ANTIMICROBIAL ACTIVITY OF FIVE RARE SPECIES OF *DIOSPYROS* AND THEIR ISOLATES

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Summary

The study was premeditated to evaluate five rare species of *Diospyros malabarica*, *Diospyros oocarpa*, *Diospyros nigrisence*, *Diospyros candolleana and Diospyros montana* for their antimicrobial and anti fungal activity.

The root and leaf extracts of all five *Diospyros* species were tested for anti antimicrobial and anti fungal activity, the results revealed that root and leaf extracts of *D. candolleana* had the highest antibacterial potential, followed by *D. malabarica*, *D. oocarpa, D. nigrisence and D. Montana.*

With regard to the isolates all the isolates showed the zone of inhibition, where as 8'-Hydroxyisodiospyrin showed highest antibacterial efficiency.

Key Words: *Diospyros*, Antimicrobial, Antifungal, Ebenaceae
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Introduction

Diospyros is an Ebenaceae member and represented by 350 species in the world. Nearly 90 species are distributed in India and all are mostly trees and rarely shrubs in which few species are endemic[1].

Diospyros species have been found to be rich source of napthoquinones and naphthalene based aromatic compounds [2-5.]and almost all plants in the genus possess interesting biological properties[6-9] and the author has made a detailed survey on the species which resulted many species and it is very interesting to note that majority of species exhibited most significant results, hence the author has proposed to evaluate their antimicrobial potentials.

Materials and methods

Collection and preparation of plants

The roots of *D.oocarpa* were collected at Amboli ghat, the roots of *D. nigriscence* were collected from Tillari ghat of Maharastra state, and the roots of *D. condolleana* were collected at Ram ghat of Goa, India.

They were identified by Prof. Jadav Taxanomist, Dept of Botany, Shivaji Uviversity, Kolhapur, Maharashtra, India a voucher specimen (SGDO-1, SGDN-2 and SGDC-3) has been deposited at the herbarium, College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

Extraction and Isolation

The plant materials were dried in shade at ambient temperature, finely powered in willy mill and exhaustively extracted with chloroform. The extracts so obtained, were then concentrated under *vacuum* to get the corresponding residues. The residues were loaded in column chromatography over silica gel (Acme 100-200 mesh) and eluted with solvents of increasing polarity of petroleum ether, chloroform and methanol.

The root extract of *D. oocarpa* afforded Habibone, 8'-Hydroxyisodiospyrin and Diospyrin, the root extract of *D. nigresence* afforded Diospyrin , 4-Hydroxy-5methoxy-2-naphthaldehyde and 5-Hydroxy-4-methoxy-2-naphthaldehyde, and the root extract of *D .candolleana* afforded 5-Hydroxy-4-methoxy-2-naphthaldehyde , 4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde, 2-Methyl anthraquinone and Diospyrin.

Antimicrobial activity

The Root and leaf extracts of *D. malabarica D. oocarpa*, *D. nigrisence*, *D. candolleana* and *D. montana* and their isolates, Habibone (1), 8'-Hydroxyisodiospyrin (2), Diospyrin (3), 4-Hydroxy-5methoxy-2-naphthaldehyde (4), 5-Hydroxy-4-methoxy-2-naphthaldehyde (5),4-Hydroxy-3,5-dimethoxy-2 naphthaldehyde(6), and 2-Methyl anthraquinone (7) were used for antimicrobial activity.

For testing five Gram (+)ve bacteria, *Staphalococcus albus, Staphalococcus aureaus, Streptococcus fecalis, Bacillus subtlis, Corynebacterium diphtheriae* and five Gram (-)ve bacteria , *Escherchia coli, Proteas vulgaris, Klebsiella aerogenes, micrococcus luteus, Pseudomonas aeruginosa* were used. The fungi employed in the antifungal activity were *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Candida tropicalis* and *Rhizopus oryzae* using cup plate method. Ampicillin (10μg/ ml) for anti bacterial and clotrimazole, (30 μg) for anti fungal were employed as reference standard. The extracts wree tested at 100, 200 and 400 mg/ml dose level and the isolates at 25, 50 and 100 μg/ml[10].

Result and Discussion

The results revealed that the root and leaf extracts of *D. candolleana* had the highest antibacterial potential, followed by *D. malabarica, D. oocarpa, D. nigrisence and D. Montana.* The growth of *E .coli* and *S. aureaus* were inhibited by the all tested extracts. The organisms, *M. coccus luteus, C. diphtheriae*, *K. aerogenes* and *S. fecalis* are resistant to the root and leaf extracts of *D.malabarica*. On the other hand *E.coli* and *S. aureaus* were more susceptible, *P. vulgaris* and *S. fecalis* were mild resistant and others (except *C. luteus*) were more resistant to root and leaf extracts of *D. nigrisence*. *D.candolleana* root and leaf extracts displayed activity on the entire tested organism where as D. oocarpa is resistant except to E. coli.

With regard to the tested isolates, all the isolates showed inhibition zone where as, isolate (2) showed highest antibacterial efficiency followed by (5), (6) and (7) respectively with gram (+) ve bacteria.

