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ORIGINAL ARTICLE

Isolation of New Diterpene from Methanolic Extract of *Capsicum annuum* Linn. Fruits

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ABSTRACT

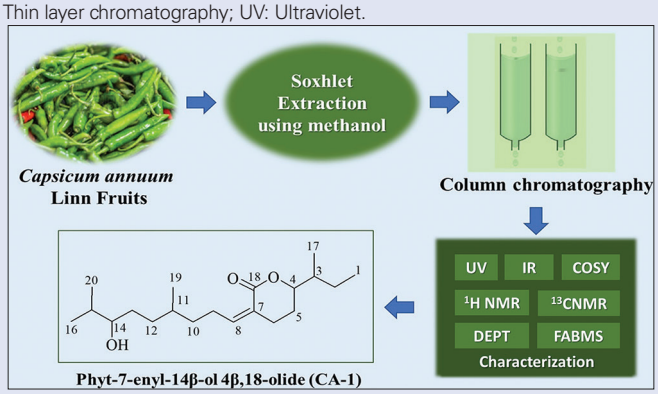
Background: *Capsicum annuum* (CA) fruits are consumed in the human diet for more than six centuries. Less research evidence for critical isolation experiment over CA fruits methanolic extract (CAFME) is an important concern for the investigators. **Objectives:** The present investigation was intended to explore the CAFME for the presence of any new phytoisolate. **Materials and Methods:** The study involved the preparation of CAFME, followed by critical isolation through normal phase silica-gel based column chromatography using various combinations of petroleum ether and chloroform. The phytoisolate was characterized using ultraviolet, Fourier transformed infrared, Nuclear magnetic resonance (^{1}H, ^{13}C, Distortion less enhancement by polarization transfer and correlation spectroscopy), and mass spectrometry. **Results:** The chromatographic critical isolation and spectrometric experiment over CAFME offered a new phytoisolate characterized as 3,11,15-trimethyl-14 β-hydroxy-n-hexadeca-7-en-4,18-olide (CA-1). **Conclusion:** The present study concludes the isolation of a new diterpene 3,11,15-trimethyl-14 β-hydroxy-n-hexadeca-7-en-4,18-olide (CA-1) for the first time in CAFME. The present study also recommends that in future this new phytoisolate should be further standardized and explored for its therapeutic properties.

Key words: Capsicum, characterization, chromatography, diterpene, phytoisolate

SUMMARY

- In the present study, the critical column chromatography of methanolic extract of *Capsicum annuum* offered a new diterpene (CA-1), that was spectrometrically characterized as 3,11,15-trimethyl-14 β-hydroxy-n-hexadeca-7-en-4,18-olide (CA-1). This phyotoisolate is reported for the first time in *Capsicum annum* fruits methanolic extract.

Abbreviations used: CA: *Capsicum annuum;* CAFs: *Capsicum annuum* fruits; CAFME: *Capsicum annuum* fruits, extract; COSY: Correlation spectroscopy; d: Doublet; DEPT: Distortion less enhancement by polarization transfer; 1D: One dimension; 2D: Two dimension; FABMS: Fast atomic bombardment mass spectrometer; FTIR: Fourier transformed infrared; g: Gram; Kg: Kilogram; m: Multiplet; MHz: Mega Hertz; *m/z*: Mass to charge ratio; λ_{max}: Maximum wavelength; NMR: Nuclear magnetic resonance; No.: Number; R_f: Retention factor; S: Singlet; Silica gel G: Silica gel gypsum; TLC: Thin layer chromatography; UV: Ultraviolet.



