

Antibacterial activity of di-butyl phthalate isolated from *Begonia malabarica*

Abstract

Natural products from medicinal plants either as pure compounds or as standardized extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. *Begonia malabarica* Lam. is one of the medicinally important herbs belonging to the family of Begoniaceae. It is reported that the leaves are used to treat respiratory infections, diarrhea, blood cancer and skin diseases. The whole plant possesses a variety of secondary metabolites. Isolation of such compounds will add more novel bioactive structures that are of interest to screen and select potential lead compounds to discover and develop drugs in the modern pharmaceutical sector. Against this backdrop, *Begonia malabarica* was extracted with various solvents and bioactive compounds were isolated using chromatographic techniques. One of the bioactive compounds isolated from it was colorless or pale yellow oily compound that is soluble in chloroform. The structure of the compound was elucidated as di-butyl phthalate with the help of spectral data such as FT-IR, MS, ¹H-NMR and ¹³C-NMR. This is the first report to the family of Begoniaceae. The compound is reported to have antibacterial and anticancer properties.

Keywords: chromatography, extraction, structural elucidation, antibacterial activity

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Introduction

Begonia malabarica L. is an important medicinal plant belonging to the family of Begoniaceae. This plant is used to treat many human ailments. Its leaves are substituted for Tamarind (*Tamarindus indica* L., Caesalpinoideae), cooked and consumed by the Palliyar tribe in Tirunelveli District of Tamil Nadu in India. Consumption of boiled leaves is practiced to treat stomach ulcer, diarrhea, stomachache, respiratory problems, and skin diseases.¹ Leaves are used as wild edible by the Kani Tribe in Kanyakumari District of Tamil Nadu in India that contain high content of phenolic and flavonoid compounds and exhibit free radical scavenging activity.² Their counterparts in Tirunelveli District of Tamil Nadu consume leaves to treat venereal diseases and cool the body.³ Plant is rich with vitamin C and its juice is consumed for blood purification and for treating fever.⁴ Leaf juice with a pinch of salt is consumed to treat giddiness and leaf paste is applied to treat foot sores.⁵ The secondary metabolites including terpenes, polyphenols, alkaloids and some glycosides are largely responsible for the individual properties of the plants such as aroma, flavour, color and medicinal actions.⁶

There are a few reports of occurrence of phthalates in plants. Ten compounds including dioctyl phthalate, butyl isobutyl phthalate, n-heptacosane, lophenol, docosanyl ferulate, tetracosanyl ferulate, hexacosanyl ferulate, octacosanyl ferulate, pterostilbene, and 4'-methoxy-3',7-dihydroxy-flavone were reported from petroleum ether and ethyl acetate extract fractions of *Dracaena cochinchinensis*.⁷ Di-(2-ethyl) hexyl phthalate was reported from the leaves of *Cassia auriculata*.⁸ Bis(2-methylheptyl) phthalate was reported from the aerial parts of *Hypericum hyssopifolium*.⁹ Di-isooctyl phthalate was reported in *Limonium bicolor*.¹⁰ Bis (2-ethylhexyl) phthalate was reported from the roots of *Euphorbia hylonoma*.¹¹ GC-MS analysis of essential oil from the skin of water caltrop showed the presence of diethyl phthalate.¹² Root exudates of banyard grass, *Echinochloa crus-galli* also reported to have diethyl phthalate.¹³ Di(2-ethylhexyl) phthalate was reported after its isolation from the leaves, root and bark of *Achornea cordifolia*.¹⁴ Isolation of di-butyl phthalate is reported

here for the first time to the family of Begoniaceae from *Begonia malabarica* by us.

Materials and methods

Collection of plant material

The plant material was collected from Tirunelveli district of Tamil Nadu in India and used for scientific investigation. Voucher specimens (MBV & CR 1293) were prepared and deposited in the Herbarium of the Centre for Research and Development of Siddha-Ayurveda Medicines (CRDSAM), Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India, after authentication by Prof. MB Viswanathan, for reference.

Extraction of plant material

Whole plants were collected, shade-dried, powdered and hot-extracted using Soxhlet apparatus. The extract thus collected was filtered and concentrated at 60°C in a Rotary Evaporator (Yamato VR300-RE300-CF300-BM510, Muromachi, Japan).

