Studies on the Antimicrobial Potential of *Berberis tinctoria* Lesch Root and Root Bark

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Chloroform and methanol extracts of *Berberis tinctoria* Lesch (Berberidaceae) root and root bark were investigated for antibacterial potential against *Bacillus subtilis* NCIM-2349, *Bacillus coagulans* NCIM-2323 *Staphylococcus aureus* NCIM-2492, *Escherichia coli* NCIM-2345 and antifungal potential against *Candida albicans* 6, 8, 11 and 27 (different strains of *Candida albicans*), *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus xylinum* and *Aspergillus fumigatus*. Both the extracts showed significant antibacterial and antifungal activity against all the microorganisms tested and the effect so produced was comparable to those of the standards, ampicillin and clotrimazole. However, the antifungal activity of the chloroform extract of the root was found to be negligible against *Aspergillus species* tested.

Berberis tinctoria Lesch (Berberidaceae) is a shrub, very variable in size and form, in the open often 2 to 3 feet high, but in the forest sometimes reaching a height of 15 feet with thick stem and long scandent branches bearing numerous slender leafy twigs[1]. It is locally called as *Oosikala* and medicinally used by the Kurumbas, the Nilgiri tribe, for stomach-ache (root paste). The aqueous root paste along with honey is used as an antimicrobial agent against skin disease[2]. The wood, root bark and extract have been used in skin diseases, menorrhagia, diarrhoea, jaundice and in affections of the eyes[3]. The root and root bark of the plant is rich in alkaloid content, berberine, the principal alkaloid[4,5]. The 50% ethanol extract of the aerial parts of this plant possessed diuretic activity at a dose level of 510.7 mg/kg and was found to have cardiovascular activity[6]. The present study is taken up to screen antibacterial and antifungal activity of the methanol and chloroform extracts of the root and root bark of *B. tinctoria* Linn.

The plant *Berberis tinctoria* was collected from Doddabetta forest area, Udhagamandalam, Nilgiri district, Tamil Nadu in the month of June 1998. It was identified by the Botanical Survey of India, Coimbatore and a voucher specimen was deposited in the Department of Pharmacognosy for future reference. The underground root part was separated from the aerial parts and the root bark was then peeled out from the root. Both root and root bark were air/shade-dried and mechanically powdered separately to obtain a coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using chloroform and methanol. Solvent elimination under reduced pressure afforded the chloroform (1.39% root, 0.60% root bark) and methanol (16.02% root, 18.98% root bark) extracts, respectively. The resulting extracts were then subjected to antibacterial and antifungal testing. All bacterial strains were obtained from National Chemical Laboratory, Pune. All the fungal stains were obtained from Calicut Medical College, Calicut, Kerala.

Chloroform and methanol extracts were screened for antifungal and antibacterial activity by the cup plate method. The extracts were prepared by dissolving each of 100 mg of chloroform and methanol extract in 10 ml of dimethyl sulphoxide (DMSO). A standard drug, clotrimazole (1 mg/ml) was prepared in DMSO for antifungal activity and 1 mg/ml solution of standard ampicillin in DMSO for antibacterial activity.

The antimicrobial activity was evaluated by the agar diffusion method employing 24 h culture of twelve different test organisms *viz. Candida albicans* strains 6, 8, 11 and 27, *A. flavus*, *A. niger*, *A. xylinum*, *A. fumigatus*, *B. subtilis*, *B.*

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coagulans, S. aureus and *E. coli*. The test organisms were seeded into sterile nutrient agar medium/Sabouraud's Dextrose Agar (SDA) medium by uniformly mixing 1 µl of the inoculum with 20 ml of sterile melted nutrient agar cooled to 48º to 50º in a sterile petridish. When the nutrient agar/SDA medium solidified, 6 holes of uniform diameter (5 mm) were made using a sterile borer. Chloroform and methanol extracts (0.1 and 0.2 ml of each) as well as the standard solution (clotrimazole 0.2 ml of 1 mg/ml and ampicillin 0.2 ml of 1 mg/ml) and the DMSO solvent control were placed in each hole separately under aseptic condition. Agar medium was used for antibacterial activity and SDA medium was used for antifungal activity. The plates were then maintained at room temperature for 2 h to allow the diffusion of the solution into the medium. All the bacterial plates were then incubated at 37° for 18 h and the fungal plates at 28° for 48 h and the zone of inhibition measured (for each zone an average of three independent determinations were noted).

TABLE 1: ANTIFUNGAL ACTIVITY OF THE ROOT AND ROOT BARK OF *BERBERIS TINCTORIA* LESCH.

