Research Article

Isolation and Characterisation of a Diethyl Pthalate, an Bioactive Compound from *Cassia Auriculata* L

Sucheta Gaikwad, Swati Devare, Asha Kale, Nirmala R. Deshpande, Jyoti P.Salvekar
Dr. T. R. Ingle Research Laboratory, Department of Chemistry, S. P. College, Tilak Road, Pune – 411 030, Maharashtra, India.
***Corresponding author's E-mail:** suchetag2000@yahoo.com

Accepted on: 03-01-2013; **Finalized on:** 28-02-2013.

ABSTRACT

An Ayurvedic medicinal plant *Cassia auriculata* L, belongs to family Cesalpineaceae. All parts of the plant have medicinal property. Phytochemical screening of *Cassia auriculata* L leaves revealed the presence of starch, proteins, sugars, alkaloids, steroids and tannins. The aim of this study is to identify and characterize the bioactive principle from the leaves of the plant. For isolation of the compound the air shade dried and pulverized leaves material was subjected to hot solvent extraction with Acetone. This extract was subjected to column chromatography. The isolation and purification afforded thick viscous oil, which was subjected to physical, chemical and spectral identification by MS, IR, ^{1}H-NMR, ^{13}C-NMR, etc. The compound was concluded as di-ethyl phthalate.

Keywords: *Cassia auriculata* L, Cesalpineaceae, isolation, diethyl pthalate, thermal parameters.

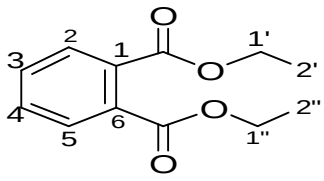
INTRODUCTION

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. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest in plant chemistry has grown throughout the world. Research studies leading to extraction, isolation, identification and biological study of plant constituents have now formed the major field of the study.

Literature survey revealed that, The phytochemical investigation of *Phyllanthus muellerianus* showed presence of bis(2-ethyloctyl)phthalate and bis(2-ethylicosyl)phthalate(VI)[1]. Di-isooctyl phthalate (VII) had been reported from *Limonium bicolor* Kuntze and *Dracaena cochinensis* (Lour.)[2]. Butyl(VIII) and isobutyl phthalates were also reported from *D. cochinensis.* Di-(2-ethyl) hexylphthalate was isolated from the *Cassia aurriculata* leaves Linn[3]Bis (2-ethylhexyl) phthalate was reported from the roots of *Euphorbia hylonoma* Hand.-Mazz[4].Dibutyl phthalate had been isolated from plants, marine algae, bacteria and fungi[5]. The presence of dibutyl phthalate from *Mimusops elengi*[6]*, Leea indica*[7]*, Alstonia scholaris, Torreya grandis, Achyrathes bidentata, Rheum glabricaule*[8] has been reported. Leaves of Mironovskaya 808 wheat was also reported to produce dibutyl phthalate as secondary metabolite[9].

MATERIALS AND METHODS

Air shade dried and powdered leaves material (300 gm) was used for detailed screening. Extractive values were determined using solvents ranging from non polar hexane to polar methanol at refluxing temperature for twenty four hours. The extracts were examined for their antimicrobial and antioxidant potentials. Acetone extract was found to be more potent, so it (4.550 g) was broad fractioned on silica gel (60-120,170 g). Fractionation was carried out using gradient polarity of solvents from hexane to methanol, which resulted in seven fractions. The fractions were monitored by TLC. Ethyl acetate indicated presence of viscous liquid along with some unidentified compounds. Total eight fractions were collected. Hexane ethyl acetate fraction was further purified by re-column chromatography obtained as pure transparent colorless liquid (61 mg) as phthalate has been reported for the first time**.** The structure was determined by modern spectral techniques. The synthesized molecule show bioactivity as already reported.



Diethyl pthalate

RESULTS AND DISCUSSION

The Compound isolated as a colourless transparent liquid. The LCMS spectrum indicates 99.74% purity. It exhibits a molecular ion peak at *m/z* 223 $[M+1]^+$ which is a base peak. Typical fragment at 148.9 *m/z*(64%) is noted for phthalate moiety which is in accordance with the molecular formula $C_{12}H_{14}O_4$.

