ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF EKDANIA LEAF EXTRACTS

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Summary

Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties like Analgesic, Antiinflammatory, Antioxidant, hypoglycemic agents and many more. Five different crude extracts: petroleum ether, chloroform, ethyl ether, ethanol and aqueous extract of *Bridelia patens* have been studied for both *in vitro* antibacterial and antifungal activites. The different extracts showed remarkable inhibitory action against various gram positive and gram negative bacteria and two fungal species.

Key words: *Bridelia patens*, Antibacterial, Antifungal.

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Introduction

Bridelia patens (Euphorbiaceae) is a traditional perennial herbaceous medicinal plant commonly known as Ekdania, gondai, kasai (1, 2). It is a tall tree with blackish brown, irregularly fissured bark, leaves are elliptic oblong, sometimes obovate, obtuse or subacute, entire, slightly crunulate, tomentose beneath. Flowers creamy-white, in terminal panicles of erect or drooping spikes. Drupes subglobose, smooth, glabrous, seated on the enclosed perianth, greenish purple when ripe (3). It is used in the treatment of Rheumatism and gynecological problems. The root and bark are valuable astringent. It is also used in vata, lumbago and hemiplegia and finally used as a fodder for animals (4, 5). A survey of literature revealed no methodical reports on antibacterial and antifungal activity of various extracts of *Bridelia patens* leaves. Bark contains tannins, triterpene ketone, decanoic acid octadecyl ester, stigmasterol, dehydrostigmasterol. Fruits contain β-sitosterol, gallic and ellagic acids. From leaves crude protein has been reported (6). The present study is therefore an attempt to assess efficacy of this indigenous herb in its different concentrations against various gram positive and gram negative bacteria and fungi.

Material and Methods

Collection of plant material: The plant was collected from the wild sources of Shirpur forest, Maharashtra, India in the month of May 2008. The plant was identified and authenticated from standard resources.

Preparation of various extracts : Air-dried powdered leaves (1 kg) were exhaustively extracted by Soxhlet's apparatus successively by increasing order of polarity with petroleum ether, chloroform, ethyl acetate and ethanol. The aqueous extract was prepared by cold maceration of 250 g of the shade-dried leaf powder in 500ml of chloroform water (1:99) for 7 days. The various extracts obtained were filtered, concentrated, dried in vacuum and the residue stored in a refrigerator at 2-8˚ C for use in subsequent experiments.

Preliminary phytochemical screening

The dry extracts were subjected to various chemical tests (7, 8) to detect the presence of different phytoconstituents. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, Flavonoids, phenols, steroids, saponins and tannins.

Antibacterial and antifungal studies

The various extracts were tested for their effect on gram +ve bacteria such as *Staphylococcus aureus, Bacillus subtilis* and gram – ve bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. Fungi used for the present study were *Aspergillus niger* and *Candida albicans.* Minimum inhibitory concentration of the extracts was evaluated by cup plate diffusion method for antibacterial and antifungal activity (9, 10). 0.1 ml of overnight grown nutrient broth culture of the bacteria was transferred aseptically to sterile glass Petri dish. Sterile molten nutrient agar (45˚C) was then poured, mixed uniformly rotating the plate and allowed to solidify. Cups were made out in the centre of the seeded nutrient agar plate using a sterile cork borer (6mm). The various extracts of the *Bridelia patens* leaf of different concentrations viz. 50, 100, 200, 400 mg/ml were made using dimethyl sulphoxide (DMSO) as a diluting solvent.

The samples were added with a sterile micropipette to each of the cups. The plates were then incubated at 37˚ C for 24 hrs. Plates with cups containing only DMSO served as a control. Antibacterial actions of various extracts were compared with the known antibiotic like Streptomycin. The diameters of the inhibitory Zones were recorded after incubation and average values of these observations were recorded. Antibacterial activity of various extracts of *Bridelia patens* leaf is given in Table 1.

