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Phytochemical and antimicrobial activity of extracts, fractions and betulin, 7-methyl juglone obtained from *Diospyros paniculata*

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Abstract

The *Diospyros paniculata* Dalz (Ebenaceae) has been used in folk medicine to treat several ailments including burns, gonorrhea, biliousness, blood poisoning, rheumatism and ulcer. In this work, we evaluated the phytochemical and antimicrobial activity of methanol extract (ME), some fractions of methanol extract [hexane (HF), chloroform (CF) and butanol fraction (BF)], as well as two pure compounds denoted as betulin (compound I), 7-methyl juglone (compound II) obtained from the stem bark of *Diospyros paniculata*. The compounds were isolated and identified by chromatographic and spectroscopic methods. The antimicrobial efficacy was tested against four types of bacteria (both Gram positive and Gram negative) and three types of fungi.

The hexane fraction was found to be very efficient against *Shigella dysenteriae* responsible for diarrhoea, chloroform fraction exhibited efficacy against *Candida albicans*. Compound I, which seems to be main active principle of hexane fraction, showed promising antibacterial effect, being more potent than ciprofloxacin while compound II from chloroform fraction showed antifungal activity, comparable to that of nystatin. Minimum Inhibitory Concentration values of both compounds I, II were found to be 30 and 75 μg/ml, respectively.

Keywords: *Diospyros paniculata*, antibacterial, antifungal, betulin, 7-methyl juglone, ciprofloxacin, nystatin

1. Introduction

The family Ebenaceae with about 500 species, is widely spread in tropics and subtropics. It consists of 6 genera namely *Diospyros, Euclea, Maba, Onotheca, Haphidanthe, Royena and Tetraclis*. The genus *Diospyros* with more than 350 species is of importance both numerically and economically. *Diospyros* Linn. consists of trees and shrubs chiefly tropical and widely distributed in both the hemispheres. About 41 species occur in India, mostly in evergreen forests of Deccan, Assam and Bengal and a few are in northern India. The characteristic features

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of the *Diospyros* species are: trees, rarely shrubs, leaves alternate; flowers green, white or yellow (few to many). The sapwood is white and soft and heartwood is black and hard. The genus is of great economic importance with many species yielding edible fruits, ebony and valuable timbers [1].

Diospyros species have long been known for their medicinal uses. Almost all the parts of these plants have been used as medicine e.g., the leaves are good for lumbago, fruits are carminative, astringent and cure biliousness and vata in Ayurveda, seeds are sedative, whereas bark is bitter, astringent and febrifuge .

In an attempt to establish a scientific basis for their folkloric, ethnomedicinal uses, one of frequently used Indian medicinal plant *Diospyros paniculata* Dalz (Ebenaceae) has been selected for phytochemical and microbiological studies.

Diospyros paniculata is a moderate sized handsome tree attaining a height of 50 ft and a diameter of 1.25 m. The fruits are green and ovoid, about 1 in long. The wood is whitish grey, occasionally with narrow stripes of black. This plant does not yield black heartwood. Bark is soft and moderately heavy (wt. 46 lb /cu ft). Leaves of the tree are used as fish poison; dried and powdered fruits are applied to heal burns; Decoction of the fruit is used in gonorrhoea, biliousness and blood poisoning; powdered stem bark is used for rheumatism and ulcer [1].

In the previous studies, 7-methyljuglone, plumagin, diospyrin and isodiospyrin have been isolated from stem bark of *Diosyros paniculata* [2].

2. Materials and Methods

2.1 Plant Material

Stem bark of *Diospyros paniculata* Dalz. (Ebenaceae), commonly known as Karunduvari (Tamil) was collected in Ranchi, India, during July 2005 and authenticated by Dr. M. P. Singh, Head, Department of Forest Science, Birsa Agriculture University, Ranchi (India). A voucher specimen (no. PG- MPH/ 16/04) has been deposited in the herbarium of Birla Institute of Technology, Mesra, Ranchi.

2.2 Phytochemical analysis

Dried stem bark of *Diospyros paniculata* (1200 g) was powdered and subjected to hot extraction with methanol. After filtration, the solvent was removed by rotatory evaporation under reduced pressure, yielding semisolid methanol extract.

The methanol extract (42 gm) was suspended in methanol and successively partitioned with n-hexane, chloroform and n-butanol to provide respective fractions. The n-hexane and chloroform fractions were chromatographed on silica gel column and eluted with mixture of n-hexane chloroform (9:1) with increasing polarity. Similar fractions which showed a positive reaction with anisaldehyde-sulphuric acid reagent were combined and rechromatographed as in previous case, giving a pure compound, which was named as compound I. Similarly, compound II (naphthoquinone in nature) was obtained after chromatographic separation of chloroform fraction using same solvent system.

