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Isolation of new phytometabolites from *Alpinia galanga* willd rhizomes

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Abstract: The current research was aimed to isolate newer phyto-metabolites from rhizomes of *Alpinia galanga* plant. Study involved preparation of *Alpinia galanga* rhizome methanolic extract, followed by normal phase column chromatography assisted isolation of new phytometabolites (using different combinations of chloroform and methanol), and characterization (by UV, FTIR, ^{13}C-NMR, ^{1}H-NMR, COSY, DEPT and Mass spectrometry). The isolation and characterization experiment offered two phytometabolites: An ester (Ag-1) and tetrahydronapthalene type lactone (Ag-2). Present study concludes and reports the two phytometabolites, benzyl myristate (Ag-1) and 3-Methyl-6α, 8β-diol-7-carboxylic acid tetralin-11, 9β-olide (Ag-2) for the first time in *Alpinia galanga* rhizome. The study recommends that these phytometabolites Ag-1 and Ag-2 can be utilized as effective analytical biomarkers for identification, purity and quality control of this plant in future.

Keywords: Phytometabolites, methanol, extraction, chromatography, galangal, isolation.

INTRODUCTION

Plants represent huge source for bioactive compounds that serve in many folk remedies and are of high medicinal value from prehistoric era (Yuan *et al.*, 2016; Vakiloddin *et al.*, 2015). The scientific evidence suggests *Alpinia galanga* (AG) rhizome as one of the therapeutically important plant (Mundugaru *et al.*, 2018). The AG Willd belonging to Zingiberaceae family is the common dietary component that is utilized in medicines, culinary and cosmetics for centuries in Chinese, Ayurveda, Thai and Unani system of medicine (Chudiwal *et al.*, 2010; Jantan *et al.*, 2003; Yang and Eilerman, 1999). It is commonly found in Indonesia, India, China, Arabic gulf areas, Malaysia, Egypt and Sri Lanka (Karunarathne *et al.* 2018; Chouni and Paul, 2018). The AG rhizome has hot/spicy taste, pungent/aromatic odour, that creates its importance in food and local medicine. Various pharmacopoeias report AG to possess high therapeutic efficacy (Jain *et al.*, 2007). AG is therpeutically used as anti-eczema, anti-ulcer, anti-fungal, anti-tumor, antidiuretic, anti-rheumatic, anti-inflammatory, diabetes and heart diseases. The AG rhizome is commonly used in spices or as source for essential oil throughout its distribution area (Hamad *et al.*, 2016; Shetty and Monisha, 2015). The AG rhizome is claimed to contain a big pool of flavonoids. The AG rhizome contains essential oil, namely: galangin (3,5,7-trihydroxy flavone); pinene; bornyl acetate; alpinin; kaempferide; kaempferol; 8-cineole-D-glucopyranoside; eugenol acetate; methyl cinnamate; *α*-terpineol; chavicol acetate; 1,8-cineole; (1R,3S,4S)-trans-3-hydroxy-l; cineole; *p*-methane-1,8-epoxy-acetoxychavicol acetate; camphene; 3-dioxy 4-methoxy flavone; 3-hydroxy-l,8-cineole glucopyranosides; 4-terpineol; camphor; 7-hydroxy-3,5-dimethoxy flavone; galangin; l'S-l'-acetoxy chavicol acetate; pineol; l'S-l'-acetoxy eugenol acetate; 1'-acetoxy chavicol acetate; diterpenes (galanga A and B); 1'-acetoxy eugenol acetate; borneol; D-camphor; chavicol; trans coniferyl diacetate; trans-*p*-coumaryl diacetate; di-(*p*-hydroxy-cis-styryl) methane; trans (3-faranesene, 4-hydoxy benzyldehyde; 1'-hydroxy chavicol acetate; *p*-hydroxy cinammaldehyde; isorhamnetin, kaempferol-4'-methyl ether, kaempferol-7'-methylether, *p*-cymene; methyl eugenol, 3-carene; *α*-thujene; *α*-pinene; myrcene; *β*-pinene; fenchyl acetate; *α*-humulene; zerumbone; galanolactone; trans-cinnamic acid; galangogalloside; and (E)-*β*-17,12-labdiene-15,16-dial) (Chouni and Paul, 2018; Shukla *et al.*, 2017; Zhang *et al.*, 2016; Shetty and Monisha, 2015; Kaushik *et al.*, 2011; Mallavarapu *et al.*, 2002; Poonsri *et al*., 2019; Jaju *et al.*, 2009; Kubota *et al.*, 1999). Many chemical investigations of AG rhizomes reported isolation of phyotometabolites from AG by column chromatography (using various combinations of different solvent system based on polarity) and structural elucidation of phytometabolites using various spectral techniques. For example, critical isolation of *β*-sitosterol diglucoside and *β*-sitosteryl arabinoside from AG rhizome using ethyl acetate and methanol (49:1) as eluent and characterization using different spectrometric techniques (Jaju *et al.*, 2009a);