Extracts tested	Conc.	Diameter of the zone of inhibition (mm)							
	mg/well	*A. fl*	*A. ni*	*A. xy*	*A. fu*	*C. a* 6	*C. a* 8	*C. a* 11	*C. a* 27
Methanol extract of root	1	20.5	25.5	22.0	23.0	24.0	24.5	22.5	22.5
	2	21.0	24.0	23.0	25.0	27.0	25.0	26.	24.5
Methanol extract of root bark	1	22.5	26.2	24.0	25.0	29.0	26.5	24.5	23.5
	2	26.2	31.2	25.5	28.0	31.5	28.5	28.0	26.5
Chloroform extract of root	1	-	-	-	-	17.0	16.5	18.0	15.5
	2	-	-	-	-	18.5	18.0	18.5	16.0
Chloroform extract of root bark	1	19.0	18.5	21.5	21.0	21.5	22.0	20.5	20.0
	2	21.5	21.5	23.0	33.0	24.5	26.5	25.5	23.5
Clotrimazole	0.2	28.0	29.0	27.0	27.5	27.0	27.5	27.0	27.5

Different extracts were tested against various microorganisms that include, *Aspergillus flavus* (*A. fl*), *Aspergillus niger* (*A. ni*), *Aspergillus xylinum* (*A. xy*), *Aspergillus fumigatus* (*A. fu*), *Candida albicans* strains 6, 8, 11, 27 (*C. a* 6, *C. a* 8, *C. a* 11, *C. a* 27).

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE ROOT AND ROOT BARK OF *BERBERIS TINCTORIA* LESCH.

Extracts tested	Conc.	Diameter of the zone of inhibition (mm)			
	mg/well	*B. subtilis*	*B. coagulans*	*S. aureus*	*E. coli*
Methanol extract of root	1	17.0	18.0	18.0	16.0
	2	17.5	18.0	23.0	16.0
Methanol extract of root bark					
	1	23.0	21.5	22.5	23.5
	2	25.0	24.5	24.5	26.5
Chloroform extract of root	1	21.0	18.0	17.0	21.0
	2	23.5	20.5	22.0	23.0
Chloroform extract of root bark	1	21.5	18.0	21.0	21.5
	2	25.5	24.0	26.0	26.5
Ampicillin	0.2	24.0	24.5	23.5	27.0

Different extracts were tested against various microorganisms that include, *Bacillus subtilis* (*B. subtilis*), *Bacullus coagulans* (*B. coagulans*), *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

In Nilgiris, the ethnic tribe Kurumbas use the plant *B. tinctoria* root and root bark for stomach ache and the aqueous root paste for skin diseases. Results of preliminary antifungal and antibacterial screening of methanol and chloroform extracts of *B. tinctoria* (Table 1 and 2) revealed that the extracts were active against all the organisms, except the chloroform root extract against the fungi tested. The root bark extracts showed better activity against the organisms than that of the extracts of the root. The methanol extract showed higher activity than the chloroform extract.

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REFERENCES

1. Fyson, P.F., In; the Flora of the Nilgiris and Pulney Hill Tops, Vol. I, I Edn., Bishen Singh Mahendra Pal Singh, Dehra Dun, 1974, 13.
2. Raghunathan, K. and Ramadas, V.N.K., In; Tribal pockets of Nilgiris. Recording of the Field Study on Medicinal Flora and Health Practices, 2nd Edn., Indian Institute of History of Medicine, Hyderabad, 1978, 103
3. Kiritikar, K.R. and Basu, B.D., In; Indian Medicinal Plants, Vol 1, 2nd Edn., International Book Distributors, Dehra Dun, 1987, 103
4. Chopra, R.N., Nayar, R.N. and Chopra, I.C., In; Glossary of Indian Medicinal Plants C.S.I.R, New Delhi, 1992, 32.
5. The Wealth of India, Vol. 2 B, C.S.I.R., New Delhi, 1988, 118.
6. Abraham, Z., Bhukuni, S.D., Garg, H.S., Goel, A.K., Melhotra, B.N. and Patnaik, G.K., **Indian J. Exp. Biol.**, 1986. 24, 46.
7. Pharmacopoeia of India, Vol. 2, 3rd Edn., Controller of Publications, New Delhi, 1985, A 85.

Development of Dissolution Medium for a Poorly Water Soluble Drug, Celecoxib

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Celecoxib, a non-steroidal antiinflammatory drug is poorly water soluble. In the present study a new dissolution medium was developed, as there is no official dissolution medium. The composition of the medium was selected on the basis of solubility data of celecoxib at 37°. Solubility data revealed that water consisting of 2% w/v sodium lauryl sulphate could be a suitable dissolution medium. The discriminating power of the selected dissolution medium (2% w/v sodium lauryl sulphate in water) relative to other dissolution mediums was evaluated and the results further justified the usage of 2% w/v sodium lauryl sulphate in water as dissolution medium for celecoxib.

Celecoxib (CB), N-(4-sulphonilamide),3-trifluoromethane,5-(4-tolyl) pyrazole, is a new non-steroidal antiinflammatory drug (NSAID)[1]. It is a selective inhibitor of the cyclooxygenase-2 (COX-2) and exhibits many of the pharmacological actions of prototypical NSAIDs, including antiinflammatory, analgesic and antipyretic activity[2]. It is not official in any pharmacopoeia.

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The testing of pharmaceutical dosage forms for *in vitro* drug release and dissolution characteristics is very important for ensuring batch to batch quality control and to optimize formulations during product development. Drugs that are practically insoluble (less than 0.01%) are of increasing