Isolation

The concentrated extract (5 g) was dissolved with 30 ml methanol, added 100 ml petroleum ether for liquid-liquid separation and collected methanol-soluble portion only for further analysis. Then, chloroform and water (50:50) was added, collected water-soluble portion, concentrated in the Rotary Evaporator and chromatographed on a silica gel column (100-200 mesh). Elution process was performed using a solvent mixture of chloroform/methanol with increasing amount of methanol. Successive fractions were collected and dried using the Rotary Evaporator. The profile of column fractions was monitored by thin layer chromatography (TLC) using pre-coated TLC plate of silica gel 60 F₂₅₄ (MERCK, Darmstadt, Germany) for 0.2 mm thickness to confirm the similarities of elutes. The number and color of the spots were visualized through UV Chamber and recorded their R_f values.

HPTLC Analysis

High Performance Thin Layer Chromatography (HPTLC) equipped with CAMAG (Switzerland) Linomat V sample applicator fitted with a 100 μ l syringe (Hamilton Bonaduz, Switzerland) was used to develop fingerprinting profile. Samples were loaded with nitrogen gas supply for simultaneous drying of bands. A 10 μ l as 6.0 mm band length in 10 x 10 Silica gel 60 F₂₅₄ TLC plate was loaded using syringe and kept in TLC twin trough developing chamber (after saturation with solvent vapour) with respective mobile phase. The plate was developed in the respective mobile phase chloroform and methanol (9:1) up to 70 mm and dried using hot air oven to evaporate solvents from the plate. Then, the plate was fixed with the scanner stage for scanning at 254 nm. The peak table, peak display and peak densitogram were identified. Finally, the plate was kept in photo documentation chamber and captured images at UV 366 and 254 nm.

Structural characterization

Melting point and m.m.p. of the compound were recorded. Spectral data were collected by using UV-Visible Spectrophotometer (1800, Shimadzu, Kyoto, Japan), Fourier Transform-Infrared Spectroscopy (FT-IR) (equipped with L1600400 Spectrum Two DTGS, Perkin Elmer, Massachusetts, USA), ¹H NMR and ¹³C NMR AV 400-Bruker 400 MHz High Resolution Multinuclear FT-NMR Spectrophotometer (Massachusetts, USA), and GC-MS 7890 A, MS 5975 equipped with HP 5 MS column (Agilent, California, USA). The data thus collected were used to elucidate the structure of the compound.

Antibacterial activity

Agar-well diffusion method (15) was followed. Muller-Hinton Agar (MHA) (HiMedia, Mumbai, India) plates were swabbed (sterile cotton swab) with 24 h old broth culture of the respective bacteria. A sterile cork-borer was used to place wells, each measuring 8 mm in diameter in each of the plate. Test sample was loaded into the wells with 100, 50, 25, 12.5 and 6.25 mg ml⁻¹ concentration using sterilized dropping micropipettes. The plates were incubated at 37°C for 18-24 h. Experiments were conducted thrice and diameters of the inhibition zones were recorded and averaged to measure antibacterial activity.

Results

The compound was isolated by column chromatography using chloroform and methanol (9:1) ratio as elute and the purity of the compound was confirmed by HPTLC (Figure 1). A single fluorescent blue spot was visualized under UV Chamber at 254 nm with R_f of 0.55.

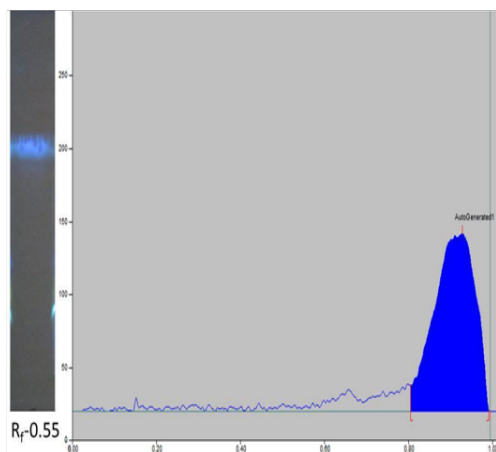


Figure 1 HPTLC chromatogram of dibutyl phthalate.