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isolation of new galangoflavonoside from AG rhizomes via column chromatography utilizing ethylacetate-methanol (9:1) eluent and structural elucidation using various spectrometric methods like: IR, UV, Mass, ^{13}C-NMR and ^{1}H-NMR (Jaju *et al.,* 2009b); and isolation of two new phenyl propanoids namely (E)-4-acetoxy cinnamyl ethyl ether and (E)-4-hydroxycinnamaldehyde using Silica gel based column chromatography and spectroscopic characterization (Ling *et al.,* 2012). Although several studies reported a large number of phytometabolites in greater galangal rhizomes using various solvents system, but still very less data is available over critical isolation of AG rhizomes methanolic extract using narrow range of eluting solvents system, especially chloroform-methanol. Based on these facts it was hypothesized that by performing critical isolation over AG rhizome, current study would explore some newer phytoisolates. Based on these findings, present study was aimed to isolate newer phyto-metabolites from rhizomes of *Alpinia galanga* plant.

MATERIALS AND METHODS

General

The melting point of compounds was recorded using 1013 melting point apparatus with temperature control (PERFIT, Ambala, India) and was uncorrected. Infrared (IR) spectral data were acquired using Bio-Rad FTS 3000, Nippon Bio-Rad Laboratories, Inc., Japan. The procurement of UV-Vis spectral data was procured using Perkin Elmer UV/VIS Spectrometer (Lambda Bio 20, United States) using methanol as solvent. 1D & 2D nuclear magnetic resonance spectra of ^{1}H,^{13}C, DEPT (Distortion less enhancement by polarization transfer) and ^{1}H-^{1}H COSY (correlation spectroscopy) were recorded on Bruker Avance 300 MHz NMR spectrometer (Switzerland) applying tetramethyl silane (internal standard) and $CDCl_3$ solvent. Mass spectra were acquired using Jeol D-300 system (EI/CI, Tokyo, Japan). The column chromatography was commenced using silica gel (60-120 mesh, Merck, India Ltd.). To perform thin layer chromatography, the precoated plates (Silica gel 60 F_{254}, Merck, India Ltd.) were utilized and spots measurement was done under UV cabinet.

Plant Material

The AG rhizomes were collected from the Ghaziabad province, Uttar Pradesh, India, during winter season, on 20 February 2009 and authenticated by Dr. K.C. Bhatt, National Bureau of Plant Genetic Resources, Pusa, Delhi. A voucher specimen (NHCP/NBPGR/2009/2/549) of the crude drug rhizomes was deposited in the herbarium of the Department of Pharmacognosy, R.V. Northland Institute, Dadri, Greater Noida, UP, India for future reference.

Preparation of Extract

The 1 Kg coarse powder of air-dried AG rhizomes was defatted (using petroleum ether) followed by extraction with methanol solvent using Soxhlet's apparatus for 24 hours. The obtained AG rhizome crude was concentrated to offer 109 grams of brown semisolid AG rhizomes methanolic extract (AGRME).