TABLE-1: ANTIBACTERIAL ACTIVITY OF *Bridelia patens* LEAF EXTRACT

Treatment	Conc. mg/ml	Zone of Inhibition (in mm)			
		Gram +ve		Gram –ve	
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Pet. ether extract	50	09	08	09	07
	100	08	10	10	09
	200	10	09	12	11
	400	13	14	15	17
Chloroform extract	50	08	09	07	08
	100	10	10	08	10
	200	10	13	10	12
	400	12	16	11	13
Ethyl acetate extract	50	09	08	09	08
	100	12	11	08	10
	200	13	15	12	13
	400	17	19	13	15
Ethanolic extract	50	09	10	09	10
	100	13	16	14	13
	200	18	18	20	18
	400	21	20	24	22
Water extract	50	07	09	08	09
	100	08	10	10	11
	200	10	11	12	12
	400	12	16	14	15
Streptomycin	100	24	22	20	20

In case of antifungal activity, the different fungal species were subcultured on sterile Sabouraud's broth. Suspensions of sub cultured organisms were made following the above- mentioned procedure adopted for antibacterial activity. The plates of fungi were incubated at 25˚C for 3-4 days. Antifungal activity of various extracts of *Bridelia patens* is given in Table 2.

TABLE- 2: ANTIFUNGAL ACTIVITY OF *Bridelia patens* LEAF EXTRACT

Treatment	Conc. mg/ml	Zone of Inhibition (in mm)	
		C. albicans	*A. niger*
Pet. ether extract	50	10	11
	100	11	13
	200	12	16
	400	18	18
Chloroform extract	50	10	11
	100	12	13
	200	15	18
	400	19	23
Ethyl acetate extract	50	09	10
	100	11	12
	200	15	16
	400	19	21
Ethanolic extract	50	09	10
	100	13	16
	200	18	20
	400	22	25
Water extract	50	08	07
	100	09	11
	200	08	07
	400	10	09
Amphotericin	10	21	22

Estimation of total phenolics: The total phenolic contents of ethanol extract was determined with Folin-Ciocalteu reagent according to Slinard & Singleton (11) and slightly modified. The stock solution of extract 1mg/ml in water was prepared. From the stock solution, 5 ml was transferred to a 25 ml volumetric flask and made up with distilled water. Out of this 5 ml of sample and 2 ml of standard was taken in 25-ml volumetric flask, to this 10 ml of distilled water, and 2ml of phenol reagent (20%v/v) was added, and then the volume was made up with 29% sodium bicarbonate. The mixture was kept in the dark for 20 min. and the absorbance was read at 760 nm. The total phenolic content was calculated as gallic acid and expressed as percent of gallic acid detected. Standard used was gallic acid.

Results and Discussion

The five different crude extracts viz. petroleum ether, chloroform, ethyl ether, ethanol and aqueous extract of *Bridelia patens* leaf were tested against various gram +ve and gram –ve bacteria. The results illustrated in Table 1 revealed the ethanolic extract of *Bridelia patens* as most active against *S.aureus, E.coli* and *P. aeruginosa* in the dilution of 100 mg/ml. The ethyl acetate and chloroform extracts showed less activity than ethanol extract, but showed more activity than Pet. ether and water extracts.

Table 2 revealed that the ethanolic and chloroform extracts are more active against *C. albicans* and *A. niger*, whereas petroleum ether and ethyl acetate showed moderate activity. No activity was found in aqueous extract. Thus, it can be concluded that while screening of various extracts of *Bridelia patens* leaf against various gram +ve and gram – ve bacteria and fungi, ethanol extracts exhibited very satisfactory inhibitory activity. Further studies involving the isolation, characterization and purification of the chemical compounds of the plant and screening for antibacterial and antifungal may result in the development of a potent entity which will be of lower toxicity and a high therapeutic value to the mankind. These activities may be due to the presence of phytoconstituent present in the extract and the exact constituent responsible for the activity can be confirmed with the help of isolation techniques.

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