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INTRODUCTION

Capsicum annuum (CA) Linn. belonging to the genus capsicum with *Solanaceae* family has been consumed in the human diet for more than six centuries.[1] It is a perennial herb that is commonly found in central and south America. Based on flavor and size, the CA is called with several names. The larger and sweeter varieties of CA are called sweet pepper in the UK and red or green or bell pepper in the USA; whereas the smaller and hotter varieties are known as chile, chillis, or chili peppers.[2] The CA Linn. is an annual herbaceous plant that possesses glabrous, lanceolate leaves, white color fruits, and flowers.[3] The CA fruits (CAFs) are commonly used in food preparations as spices attributed to their pungent taste (due to capsaicin presence in fruits, seeds, and placental tissue). The CAFs are indicated for various medicinal purposes, such as stomach pain, asthma, gout, arthritis, anorexia, seiatica, dyspepsia, flatulence, cardiac debility, cough, malaria, cholera, muscle spasm, neuralgia, lumbago, and chilblains.[4,5] The CAFs are reported to contain capsaicinoids (0.1%–2%), that are responsible for characteristic pungent taste and possess high therapeutic value in gastric ulcer and rheumatoid arthritis.[6,7] Study suggests most varieties of CAFs to comprise key capsaicinoids like capsaicin and dihydrocapsaicin (1:1); and few capsaicinoids like homodihydrocapsaicin, homocapsaicin, and nordihydrocapsaicin (ranging from 1% to 38%).[8] The crude extract of CAFs known as capsicum oleoresin contains at least 100 different volatile chemical constituents.[9] Other chemical constituents found in

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CAFs include carotenoids (like capsanthin, capsorubin, carotene, lutein, etc.), saponin alkaloid (capsicidin), fats (9%–17%), protein (12%–15%), vitamins (A, B and C), xanthins (Cycloviolaxanthin, (8S)-capsochrome, 5,6-epoxycapsanthin, karpoxanthin, cucurbitaxanthin A and B, violaxathin, 3,6-epoxycapsanthin), steroidal alkaloidal glycosides (solanine, solanidine, solasodine), steroidal glycosides (capsicoside A-D), coumarins (Scopoletin, a coumarin) and volatile oils.[10,11] Although several studies reported the presence of a wide range of phytochemicals in CAFs using various solvents system, still very less research data is available over the critical isolation of CAFs methanolic extract (CAFME) using narrow range of eluting solvents system, especially over petroleum ether-chloroform. Based on these facts, it was hypothesized that by performing critical isolation over CAFs, the present study would explore some newer phytoisolate. Hence based on these findings, the present study was designed to prepare methanolic extract of CAFs, followed by its critical isolation (by normal phase column chromatography using various combinations of petroleum ether and chloroform) and spectrometric characterization (using ultraviolet (UV), Fourier transformed infrared (FTIR), Mass and 1D and 2D nuclear magnetic resonance [NMR] spectroscopy).

Experimental

General

The reagents, solvents, and chemicals used in the present study were of analytical standard, obtained from Merck and Sigma Aldrich and were used without purification. The normal phase column chromatography of phytosiolate involved the use of Silica gel G (60–120 mesh). The thin-layer chromatography (TLC) of phytoisolate was performed over silica gel G plates. The UV spectrum of phytoisolate was recorded at 200–400 nm using Shimadzu UV-160A UV visible spectrophotometer. Fourier transformed infra-red spectrum was recorded at 500–4000 cm^{-1}, using Bruker FTIR spectrometer The 1D and 2D NMR spectra (^{1}H,^{13}C, Distortion less enhancement by polarization transfer [DEPT] and correlation spectroscopy [COSY]) were recorded on Avance 300 MHz spectrometer using $CDCl_3$ (solvent) and TMS (internal standard) expressing coupling constants in Hertz. The mass spectrum was recorded using fast atomic bombardment mass spectrometer (FABMS) spectrometer.

Plant material and extract preparation

The CAFs were procured from the province of Ghaziabad, Uttar Pradesh, India, and were authenticated by Dr. K. C. Bhatt, National Bureau of Plant Genetic Resources, Pusa Campus, Delhi, India. A voucher specimen (NHCP/NBPGR/2009/2/551) of CAFs was deposited in the herbarium of the Department of Pharmacognosy, R. V. Northland Institute, Dadri, Greater Noida, Uttar Pradesh, India, for future reference. The CAFs were collected, air-dried under the shade, and powdered. The dried powder of CAFs (1 kg) was subjected to exhaustive extraction with 95% methanol in a Soxhlet apparatus for 50 h. The obtained CAFs crude was concentrated with rotary evaporator to offer 96 g of dark brown CAFME.