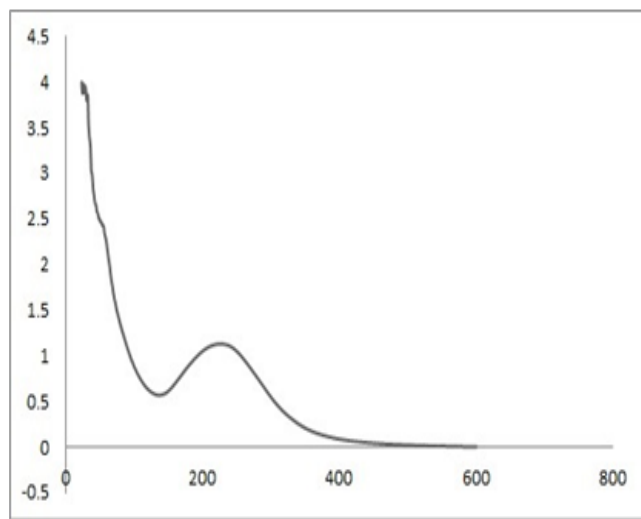


Figure 2 UV-Vis spectrum.

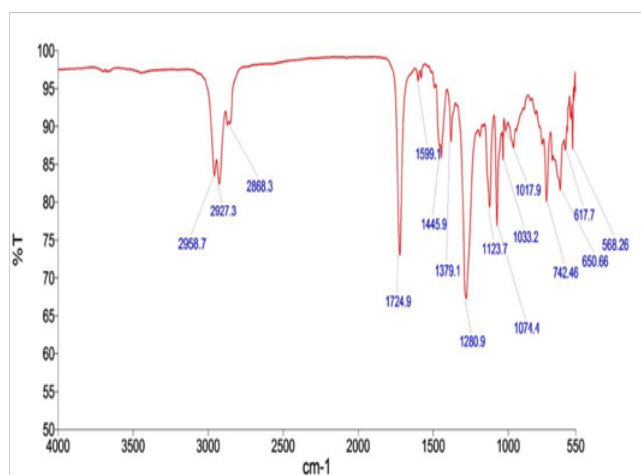


Figure 3 FT-IR spectrum.

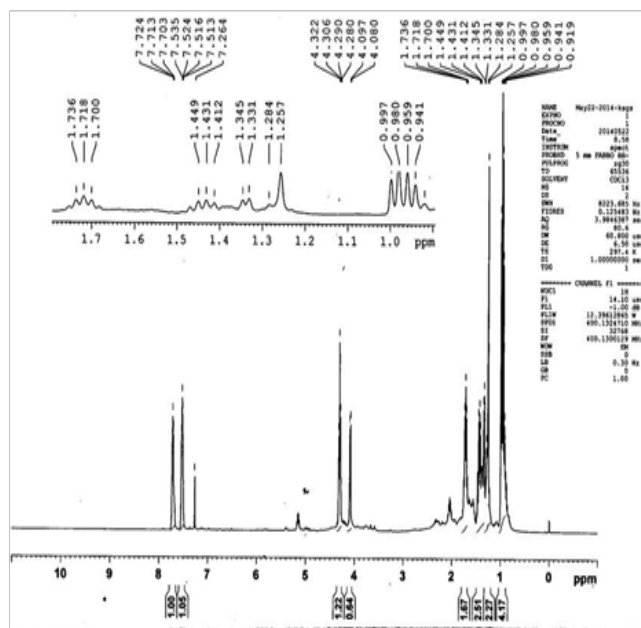


Figure 4 ¹H NMR spectrum.