Isolation and Purification

The procedure for isolation and purification was done as per the established procedure with slight modification (Jaju *et al.,* 2009a). Briefly, the AGRME (100 g) was solubilized in minimum methanol in a China dish, followed by adsorption over silica gel (60-120 mesh) for preparation of a slurry. Prepared AGRME slurry was air dried and big lumps were broken by rubbing and finally passed through sieve (No. 8) for uniform particle size. Next, the dried slurry was subjected to normal phase chromatography using petroleum ether packed silica gel column. Column was critically eluted using various combinations of chloroform and methanol (99.5:0.5, 99:1, 98.5:1.5, 98:2, 97.5:2.5, 97:3, 96.5:3.5, 96:3, 95.5:4.5, 95:5). Fractions were collected separately and checked for homogeneity using TLC. The fractions with same Rf value were mixed and concentrated. Finally the concentrates were purified with suitable solvent systems to yield two phytoisolates Ag-1 and Ag-2. The purified phytoisolates were subjected to UV, FT-IR, NMR (^{1}H, ^{13}C, DEPT and COSY) and Mass spectrometric characterization studies. The structure of phytoisolates Ag-1 and Ag-2 were established based on their physical and characterization data.

RESULTS

Benzyl Myristate (Ag-1)

Elution of 100 grams of AGRME with chloroform and methanol (98:02) V/V offered yellow crystals of Ag-1, recrystallized using methanol, yield (0.00202 %, 202 mg); Rf value: 0.58 (dichloromethane and methanol, 4:1); m.p. 76-78 °C; Rf value 0.58; UV λmax (MeOH): 222, 230 nm; IR n_{max} (KBr): 2926, 2853, 1725, 1632, 1457, 1267, 1012 and 832 cm^{-1}; ^{1}H-NMR (DMSO d-$_6$): δ 0.91 (3H, t, CH_3-14'), 1.16 (20H, br 10 × CH_2), 1.99 (2H, m, H_2-3'), 2.24 (2H, t, H_2-2'), 3.84 (2H, br, H_2-7), 6.71 (1H, m, H-4), 6.89 (2H, m, H-3, H-5), 7.07 (1H, m, H-6) and 7.19 (1H, m, H-2); ^{13}C NMR (DMSO d-$_6$): d 15 (C-14'), 23 (C-13'), 25 (C-12'), 27 (C-11'), 28 (C-10'), 29 (C-9'), 30 (C-8'), 31 (C-7'), 32 (C-6'), 35 (C-5'), 38 (C-4'), 52 (C-3'), 57 (C-2'), 80 (C-7), 111 (C-3), 115 (C-4), 116 (C-5), 117 (C-6), 122 (C-2), 148 (C-1) and 172 (C-1'); EI/CI-MS m/z: 318 $[M]^+$ ($C_{21}H_{34}O_2$).

3-Methyl-6α,8β-dihydroxy-7-carboxylic acid tetralin-1,9β-olide (Ag-2)

Elution of 100 grams of AGRME with chloroform and methanol (96:04) offered pale yellow crystals of Ag-2,

recrystallized using methanol (yield 0.00131%, 131 mg); Rf value: 0.46 (dichloromethane and methanol, 4:1); m.p. 118-119 °C; UV λ_{max} (MeOH): 216, 243 nm; IR n_{max} (KBr): 3439, 3278, 3241, 2948, 2833, 1762, 1706, 1632, 1542, 1439, 1362, 1251, 1036 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.33 (3H, s, CH_3-12), 2.82 (1H, m, H-7β), 3.29 (1H, m, H-8α), 3.41 (1H, d, H-6β), 4.25 (1H, d, H-9α), 7.01 (1H, d, H-4), 7.08 (1H, d, H-2); ^{13}C-NMR ($CDCl_3$): δ 29 (C-12), 53 (C- 7), 67 (C-8), 69 (C-6), 78 (C-9), 110 (C-2), 111 (C-3), 122 (C-10), 122 (C-4), 140 (C-5), 148 (C-1), 170 (C-11), 180 (C-13); EI/CI-MS m/z: 264 $[M]^+$ ($C_{13}H_{12}O_6$).