Isolates of (5),(1) and (2), showed maximum zone of inhibition, whereas isolate (4) has showed less than that of the standard, and compounds (3), (6) and (7) were resistant to almost all gram (-) ve bacterial strains.

The antifungal activity results demonstrated that all the extracts showed strong activity against *Aspargillus fumigatus, Candida albians* and *Rhizopus oryzae* and moderate activity against *Aspergillus niger* and *Candida tropicalis*.in case of isolated campounds, compound (4), (5),(6) and (2) exhibited more activity than the other tested compounds. The compound (7) showed negligible activity on all the tested organisms.

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Table No 1 Antibacterial Activity of *D. malabarica* D. *oocarpa*, *D. nigrisence*, *D. candolleana* and *D. montana* against Gram Positive Bacteria

Organism ⟶	*S. albus*			*S aureaus*			*S. fecalis*			*B. subtlis*			*C.diphtheriae*		
Concentration ⟶	100	200	400	100	200	400	100	200	400	100	200	400	100	200	400
Chloroform root ext. of *D. malabarica*	11	12	12	14	18	23	1	4	7	14	14	15	9	11	11
Chloroform leaf extract of *D. malabarica*	10	13	13	15	16	21	3	2	6	13	12	14	6	11	12
Chloroform root extract of *D. Condollena*	16	20	23	18	18	20	13	13	14	16	16	18	8	9	12
Chloroform leaf extract of *D. Condollena*	17	19	22	16	18	20	15	16	15	16	16	17	5	7	11
Chloroform root extract of *D. nigresensis*	9	11	11	13	16	19	12	12	12	4	6	7	4	9	9
Chloroform leaf extract of *D. nigresensis*	11	10	11	13	13	17	11	12	11	10	11	4	5	2	5
Chloroform root extract of *D. oocarpa*	-	-	-	7	7	7	5	-	-	-	-	-	-	1	1
Chloroform leaf extract of *D. oocarpa*	3	3	6	1	3	6	5	1	5	1	2	4	5	0	3
Chloroform root extract of *D. montana*	7	11	12	3	7	10	1	-	8	-	-	-	10	10	10
Chloroform leaf extract of *D. montana*	13	13	11	11	10	10	-	1	7	1	-	-	7	7	10
Control- Chloroform	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Standard : Ampicillin (10µg/ ml)	15			12			13			16			9		

Figures indicate Zone of Inhibition in mm. Inactive (-): moderately active (7-13): highly active (> 14)

Table No. 2 Antibacterial Activity of *D. malabarica* (MHDM) *D. oocarpa* (MHDO), *D. nigrisence* (MHDN), *D. candolleana* (GADC) and *D. Montana* (KADM) against Gram Negative Bacteria

Strain of the Organism ⟶	*E. coli*			*P. vulgaris*			*K. aerogenes*			*C. luteus*			*P. aeruginosa*		
Concentration ⟶	**100**	**200**	**400**	**100**	**200**	**400**	**100**	**200**	**400**	**100**	**200**	**400**	**100**	**200**	**400**
Chloroform root ext. of *D. malabarica*	14	15	18	9	11	12	14	14	15	-	-	-	1	1	-
Chloroform leaf extract of *D. malabarica*	13	14	17	8	10	12	13	12	14	6	9	1	5	8	8
Chloroform root extract of *D. Condollena*	22	21	22	29	29	31	8	9	12	6	10	11	13	15	15
Chloroform leaf extract of *D. Condollena*	18	20	22	26	26	28	6	8	11	6	9	12	13	13	14
Chloroform root extract of *D. nigresensis*	17	20	18	8	12	12	1	7	7	-	-	-	9	10	10
Chloroform leaf extract of *D. nigresensis*	15	15	17	9	9	11	2	4	9	1	1	-	5	6	9
Chloroform root extract of *D. oocarpa*	19	21	21	-	-	-	-	-	-	-	1	1	-	1	1
Chloroform leaf extract of *D. oocarpa*	18	19	20	-	1	-	0	1	1	-	1	1	1	1	1
Chloroform root extract of *D. montana*	12	12	12	-	-	-	10	10	10	10	10	11	5	0	2
Chloroform leaf extract of *D. montana*	17	13	13	1	2	1	6	7	10	10	10	12	3	3	1
Control- Chloroform	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Standard : Ampicillin (10µg/ ml)	22			32			18			16			9		

Figures indicate Zone of Inhibition in mm.

Inactive (-): moderately active (7-13): highly active(> 14)

Table No.3 Antibacterial Activity of isolates of *D. oocarpa*, *D. nigrisence* and *D. candolleana* against Gram Positive Bacteria

Strain of the Organism ⟶	*S. albus*			*S.aureaus*			*S. fecalis*			*B. subtlis*			*C. diphtheriae*		
Concentration ⟶	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
Habibone	16	16	14	10	10	10	14	15	19	29	35	28	14	16	16
8'-Hydroxyisodiospyrin	32	31	32	27	21	28	23	22	25	30	31	31	18	20	20
Diospyrin,	10	12	12	11	8	7	13	12	14	13	13	11	10	9	9
4-Hydroxy-5methoxy-2-naphthaldehyde	18	19	22	14	14	12	13	12	13	15	19	22	22	20	12
5-Hydroxy-4-methoxy-2-naphthaldehyde	30	28	22	12	8	13	22	22	19	28	27	27	40	26	26
4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde	12	12	12	10	9	11	12	13	12	15	13	10	10	10	9
2-Methyl anthraquinone.	18	18	15	30	28	22	23	21	21	22	21	17	30	28	31
Control- Chloroform	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Standard : Ampicillin (10μg/ ml)	15			12			13			16			9		

Figures indicate Zone of Inhibition in mm.