The IR spectrum displays a characteristic absorption frequency at 1729 cm^{-1} for ester carbonyl groups. The absorption bands at 1592 cm^{-1}, 1474 cm^{-1} and 1453 cm^{-1} are noticed for aromatic stretching frequency. Bands at 1285 cm^{-1}, 1127 cm^{-1} and 1073 cm^{-1} are noticed for -C-O- stretching and at 745 cm^{-1} represents ortho di-substitution of benzene ring.

^{1}H NMR spectrum, has displayed a downfield triplet at δ 1.39 (t, *J* = 8 *Hz,* 6H) for (H 2' and H 2") methyl protons which are attach to oxy methylene group. A downfield quartet at δ 4.39 (q, *J* = 6 *Hz,* 4 H) is noticed for (H 1' & H 1") protons. The doublet of doublets at δ 7.75 (dd, *J* = 10 & 2 *Hz,* 2 H) and δ 7.55 (dd, *J* = 10 & 2 *Hz*, 2 H) are indicated for (H 2 & H 5) and (H 3 & H 4) aromatic protons respectively.

^{13}C NMR spectrum, displays presence of six signals. This is in accordance with twelve carbon atoms, indicating two sets of identical and equivalent carbon atoms. Each signal corresponds two carbon atoms. A quartet at δ 8.86 is assigned to (C 2' and C 2") methyl carbon atoms. The triplet at δ 56.39 is noticed for (C 1' & C 1") for ethoxy methylene carbon atoms. The downfield doublets at δ 125.72 and δ123.60 are observed for (C2 & C5) and (C3 & C4) aromatic carbon atoms. A singlet at δ 126.97 is indicated for (C1 and C6) tetra substituted aromatic carbon atoms. The most downfield singlet at δ162.42 is assigned for ester carbonyl carbons.

DEPT pulse sequence demonstrates the presence of four methine, two methylene, two methyl carbon atoms and by the difference four quaternary carbon atoms

CONCLUSION

The molecule has diverse applications. Reports have recorded very few plants produce such bioactive secondary metabolite, diethyl phthalate. This important metabolite has been isolated for the first time from this genus- family and the species. Leaves extract of this annual perennial shrub may be employed for various applications to mankind. Thus this plant may be useful for the society.

Acknowledgement: Authors are thankful to the Principal S. P. College Pune and the Head, Department of Chemistry, S. P. College, Pune, Maharashtra, India for providing the necessary laboratory facilities for the work.

REFERENCES

1. R. N. Roy, S. Laskar, S. K. Sen, Dibutyl phthalate, the bioactive compound produced by Streptomyces albidoflavus, Microbiol Res.. 161(2), 2006,121-6.
2. Saleem M, Nazir M, Akhtar N, Onocha PA, Riaz N, Jabbar A, Shaiq Ali M, Sultana N New phthalates from Phyllanthus muellerianus (Euphorbiaceae).. *J Asian Nat Prod Res.* Nov,11, 2009,974-7.
3. Wei H , Wen D, Liu X , Tang R, *Zhongguo Zhong Yao Za Zhi*, constituents of petroleum ether and ethyla acetate fraction of *Dracaena chochinensis .J. Chil. Chem. Soc* 23, 616-618, 1998.
4. N. Rao , M. Kumar, VS Dandapani, R Krishna ,Hayashi, *Fitotp,* 71, 2000,82-83.
5. Ruan HL, Zhang YP, ,Wu JZ, Studies on constituents of roots of *Euphorbia hylonoma Zhongguo Zhong Yao Za Zhi*, 31, 2006,742-744,.
6. M. Namikoshi, T. Fujiwara, T. Nishikawa,K. *Marine Drugs*, 4, 2006, 290-297.
7. A D Ruikar, TV Gadkari,U D Phalgune, VGPuranik, N R Deshpande, Studies on aerial part of *Artimisia pallens* for the phenol flavonoid content *Chem.of Nat.Comp.*, 213 2010, 6.
8. G.V. Srinivasan, P. Sharanappa, N K Leela, C T Sadashiva, and K K Vijayan, Anti-microbial Activity and Biochemical Constituents of Two Edible and Medicinal Mushrooms of Mid-Western, Uganda *Nat.Prod.Radiance*, 8, 2009, 488-493.

Source of Support: Nil, **Conflict of Interest:** None.