Isolation and purification

The CAFME (90 g) was subjected to critical isolation by dissolving in a minimum amount of methanol in a China dish and then adsorbed on silica gel (60–120 mesh) slowly for preparation of a slurry. The CAFME was air-dried, powdered and passed through sieve (No. 8) to get uniform particle size. A clean dried column plugged on the lower side with a piece of nonabsorbent cotton was fitted in vertical position on a stand. Column was then half filled with petroleum ether. Silica gel for column chromatography (60–120 mesh) was then poured in small portions and allowed to settle down to form the stationary phase. The dried CAFME slurry was loaded over the column and elution of the column was carried out successively with various combinations of petroleum ether:chloroform (10:90, 9:91, 8:92, 7:93, 6:94, 5:95, 4:96, 3:97, 2:98, 1:99, 0:100). The homogeneity of collected fractions was checked using TLC. The fractions having the same retention factor (R_f) values were combined and concentrated. The concentrate was purified using a suitable solvent system to offer CA-1. The purified phytoisolate was subjected to UV, FT-IR, NMR (^{1}H, ^{13}C, DEPT, and COSY) and FAB-Mass spectrometric characterization studies. The structure of phytoisolate CA-1 was established based on the physical and characterization data.

RESULTS

The isolation experiment offered a new isolate CA-1 using petroleum ether and chloroform (2:98) solvent system. Results of physical and characterization studies over CA-1 are presented as follows:

Physical data

Quantity: 0.0018% (167 mg from 90 g CAFME); colour: pale yellow; state: semisolid; eluent system: 9th fraction of petroleum ether and with Chloroform (2:98); R_f value: 0.46 (chloroform:methanol, 3:1).

Characterization data

UV maximum wavelength (MeOH): 229 nm; IR ν_{max} (KBr) in cm^{-1}: 3409, 2926, 2846, 1733, 1645, 1442, 1383, 1256, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ value in Hz: 0.85 (3H, d, *J* = 6.4, CH_3-1), 0.99 (3H, d, *J* = 6.8, CH_3-19), 1.03 (3H, d, *J* = 6.6, CH_3-17), 1.10 (3H, *J* = 6.7, CH_3-20), 1.15 (3H, d, *J* = 6.5, CH_3-16), 1.19–1.80 (9H, m, CH-5 and 4 x CH_2), 2.01 (2H, m, CH_2-5), 2.05 (1H, m, CH-3α), 2.23 (1H, m, CH-11), 2.36 (2H, m, CH_2-9), 2.72 (2H, m, CH_2-6), 3.60 (1H, m, CH-14α), 4.06 (1H, m, CH-4α) and 5.3 (1H, m, CH-8); ^{13}C NMR ($CDCl_3$) δ value in Hz: 14.5 (C1), 22.2 (C2), 39.6 (C3), 78.9 (C4), 58.3 (C5), 50.0 (C6), 140.2 (C7), 120.2 (C8), 30.5 (C9), 30.6 (C10), 42.7 (C11), 21.2 (C12), 21.4 (C13), 68.2 (C14), 31.5 (C15), 18.3 (C16), 16.5 (C17), 166.4 (C18), 20.2 (C19), 18.3 (C20); FABMS *m/z*: 324 [M] + ($C_{20}H_{36}O_3$), 86 (base peak), 281, 267, 251, 239, 157, 129, 57.