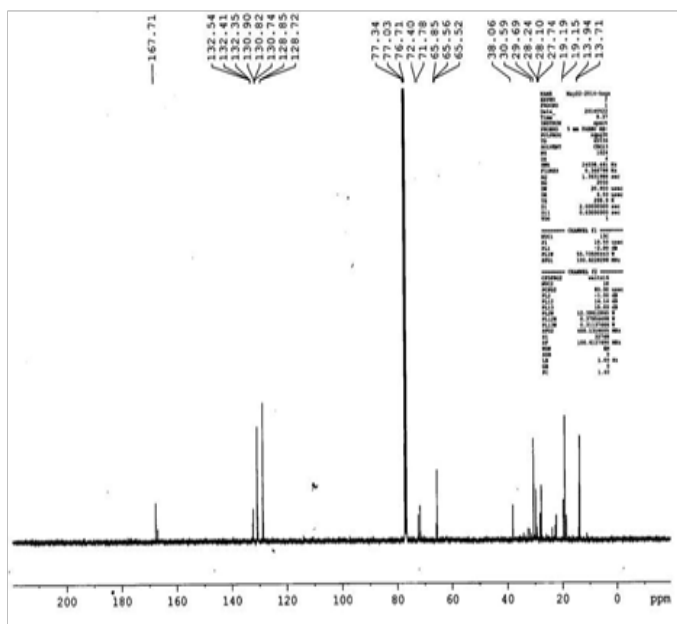
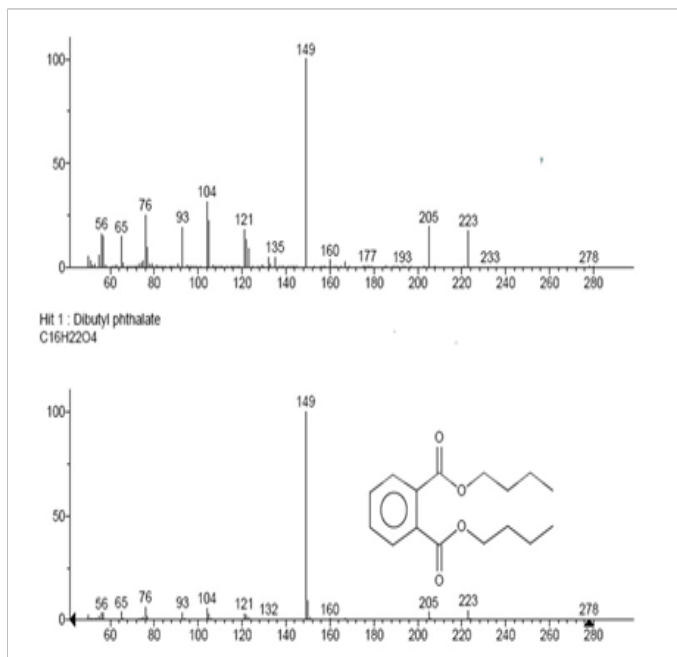
Figure 5 ^{13}C NMR spectrum.

Figure 6 Mass spectrum.

Dibutyl phthalate ($\text{C}_{16}\text{H}_{22}\text{O}_4$), pale yellow oily compound, yield: 500 μl (0.01%). Boiling point: 340-343°C, UV (λ_{max}): in chloroform 231 (Figure 2). FT-IR (cm^{-1}): 1724.9 (ester carbonyl), 1280.9 and 1074.4 (-C-O-stretching), 742.46 (ortho disubstitution) (Figure 3). ^1H NMR (δ ppm): $\text{CDCl}_3\text{-d}_6$, 400 MHz, 7.724 (2H, *dd*), 7.535 (2H, *dd*), 4.322 (4H, *t*, -O- CH_2), 1.736-1.449 (4H, *m*, - CH_2), 1.345-1.284 (4H, *m*, - CH_2), 0.919 (6H, *t*, - CH_3) (Figure 4). ^{13}C NMR (δ ppm): $\text{CDCl}_3\text{-d}_6$, 400 MHz, 167.71 (C=O), 132.54 (C), 130.90, 128.85 (CH, Aromatic), 65.85 (-O- CH_2), 30.59, 19.17 (- CH_2), 13.82 (- CH_3) (Figure 5). Mass (m/z): 278 (M^+ peak) (Figure 6).

Antibacterial activity

Dibutyl phthalate (Figure 7) showed 9 mm zone of inhibition against *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Vibrio cholerae* and *P. aeruginosa* at the concentration of 100 mg ml^{-1} . Similar zone of inhibition recorded at all the concentrations of *E. coli* and *Pseudomonas aeruginosa*. Eight mm zone of inhibition was recorded against *Streptococcus pneumoniae* at 50, 25 and 12.5 mg ml^{-1} . *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Shigella flexneri* also showed 8 mm zone of inhibition at 25, 12.5 and 6.25 mg ml^{-1} concentrations.

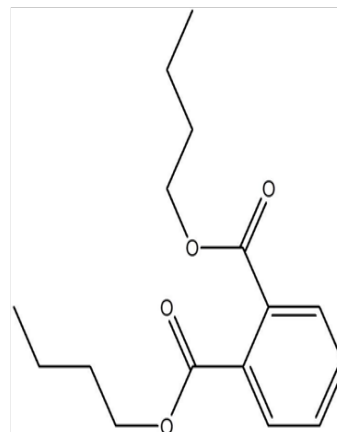


Figure 7 Structure of dibutyl phthalate.

