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ISOLATION AND CHARACTERIZATION OF DIBUTYL PHTHALATE FROM LEAVES OF *Ipomoea carnea*

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Ipomoea carnea, belonging to the family Colvolvulaceae and commonly called Mahananda in Marathi, is a nonwoody plant, representing an annually renewable source of high- quality fibers. It can be successfully grown in temperate and tropical climatic conditions without requiring much attention [1]. It is frequently found in planes and lowlands near water sources [2]. It is an ornamental plant due to its variety of flowers that are pale rose, pink or light violet, and whitish blue [3]. The antifungal activity of the chloroform and methanol extract of *I. carnea* showed activity at concentrations of 5–20% against eleven pathogenic and nonpathogenic fungi [4]. The water extract of *I. carnea* in combination with other extracts was tested for its HIV type I reverse transcriptase inhibitory activity [5]. The antimicrobial activity of metal complexes prepared from the leaf proteins of *I. carnea* has been reported [6].

The entomological activity of the ethyl acetate extract of the plant was tested against two mosquito species at the 4th instar larval stage of *Aedes aegypti*, which causes Dengue fever, as well as *Culex quinquifasciatus*, which is a vector of filariasis. The experiments show that the ethyl acetate extract has significant larvicidal activity. Efforts were made towards the isolation of molecules from the same extract. Column chromatography was carried out using nonpolar *n*-hexane and polar ethanol as solvent systems. The *n*-hexane fraction of the ethyl acetate extract furnished a mixture of crude products. An aromatic ester, dibutyl phthalate (**1**), was isolated and purified by chromatographic techniques. This compound was isolated for the first time from this plant source.

Dibutyl phthalate (DBP) has been isolated from marine algae [7–9], bacteria [10, 11], fungi [12], roots of *Achyrathes bidentata* [13], and *Rheum glabricaule* [14] as a secondary metabolite. The leaves of "Mironovskaya 808" wheat [15], *Alstonia scholaris* [16], and *Torreya grandis* [17] showed presence of dibutyl phthalate. DBP was found to be present in the pericarp of *Trichosanthes rosthornii* [18].

The compound isolated is a colorless transparent liquid. HPLC-LC-MS of the compound exhibited a molecular ion peak at *m/z* 279 in positive mode, which matches the molecular formula $C_{16}H_{22}O_4$.

IR spectrum (nujol, ν, cm^{-1}): 1728 (C=O); 1600, 1579 (Ar). ^{1}H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.98 (6H, t, J = 5, CH_3-5′, 5″), 1.47 (4H, m, CH_2-4′, 4″), 1.74 (4H, m, CH_2-3′, 3″), 4.33 (4H, t, J = 5, CH_2-2′, 2″), 7.73 (2H, dd, J = 10, 5, H-2, 5); 7.56 (2H, dd, J = 10, 5, H-3, 4). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ): 13.70 (q, C-5′, 5″), 19.18 (t, C-4′, 4″), 30.59 (t, C-3′, 3″), 65.55 (t, C-2′, 2″), 128.84 (d, C-2, 5), 130.89 (d, C-3, 4), 132.35 (s, C-1, 6), 167.69 (s, C-1′, 1″)

Saponification of the isolate led to new peaks of the hydrolyzed products with decrease in the intensity of the molecular ion peak. MS chromatograms of the hydrolyzed products confirmed the isolated compound **1** as dibutyl phthalate.

The plant, *Ipomoea carnea*, was collected from the river side of Pune, Maharashtra, India. It was authenticated by comparison with a herbarium voucher specimen deposited at Botanical Survey of India, Pune, India. Its authentication number is E LICAI BSI/WC/Tech/2009/96.

The air shade-dried and pulverized material (100 g) was refluxed with ethyl acetate for 48 hours on a steam bath. The solvent was recovered under reduced pressure. The sticky thick extract (B) was found to be 3.4%. The extract (B, 2.350 g) was column chromatographed using silica gel (60–120, 1:30). It was fractionated using gradient elusion from *n*-hexane (I – 0.310 g), *n*-hexane–ethyl acetate (II – 9.5:0.5, 1.034 g; III – 9:1, 0.02 g, IV – 8:2, 0.120 g) with successive by increasing polarity of solvents (V – chloroform, 0.04 g; VI – ethyl acetate, 0.311 g; VII – acetone, 0.285 g; VIII – ethanol, 0.310 g). Methanol was used as terminal eluate (IX – 0.08 g). The collected volume of each fraction was 200 mL. The progress of the column chromatographic separation was monitored by TLC. Fractions of similar composition were combined to obtain nine (I–IX) fractions.

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TABLE 1. LC-MS of the Hydrolytic Degradation Products (Values fragments, V. f.)

Product	RT, min	V.f. amu, *m/z*	Product	RT, min	V.f. amu, *m/z*
I	9.5	279 (M + 1)	IV	6.4	195
II	8.2	237	V	4.8	181
III	6.7	223			

TLC-guided fractions I and II were mixed together (1.20 g), which were showed minor impurities along with major component. The mixed fractions were re-column chromatographed using silica gel (60–120, 1:50) and eluted with gradient polarity of solvents (*n*-hexane–ethyl acetate (9.5:0.5→5:5 Fr. K), ethyl acetate (Fr. L), and acetone (Fr. M). Each fraction of 200 mL volume was collected. A total of 13 fractions (A to M) was collected. The fractions were monitored by thin layer chromatography. Fraction K was a mixture of compounds along with compound **1**, as shown by thin layer chromatography. It required purification.

Purification of Compound 1. The fraction was passed through a silica gel column using *n*-hexane–ethyl acetate (7:3) as eluent to yield a transparent liquid, compound **1**. Saponification of the isolate, compound **1**, was achieved by HPLC-LC-MS and to confirmed the structure. Examination of the resultant masses of the fragments led to the conclusion that the compound is dibutyl phthalate.

Saponification by HPLC-LC-MS. Approximately 100 μg of compound **1** was dissolved in 100 μL of methanol and diluted to 1000 μL with 0.1 N NaOH and then sonnicated. The sample was injected in into an LC-MS system at 0, 1, 2, 4, 6, and 8 hour time intervals to track the progress of the saponification reaction. The fragmentation pattern of the parent molecular ions to its daughter ions was recorded. The degradation products, stable ions, were analyzed by their mass spectra and confirmed by their classical fragmentation patterns. The LC-MS chromatograms for the hydrolytic fragments showed the probable fragment ion moieties. The hydrolytic degradation products along with parent molecule are shown in Table 1.

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