Inactive (-): moderately active (7-13): highly active(> 14)

Table No.4 Antibacterial Activity of isolates of *D. oocarpa*, *D. nigrisence* and *D. candolleana* against Gram Negative Bacteria

Strain of the Organism ⟶	*E. coli*			*P. vulgaris*			*K.aerogenes*			*C. luteus*			*P. aeruginosa*		
Concentration ⟶	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
Habibone	28	25	20	45	40	42	33	31	31	28	20	25	31	39	39
8'-Hydroxyisodiospyrin	24	26	26	21	30	24	20	21	20	>60	>60	>60	40	22	31
Diospyrin	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
4-Hydroxy-5methoxy-2-naphthaldehyde	18	17	22	14	14	12	13	12	13	15	19	22	22	20	12
5-Hydroxy-4-methoxy-2-naphthaldehyde	32	34	32	32	32	25	28	28	34	23	19	19	17	19	19
4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde	-	-	3	-	5	5	2	-	3	-	-	-	-	-	-
2-Methyl anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control- Chloroform	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Standard :Ampicillin (10µg/ ml)	22			32			18			16			9		

Figures indicate Zone of Inhibition in mm.

Inactive(-): moderately active (7-13): highly active(> 14)

Table No.5 Antifungal Activity of the root and leaf extracts of *D. malabarica, D. oocarpa*, *D. nigrisence*, *D. candolleana* and *D. montana*.

Strain of the Organism ⟶	*A.niger*			*A. fumigatus*			*C.albicans*			*C.tropicalis*			*R. oryzae*		
Concentration ⟶	**100**	**200**	**400**	**100**	**200**	**300**	**100**	**200**	**300**	**100**	**200**	**300**	**100**	**200**	**300**
Chloroform root ext. of *D. malabarica*	6	9	8	15	15	15	17	20	20	12	12	14	15	16	16
Chloroform leaf ext.of *D. malabarica*	9	4	9	12	15	15	15	15	19	13	13	14	10	16	17
Chloroform root ext.of *D. Condollena*	6	6	8	12	14	14	14	16	18	11	11	10	14	14	15
Chloroform leaf ext of *D. Condollena*	5	6	7	8	12	13	16	15	18	9	10	12	13	13	13
Chloroform root ext.of *D. nigresensis*	8	9	9	11	12	13	13	13	15	8	8	9	6	9	10
Chloroform leaf ext of *D. nigresensis*	7	8	8	9	11	11	13	14	14	4	4	5	9	12	10
Chloroform root ext.of *D. oocarpa*	9	10	9	15	15	16	6	15	16	-	7	7	19	20	20
Chloroform leaf ext of *D. oocarpa*	10	11	11	8	15	15	12	12	14	7	6	6	17	20	21
Chloroform root ext.of *D. montana*	7	7	7	12	15	13	10	12	14	8	8	8	16	19	18
Chloroform leaf ext of *D. montana*	5	8	7	11	12	12	14	15	15	9	11	10	15	18	18
Chloroform Control	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Standard- clotrimazole, 30 µg	21			23			18			14			20		

Figures indicate Zone of Inhibition in mm.

Table No.6 Antifungal Activity of the isolates of *D. oocarpa*, *D. nigrisence* and D. *candolleana*

Strain of the Organism ⟶	*A. niger*			*A. fumigatus*			*C. albicans*			*C. tropicalis*			*R. oryzae*		
Concentration ⟶	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**	**25**	**50**	**75**
Habibone	12	14	14	14	16	18	11	11	10	14	14	15	12	15	13
8'-Hydroxyisodiospyrin	18	17	22	14	14	12	15	19	22	22	20	12	22	20	12
Diospyrin	15	23	19	18	17	19	13	12	13	12	9	9	12	23	10
4-Hydroxy-5methoxy-2-naphthaldehyde	32	34	32	32	32	28	34	23	19	19	17	19	19	19	19
5-Hydroxy-4-methoxy-2-naphthaldehyde	28	34	23	19	19	17	19	19	19	19	17	19	19	19	19
4-Hydroxy-3,5-dimethoxy-2-naphthaldehyde	28	25	20	45	45	42	33	30	31	28	20	29	31	39	39
2-Methyl anthraquinone	-	8	9	4	4	7	-	-	-	8	7	9	2	-	-
Control- Chloroform	--	--	--	--	-	--	--	--	--	--	--	--	--	--	--
Standard : clotrimazole, 30 µg	21			23			18			14			20		

Figures indicate Zone of Inhibition in mm.