Discussion

Bark of *Mimusops elengi* was extracted with hexane, chloroform, methanol and ethanol using Soxhlet apparatus. Then, the crude extract of chloroform was fractionated using 100% toluene and toluene-ethyl acetate (75:25). These fractions were mixed together and re-chromatographed using toluene and ethyl acetate (90:10), again fractionated using hexane and ethyl acetate with increasing percentage of ethyl acetate to obtain impure dibutyl phthalate.¹⁶ Dibutyl phthalate was cold extracted with ethyl acetate and eluted with hexane and ethyl acetate (9.5:0.5) from the stem of *Ipomoea carnea*.¹⁷ Essential oil of flowers in *Leea indica* was obtained by hydro-distillation and re-extracted with diethyl ether and dried over anhydrous Na_2SO_4 . Essential oil in hexane (1:40) ratio was analysed by GC-MS that revealed the presence of 17 compounds including the identification of 8 different types of phthalates.¹⁸ In the present study, chloroform and methanol (9:1) fraction of methanol extract yielded 0.01% of dibutyl phthalate (Figure 8). The spectral data were identical to those reported in the literature¹⁹ (Figures 1-6).

Seven-day old culture of *Streptomyces bangladeshensis* mycelium was extracted twice with ethyl acetate and isolated bis-(2-ethylhexyl) phthalate from it.²⁰ Bis (2-methylheptyl) phthalate isolated from the leaves of *Pongamia pinnata* increased 60% survival of *Penaeus monodon* infected with White Spot Syndrome Virus (WSSV) at 100 $\mu\text{g g}^{-1}$ of body weight of shrimp day^{-1} .²¹ Methanol extract of *Nigella glandulifera* seeds inhibited B16F10 murine melanoma cells by 43.7% and exhibited low cytotoxicity (8.1%) at a concentration of 100 $\mu\text{g ml}^{-1}$. Dried seeds of *N. glandulifera* extracted three times with four volumes of 95% methanol and fractionated with hexane and chloroform (1:0-0:1) to yield dioctyl phthalate (DOP). The compound

also showed similar mode of action as that of *N. glandulifera* crude extract and inhibited murine tyrosine activity in B16F10 melanoma cells by 25.8% at a concentration of 10 μ M (22). In the present study, antibacterial activity of DBP showed 9 mm zone of inhibition against *S. epidermidis*, *S. pneumoniae*, *E. coli*, *M. luteus*, *K. pneumoniae*, *S. flexneri*, *V. cholerae* and *P. aeruginosa* at the concentration of 100 mg ml⁻¹ (Figure 8). All the concentrations of DBP showed 9 mm zone of inhibition against *E. coli* and *P. aeruginosa*. DBP exhibited 8 mm zone of inhibition against *S. pneumoniae* at 50, 25, 12.5 mg ml⁻¹ concentrations. At the concentrations of 25, 12.5 and 6.25 mg ml⁻¹, DBP exhibited 8 mm zone of inhibition against *S. epidermidis*, *K. pneumoniae* and *S. flexneri*.

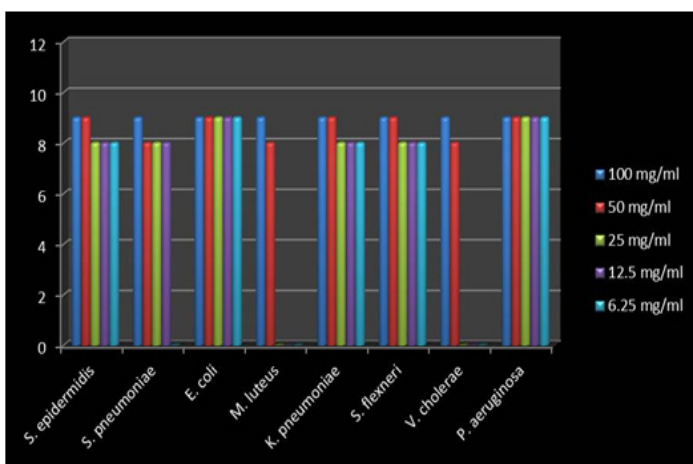


Figure 8 Antibacterial activity.

Conclusion

Dibutyl phthalate was isolated and characterized for the first time in *Begoniaceae*. It showed remarkable antibacterial activity. It can be tested against melanoma as its related compounds exhibited potential activity.

Acknowledgements

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Conflict of interest

The authors declare that there is no conflict of interest.

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