±0.00	10.25±0.96	12.50±0.82
Allopathic eye drop (Gentamycin)	19.20±0.00	11.12±0.75	16.00±0.00	-	10.00±0.82	14.00±0.82
Berberine	07.00±0.00	08.00±0.82	14.00±0.00	-	-	10.00±0.00
Ampicillin	17.75±0.96	08.25±0.50	40.00±0.00	-	-	12.50±0.00
Distilled water	-	-	-	-	-	-

N – *Nocardia* sp; Sa – *Staphylococcus aureus*; Stp – *Streptococcus pneumoniae*; Pa – *Pseudomonas aeruginosa*; Sv – *Staphylococcus viridians*; Ec – *Escherichia coli*. Values including diameter of the well (6 mm) are means of three replicates±standard deviation. The data were analyzed by ANOVA

Table 4: Minimum inhibitory concentration (mg/ml) of methanolic stem extracts of Daruharidra (*B. aristata*) and its substitutes on eye pathogens determined by modified agar well diffusion method

Extracts	MIC (mg/ml)		
	N	Stp	Ec
<i>B. aristata</i> (authentic sample 1)	14.70	14.70	14.70
<i>B. aristata</i> (authentic sample 2)	12.90	12.90	12.90
<i>B. lycium</i> (authentic sample 3)	14.98	14.98	14.98
<i>C. fenestratum</i> (authentic sample 4)	14.70	14.70	14.70
<i>M. umbellate</i> (authentic sample 5)	15.46	15.46	15.46

N – *Nocardia* sp; Stp – *Streptococcus pneumoniae*; Ec – *Escherichia coli*

and market sample 6 showed minimum of 6.2 mm for *Nocardia* sp. with an MIC of 8.44 and 6 mg/ml, respectively.

Among market samples, *E. coli* showed a zone of 24 mm as maximum with an MIC (8.44 mg/ml) for market sample 4 and minimum of 18 mm with an MIC (6 mg/ml and

23.2 mg/ml) for market samples 6 and 8. Market samples 3 and 8 showed maximum zone of inhibition (12 mm) and minimum zone of inhibition (8 mm) with an MIC of 13.76 mg/ml and 23.2 mg/ml respectively against *S. pneumoniae*. For *Nocardia* sp. market sample 8 and 6 showed maximum of 9.5 mm and minimum of 6.2 mm with an MIC of 23.2 mg/ml and 6 mg/ml, respectively [Tables 3 and 5].

All the organisms showed the zone of inhibition in the range of 11 and 12.5 mm for Ayurvedic eye drop (Ilaneer kuzhambu) where as allopathic eye drop (Gentamycin) showed zone of inhibition in the range of 10 and 19.2 mm except for *P. aeruginosa*. Berberine and Ampicillin showed sensitivity against *Nocardia* sp, *S. aureus*, *S. pneumoniae* and *E. coli* ranging between 7 and 17.75 mm [Tables 2 and 3].

All the obtained results [Tables 2 and 3] are not significantly different ($P > 0.01$) from that of authentic Daruharidra (*B. aristata*).

Table 5: Minimum inhibitory concentration of stem extracts of Daruharidra (*B. aristata*) and market samples on eye pathogens determined by modified agar well diffusion method

Extracts	MIC (mg/ml)		
	N	Stp	Ec
<i>B. aristata</i> (authentic sample 1)	15.33	15.33	15.33
Market sample 1	13.80	13.80	13.80
Market sample 2	13.76	13.76	13.76
Market sample 3	08.44	08.44	08.44
Market sample 4	16.53	16.53	16.53
Market sample 5	06.00	06.00	06.00
Market sample 6	08.13	08.13	08.13
Market sample 7	23.20	23.20	23.20
Market sample 8	14.70	14.70	14.70

N – *Nocardia* sp; Stp – *Streptococcus pneumoniae*; Ec – *Escherichia coli*

DISCUSSION

Indigenous knowledge of herbal medicine is a big source of the modern knowledge. Herbal medications have seen a resurgence of interest due to its lower incidence of adverse reactions compared with synthetic pharmaceuticals and reduced cost of plant preparations which makes the search for natural therapeutics an attractive option.^[28-30] New and effective antimicrobial agents with broad-spectrum activities from natural resources have now become the demand of the day due to the pathogenic resistance to antibiotics which are been used arbitrarily.^[31] Therefore, in the present investigation, methanolic stem extracts of Daruharidra, its substitutes and market samples were evaluated for their antimicrobial potential against the eye infecting pathogens for the first time.

In this study, all the Daruharidra samples were found to be most active in inhibiting the growth of three eye pathogens viz., *Nocardia* sp., *S. pneumoniae* and *E. coli* out of the six tested. This is in line with the findings revealed the methanolic extract of Daruharidra posses stronger and broader spectrum of action against bacterial strains.^[26,32-34] Ethnobotanically, Daruharidra have been used to cure various eye and ear infections caused by microbes. Also, the variable activities of the plant have been found to have a good antagonistic activity against human microbial pathogens.^[8]

The antimicrobial activity of all the methanolic stem extracts of Daruharidra against the tested eye pathogens may be due to the presence of common secondary metabolite Berberine, a yellow-coloured isoquinoline alkaloid. Berberine has been shown to be active against intestinal infections such as *Shigella dysenteriae*, *Salmonella paratyphi* and various *Klebsiella* species,^[35] and inhibit the intestinal secretory response of *Vibrio cholerae* and *E. coli*.^[36] Berberine was also reported to inhibit *Helicobacter pylori*.^[37] Berberine from root, stem and

leaf extracts of various *Berberis* sp also reported to posses *in vitro* antimicrobial property against Gram-positive and Gram-negative bacteria and fungi.^[38,39] Published reviews has described a majority of antimicrobial effects of the extracts of *Berberis* species have been attributed to their secondary metabolites, especially alkaloids.^[4]

CONCLUSIONS

The methanolic stem extracts of Daruharidra (*B. aristata*), its substitutes and market samples showed promising antimicrobial activity to *Nocardia* sp., *S. pneumoniae*, *E. coli* among the six clinically isolated eye infecting organisms. This result lends credibility to the folkloric use of Daruharidra in treating eye infection. Thus Daruharidra (*B. aristata*), its substitutes and market samples could be conquered for new antimicrobial agents.

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