DISCUSSION

Current investigation was intended to explore any new phytometabolite cotained in AGRME. Accurately, 100 grams of prepared AGRME was successively eluted by different combinations of chloroform and methanol critically in increasing order of polarity using column chromatography. This yielded 10 fractions (each fraction of 500 mL): fraction 1-5 (99.5:0.5), fraction 6-10 (99:1), fraction 11-15 (98.5:1.5), fraction 16-20 (98:2), fraction 21-25 (97.5:2.5), fraction 26-30 (97:3), fraction 31-35 (96.5:3.5), fraction 36-40 (96:4), fraction 41-45 (95.5:4.5), fraction 46-50 (95:5). The critical isolation was based on the earlier established protocols with minor modifications (Kurkin and Kharisova, 2014; Serrano *et. al.,* 2011, Jaju *et al.* 2009a). The fractions 16-20 when combined and subjected to preparative TLC, offered a new spot for a new phytometabolite Ag-1 with Rf value of 0.58 (dichloromethane-methanol, 4:1). The phytometabolite Ag-1 was obtained as yellow crystals using chloroform and methanol (98:2) as eluant. The IR spectrum of Ag-1 exhibited absorption band at 1722 cm^{-1} indicating presence of ester group. Based on ^{13}C-NMR and mass spectral *m/z* value, characteristic M^+ ion signal of Ag-1 at 318 was found in agreement with phenylic ester ($C_{21}H_{34}O_2$) structure. Fragment ion signal at 203 $[M-CO(CH_2)_{12}CH_3]^+$ indicated that myristic acid was esterified with aromatic alcohol. ^{1}H-NMR spectra exhibited a triplet signal at δ 0.91 accounted to 3 protons of terminal primary methyl protons (CH_3-14'). A two-protons based broad singlet signal at δ 1.16 was attributed to protons of 20H of methylene units (H_2-4' to H_2-13'). A multiplet at 1.99 was attributed to protons of methylene unit (H_2-3'). A triplet at δ 2.24 was ascribed to two protons of methylene unit attached to the ester group (H_2-2'). ^{1}H-NMR spectrum exhibited a broad signal at δ 3.84 attributed to two protons of benzylic H_2-7. The 3 multiplets at δ 6.71, 7.07 and 7.19, related to one aromatic proton of H-4, H-6 and H-2 respectively. A multiplet at δ 6.89 was related to two aryl protons of H-3 and H-5. ^{13}C-NMR spectral data of Ag-1 exhibited signals at δ 15 assigned to methyl carbon (C-14'), at δ 23 to 57 assigned to methylene carbons (C-2' to C-13'), at δ 80 attributed to oxygenated methylene carbon (C-7), at δ 111 to 148 related to aromatic carbons (C-1 to C-6) and at δ 172 attributed to ester carbon (C-1'). Based on spectral data analysis, the structure of compound Ag-1 was determined as benzyl myristate or benzyl *n*-tetradecanoate (Figure 1). This is new aromatic ester that is claimed first time in AG rhizome.

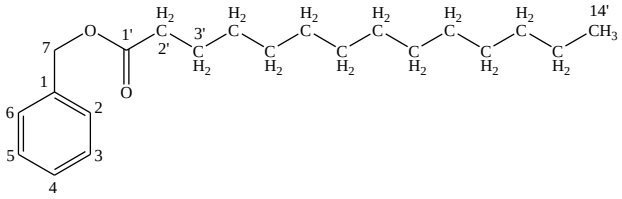

Fig. 1: Chemical structure of benzyl myristate (Ag-1)

The fractions 36-40 when combined and exposed to preparative TLC offered a new spot for a new phytometabolite Ag-2 with Rf value of 0.46 (dichloromethane-methanol, 4:1). The compound Ag-2 was obtained as pale yellow crystal using chloroform and methanol (96:4) solvent system and produced effervescence when treated with sodium bicarbonate indicating existence of carboxylic acid group. IR spectrum of Ag-2 displayed characteristic absorption bands for OH group (3439 cm^{-1}), COOH group (3241, 1706 cm^{-1}), five membered lactone ring (1762 cm^{-1}) and aromatic ring (1632, 1542, 1036 cm^{-1}). Based on mass and ^{13}C-NMR spectra characteristic M^+ ion signal of Ag-2 exhibited a signal at 264 that was found to be consistent with molecular formula of tetrahydronaphthalene-type lactone ($C_{13}H_{12}O_6$).