DISCUSSION

The present study was intended to explore any new phytoisolate present in the CAFs. For this 90 g of prepared CAFME was successively eluted with petroleum ether and chloroform using normal phase column chromatography. This yielded 10 fractions (each fraction of 500 ml) each with the following eluent system (chloroform:methanol ratio): fraction 1–5 (10:90), fraction 6–10 (9:91), fraction 11–15 (8:92), fraction 16–20 (7:93), fraction 21–25 (6:94), fraction 26–30 (5:95), fraction 31–35 (4:96), fraction 36–40 (3:97), fraction 41–45 (2:98), fraction 46–50 (1:99), fraction 51–55 (0:100). The fractions 46–50 were combined and exposed to TLC, which offered a new spot with R_f value of 0.46 (chloroform:methanol, 3:1). The fraction 46–50, when dried and subjected to preparative TLC, offered semisolid pale yellow mass CA-1. Based on the characterization techniques used in other standard investigations present study involved characterization of phytoisolate (CA-1) using UV, FT-IR, NMR (^{1}H, ^{13}C, DEPT, and COSY) and FAB-Mass spectrometric characterization studies.[12-14] Characteristic IR bands at 3411, 1737, and 1646 cm^{-1} in CA-1 FTIR spectrum revealed the presence of OH, δ-lactone, and unsaturation, respectively in CA-1. The FAB mass spectrum of CA-1 exhibiting M+ ion signal at *m/z* 324 established its molecular formula as: $C_{20}H_{36}O_3$. The presence of fragmented ions signals at *m/z* 281, 251, 239, and 129; corresponding to C_{14}-$C_{15}]+$, C_{13}-$C_{14}]+$, C_{11}-$C_{12}]^{+\cdot}$ and C_{10}-$C_{11}]^{+}$ fission fragment ions revealed the presence of OH group at C_{14} position. The

fragment ion signals at *m/z* 57, 267, 157 attributed to C_3-C_4]$^+$ fission and C_8-C_9]$^+$ fission fragment ion revealed the presence of δ-lactone nucleus at C4 (18) and vinyl linkage at C_7. The ^{1}H NMR spectrum of CA-1 exhibited one three-proton triplet at δ 0.85 and showed the presence of primary C1 methyl protons. Four three proton doublet signals at δ 0.99, 1.03, 1.10, and 1.15 revealed the presence of C19, C17, C29, and C16 secondary methyl protons, respectively. The two one proton multiplets at δ 3.67 and 4.06 revealed the presence of H-14α carbinol and H-4α-oxymethine protons. One proton multiplet signal at δ 5.35 showed the presence vinylic H-8 proton. Remained methylene and methine protons exhibited signals from δ 1.19 to 2.72. The ^{13}C NMR spectrum of CA-1 exhibited characteristic signals at δ 68.19, 78.85, and 166.42 attributed to the presence of C14 carbinol, C4 oxymethine and C18 lactone carbons. The signals at δ 120.15 and 140.22 were attributed to C8 and C7 vinylic carbons. The spectrum signaled for methyl carbons at δ 14.48, 16.51, 18.27, 18.33, 20.17, corresponding to C1, C17, C20, C16, and C19, respectively. The DEPT spectrum of CA-1 also supported the presence of 05 methyl, 07 methylene, 06 methine, and 02 quaternary carbons. The ^{1}H-^{1}H COSY spectrum of CA-1 showed the characteristic correlation of H_2-5 with H_2-6; H4 and H3; H8 with H_2-6 and H_2-9; and correlation of H14 with H_2-13, H15, H_3-16, and H_3-20. The confirmation of spectrometric characterization data results of the present study was based on the agreement with the results of other standard findings.[15,16] Based on spectrometric characterization data, the structure of phytoisolate CA-1 is elucidated as 3,11,15-trimethyl-14 β-hydroxy-n-hexadeca-7-en-4,18-olide [Figure 1]. This diterpene lactone is reported for the first time in CAFME.

CONCLUSION

The findings of the present research conclude and reports the isolation of a new diterpenic lactone 3,11,15-trimethyl-14 β-hydroxy-*n*-hexad eca-7-en-4,18-olide for the first time in the methanolic extract of CA Linn. fruits. The present study also recommends that in future this new phytoisolate should be further standardized and explored for its therapeutic properties.

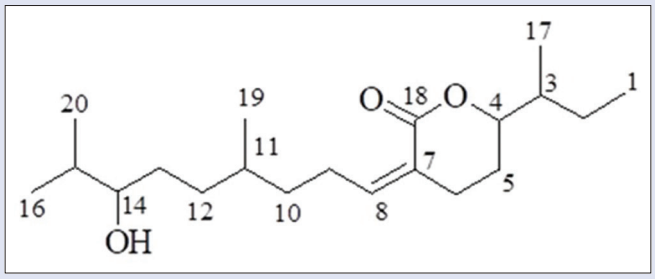

Figure 1: Chemical structure of *Capsicum annuum*-1

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Conflicts of interest

There are no conflicts of interest.

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