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Isolation of 3-n-Butyl Phthalide & Sedanenolide from *Apium graveolens* Linn.

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ABSTRACT: Phytochemical studies of *apium graveolens* extract resulted in the isolation of two phthalide compounds, 3-n-butylphthalide and sedanenolide using column chromatography. Both the compounds have been purified by gas chromatography and found out to be 89.65% and 80.5%. Their structures were established on the basis of extensive spectral data analysis(UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrum) and chemical methods.

KEYWORDS: Apium Graveolens; n-butylphthalide; sedanenolide; Phytochemistry.

INTRODUCTION

Apium graveolens Linn. (Apiaceae) has a long history of use in Ayurveda and Unani system of medicine. Apium graveolens L (Apiaceae) grows wild at the base of the north western himalyas and outlying hills in Punjab and in western India[1]. A. graveolens has been used as a food, and at various times both the whole plant and the seeds have been consumed as a medicine. Celery seeds or celery seed extracts are used as flavoring agents and also in anti rheumatic formulations as the seeds have significance as arthritic pain relief, for treating rheumatic conditions and gout. Apart from the role in rheumatism, celery seeds proved its use in asthma, bronchitis and inflammatory conditions[2-6].

Literature study revealed that apium graveolens consist of brassinosteroid like 2-deoxybrassinolide[7]; furanocoumarins and its glucosides[8] like apiumoside, rutaretin-1`-O-glucoside, coumaric acid[9] & apiumetin, rutaretin[10] ; phenolics like 3-methoxy-4,5-methylenedioxybenzoic acid(myristic acid), 8-hydroxy-5-methoxypsoralen and umbelliferone[11]; coumarins like seselin, isoimperatorin, osthenol, bergapten, isopimpinellin, apigravin[12]; flavanoids like apiin and 4`,5,7-trihydroxyflavone(apigenin)[13]; senkyunolide-N, senkyunolide-J, 3-hydroxymethyl-6-methoxy-2,3-dihydro-1H-indol-2-ol and its acetate derivative, l-tryptophan, 3-methoxy apiin, sedanolide[14]; sesquiterpenoid glucosides like celeroside A- E & Phthalide glycosides like celephthalide A-C[15,16].

Current research work is to isolate & structural elucidation of two phytoconstituents from apium graveolens.

EXPERIMENTAL WORK

General experimental procedure

Melting points were uncorrected. IR spectra were recorded as KBr pellets on Shimiadzu FTIR spectrophotometer. MS were recorded by effecting electron impact ionization at 70ev on a ESIMS analyst QS TOF (Canada) mass spectrometer. ^{1}H-NMR spectra was scanned on Bruker DRX-300 NMR (300 M Hz) instrument in $CDCl_3$ using Tetramethylsilane (TMS) as the internal standard and coupling constants (*J* values) are expressed in Hertz (Hz). Silica gel G (Qualigen, 60-120 mesh) was used for column chromatography. TLC was performed on plates coated with silica gel G (Qualigen).

Plant material

The celery seeds were collected from Punjab and were authenticated by taxonomist Dr. G K Bhat, Udupi, India. A voucher specimen is preserved in herbarium section of Sami labs as well as in the department of Pharmacognosy, school of pharmaceutical sciences, Jaipur National University.

Extraction and isolation

In the present study, the celery seeds were carefully collected and air dried. The air dried material was reduced to coarse powder. The coarse powdered material (4 Kg) was subjected to exhaustive extraction with 20% aqueous methanol thrice filtered through PPcloth.The filtrate is then concentrated completely under reduced pressure. The yield of extract obtained is 640 gms (16%). The extract had been subject to the partitioning using water: hexane system. Aqueous layer after evaporation yields 570 gms of dark green thick paste whereas the hexane layer after evaporation yields 80.5 gms of dark green thick paste.

Column chromatography

The column of 75cm length and 7cm diameter was used for separation. Silica gel of 300- 400 mesh size and 80.5 gms loading material were used in column. Column solvent is hexane:ethyl acetate(gradient type). Column is initially eluted with hexane followed by elution with 1% ethyl acetate in hexane. All the fractions were collected and concentrated according to TLC. In the 11-20th fractions, 5.88 gm mixture of 3-n-butylphthalide and thymol, in 21-30th fractions, 14 gms mixture of 3-n-butylphthalide & sedanenolide and in 31-38th fractions, 7 gms of pure sedanenolide were obtained. 11-20th fractions and 21-30th fractions were again subjected to column chromatography for isolation of pure 3-n-butylphthalide which was done successfully using isocratic system. 6-19th fractions of previous 11-20th fractions & 2-8th fractions of 21-30th fractions having pure 3-n-butylphthalide.

Gas Chromatography Analysis of Phytoconstituents

GC–MS analysis was performed on a HP Model 6890A gas chromatograph equipped with a Model 5973 mass selective detector, a split capillary inlet system (split ratio = 1/30), a Model 6890 autosampler and Enhanced Chemstation version B.01.00 software (Hewlett-Packard, Palo Alto, CA). The injection (2 µL) was made at a temperature of 250 ◦C. In 3-n-butylphthalide GC-MS Analysis, one major peak was found to be at retention time 10.454 and the purity calculated out to be 89.65%. In sedanenolide GC-MS Analysis, one major peak was found to be at R.T (retention time) 11.360, giving the mass 192 and the purity calculated is 80.5 %.