^{1}H-NMR spectrum of Ag-2 displayed a singlet signal at δ 2.33 related to three protons of CH_3; two doublets at δ 7.08 and 7.01 related to aromatic protons of H-2 & H-4; 02 doublets at δ 3.41 and 4.25 related to protons of H-6β and H-9α; two multiplets at δ 2.82 and 3.29 related to protons of H-7β and H-8α. ^{13}C-NMR spectral data of Ag-2 displayed signal at δ 29 ascribed to methyl carbon (C-12), at δ 67 and 69 assigned to carbinol carbons C-8 and C-6 respectively. Spectrum exhibited a signals at δ 78 attributed to oxymethine carbon (C-9), at δ 110-148 attributed to all aromatic carbons, at δ 170 ascribed to lactone carbon (C-11) and at δ 180 ascribed to carboxylic carbon (C-13). The carbon nature (CH_3, CH_2 and CH) was analysed by DEPT experiments (Ghavam-Haghi *et al.*, 2017; Mehta *et al.,* 2008). In DEPT analysis the supplementation of J-modulation with polarization transfer from protons to coupled carbons, enhances sensitivity. In DEPT by changing pulse width (θ) that is 45°, 90° and 135° during irradiation of 1H, different carbons exhibit different signs and strengths. When θ= 45°, the CH, CH_2 and CH_3 exhibits positive peaks; when θ=90° the CH exhibits positive peaks; whereas, when θ = 135° the CH and CH_3 exhibits positive peaks, CH_2 exhibits negative peaks and Quaternary carbons exhibits no peak in DEPT spectrum (Fuloria *et al.,* 2013). DEPT

spectrum of Ag-2 indicated presence of one methyl and six methine and six quaternary carbons each.

The ^{1}H-^{1}H COSY experiment involves use of conventional pulse sequence. COSY experiment generates chemical shift correlation spectrum between 1H and 1H protons in same coupling system (Fuloria *et al.*, 2020; Mehta *et al.*, 2008). The ^{1}H-^{1}H COSY spectral data of Ag-2 displayed correlation of H-4 with CH_3-12, H-2 and H-6; and H-8 with H-7 and H-9. Based on these evidences the structure of Ag-2 is interpreted as 3-methyl-6α,8β-dihydroxy-7-carboxylic acid tetralin-1,9β-olide (Figure 2), This is a new tetralin lactone that is reported for the first time in AGRME. The confirmation of structure of phytometabolites Ag-1 and Ag-2 critically isolated in present study was based on the agreement with other standard references (Sultana *et al.*, 2018; Fuloria and Fuloria, 2013).

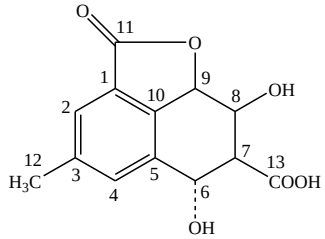

Fig. 2: Chemical structure of Ag-2

Study suggests major active constituents of AG to exhibit several biological activities, like: antifungal, antitumor, antibacterial, antiulcer, antioxidant, insecticidal, antiallergic activity and antimalarial activity (Jaju *et al.*, 2010; Poonsri *et al.*, 2019). Thereby newly isolated compounds AG-1 and AG-2 are expected to exhibit profound biological activities and medicinal value. Therefore, present study recommends that in future the isolated compounds AG-1 and AG-2 can be further investigated for *in vivo* studies for the mentioned biological activities.

CONCLUSION

Present study conclude and reports the isolation of newer phytoisolates benzyl myristate/benzyl *n*-tetradecanoate (a new aromatic ester) and 3-Methyl-6α, 8β-diol-7-carboxylic acid tetralin-11, 9β-olide (a new tetralin lactone) first time in methanolic extract of *Alpinia galanga* rhizomes. Present study recommends that these phytometabolites could be utilized as effective analytical biomarkers for identification, purity and quality assurance of this plant in future.

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