Compound I and compound II were identified on the basis of their spectral data as betulin (Fig 1) [3] and 7-methyl juglone (Fig 2) [4]. The purity of isolated compounds was examined by thin layer chromatography using silica gel precoated aluminium plates of 200 μm layer thickness (Merck, Germany). Short wave UV light, anisaldehyde-sulphuric acid, ferric chloride reagents were used to visualize spots.

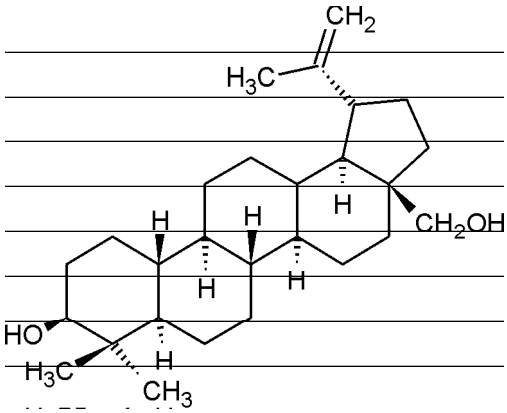

Fig. 1: Structure of betulin

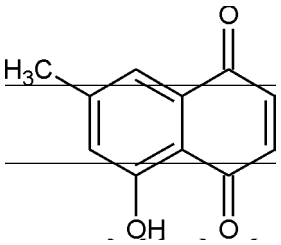

Fig. 2: Structure of 7-methyl juglone

Table1: Minimum inhibitory concentration of extracts, fractions and betulin, 7-methyl juglone obtained from *Diospyros paniculata* against bacteria and fungi

S. N.	Test organism	Minimum inhibitory concentration (MIC) µg/ml							
		ME	HF	CF	BF	Betulin juglone	7-methyl floxacin	Cipro-	Nystatin
1.	*Staphylococcus aureus* ML267	50	70	90	200	60	150	60	-
2.	*Vibrio cholerae* 1313	90	80	100	210	70	110	60	-
3.	*Escherichia coli* ATCC 10536	100	90	80	110	65	160	50	-
4.	*Shigella dysenteriae* 2	60	35	50	140	30	200	50	-
5.	*Saccharomyces cerevisiae* MTCC 36	80	70	90	100	150	120	-	80
6.	*Aspergillus niger* MTCC 281	70	60	85	140	150	110	-	65
7.	*Candida albicans* ATCC 10231	90	80	75	130	180	80	-	70

Disc of Whatmann filter paper no.1 of 6 mm diameter was used.
ME: metanol extract, HF: n-hexane fraction, CF: chloroform fraction, BF: n-butanol fraction

2.3 Microorganisms

The microorganisms, *Staphylococcus aureus* (ML267), *Vibrio cholerae* (1313), *Escherichia coli* (ATCC 10536), *Shigella dysenteriae* (2), *Saccharomyces cerevisiae* (MTCC 36), *Aspergillus niger* (MTCC 281), *Candida albicans* (ATCC 10231) were obtained from Institute of Microbial Technology, Chandigarh, India, Central Drug Laboratory, Calcutta, India and Dr. K. Patricia Carpenter, London.

2.4 Antimicrobial test

The test solutions were prepared using an aqueous solution of DMSO (dimethyl sulphoxide) 1% v/v. The dilutions for MIC

(Minimum Inhibitory Concentration) determination were serially done up to a concentration of 1000 μg/ml. MIC for antibacterial activity was determined by spot inoculation method [5] and antifungal activity was determined by agar slant method [6]. The positive control was prepared using antibiotic solution (ciprofloxacin for bacteria and nystatin for fungi) in 200 μg/ml concentration. The solvent in its pure state was used as the negative control (1% aqueous DMSO solution).

3. Results and Discussion

The results of antimicrobial studies of extracts, fractions, betulin and 7-methyl juglone are given in Table 1. From the results, it can be observed that hexane fraction was found to be very efficient against *Shigella dysenteriae*, the bacteria responsible for diarrhoea, chloroform fraction exhibited efficacy against *Candida albicans*. Compound, I which seems to be main active principle of hexane fraction, showed promising antibacterial effect, being more potent than ciprofloxacin, while compound II from chloroform fraction showed antifungal activity, comparable to that of nystatin. MIC values of both compounds I, II were found to be 30 and 75 μg/ml, respectively.

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