RESULTS & DISCUSSION

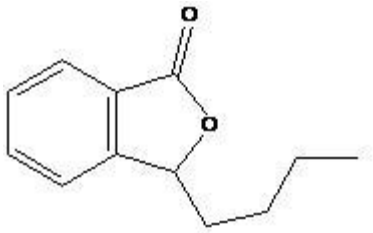

Figure 1 1, 3--n-butylphthalide

Compound **1 [**n-Butylphthalide (**Figure 1**)]: Mol. Formula $C_{12}H_{14}O_2$; Mol. Wt: 190.24. Positive reactions with iodine and anisaldehyde reagents. UV: 232 nm; MS: $[M^+]$ - 190, $[Phthalide]^+$ - 133, $[C_6H_5CO]^+$ - 105, $[C_6H_5]^+$ - 77, $[C_4H_4]^+$ - 51; ^{1}H-NMR(ppm): 0.779-0.824(t, 3H, -C<u>H</u>$_3$), 1.124-1.363(m, 4H, -C<u>H</u>$_2$), 1.944- 1.991(m, 2H, - C<u>H</u>$_2$), 5.367 - 5.406(q, 1H, phthalyl C<u>H</u>), 7.258 – 7.786(m, 5H, Ar-<u>H</u>); FTIR(cm^{-1}): 1650(C=O), 1245(C-O-C), 2926(C-H), 754(Ar- C-H) out of plane stretching, 1400(Ar C=C); ^{13}C-NMR(ppm): 170.871(O-<u>C</u>=O), 122.033, 125.671, 126.176, 129.185, 134.206,150.295(C in aromatic), 14.052, 14.323(1^0- CH_3), 16.467, 22.601, 22.872, 23.655, 25.171(2^o- CH_2), 27.059, 27.264, 27.352(3^o- CH).

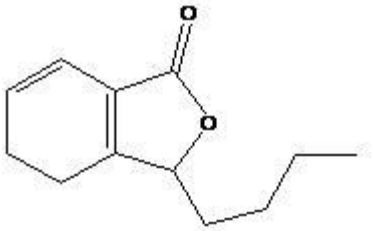

Figure 2 Sedanenolide

Compound **2[**Sedanenolide(**Figure 2)]**: Mol. Formula $C_{12}H_{16}O_2$; Mol wt.: 192.25; Active with iodine & blue color spot with anisaldehyde reagent; UV(nm): 232; MS: [M+]- 192, [Phthalyl]+ - 135, [C_6<u>H</u>$_7$CO]+ - 107, [C_6<u>H</u>$_6$]+ - 77, [C_4<u>H</u>$_4$]+ - 51; ^{1}H-NMR(ppm): 0.775-0.900(m, 3H, -C<u>H</u>3), 1.184-1.477(m, 4H, -C<u>H</u>2), 2.406-2.460 (m, 2H, -C<u>H</u>$_3$), 4.80-4.885(m,1H, - phthalyl – C<u>H</u>), 5.822 – 5.866 & 6.096-6.128(d, 4H, ring-C<u>H</u>$_2$); FTIR(cm^{-1}): 1626(C=O), 1264(C-O-C), 2900(C-H), 3050(C=C-H), 1350(Ar C=C); ^{13}C-NMR(ppm): 171.471(O-C=O), 116.902, 124.536, 128.621(C=C alkene), 11.614, 14.059, 14.323(1^0- CH_3), 20.932, 22.455, 22.623, 22.843, 22.879, 25.200(2^o- CH_2), 26.920, 27.264(3^o- CH).

Compound **1** with mass of 190.24 have been successfully isolated from *apium graveolens*. Isolated compound found to be 89.65 % pure after GC analysis with retention time 10.454. In Mass analysis, compound gives a molecular ion peak at 190 while the base peak comes out to be of phthalide ion with mass 133. Butylphthalide showed qualitative peaks for the functional groups like carbonyl at 1650, ethereal at 1245, C-H at 2926, aromatic hydrgens at 754 out of plane stretching, aromatic carbons at 1400 while the proton NMR have shown a triplet at 0.779- 0.824 for 1^o- CH_3 Group, multiplet at 1.124- 1.363 for 4 hydrogens of 2^o- CH_2 type, multiplet for two hydrogens of terminal methylene group at 1.944-1.991, quartet at 5.367 – 5.406 for one hydrogen of phthalyl ring(five membered), multiplet at 7.258 – 7.786 for 4 hydrogens of aromatic ring. While recording the carbon NMR spectral values, we found peak at 170.871 for the esterial carbon, 122.033, 125.671, 126.176, 129.185, 134.206 and 150.29 for carbons in aromatic ring, 14.052 and 14.323 for terminal primary carbon, 16.467, 22.601, 22.872, 23.655 and 25.171 for secondary carbons of butyl substituent, 27.059, 27.352 for the tertiary carbon of the five membered ring.

Compound **2** with mass of 192.25 have been successfully isolated from *apium graveolens*. Isolated compound found to be 80.5 % pure after GC analysis with retention time 11.360. In Mass analysis, compound gives a molecular ion peak at 192 while the base peak comes out to be of dihydrophthalide ion with mass 135. In case of proton NMR, peak for aromatic hydrogens were diminished and appears to be negligible while we had seen additional doublet peaks at 5.822-5.866 & 6.096-6.128 for 4 hydrogens of ring carbons. Similarly in case of FTIR spectral values, we have not observed the stretching or bending of the aromatic hydrogens. In case of carbon NMR spectrum, we have observed peaks for C=C rather than aromatic carbons. All this led us to conclude the isolation of two phytoconstituents as n-butylphthalide and sedanenolide form apium graveolens.

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