Fitoterapia 125 (2018) 78–88

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Thiotagetin B and tagetannins A and B, new acetylenic thiophene and digalloyl glucose derivatives from *Tagetes minuta* and evaluation of their in vitro antioxidative and anti-inflammatory activity

Sabrin R.M. Ibrahim[a,b,*], Hossam M. Abdallah[c,d], Ali M. El-Halawany[d], Ahmed Esmat[e], Gamal A. Mohamed[c,f]

[a] Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Al Madinah Al Munawarah 30078, Saudi Arabia
[b] Department of Pharmacognosy, Faculty of Pharmacy, Assuit University, Assuit 71526, Egypt
[c] Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[d] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
[e] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt
[f] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assuit Branch, Assuit 71524, Egypt

ARTICLE INFO

Keywords:
Thiotagetin B
Tagetannin A
Acetylenic thiophene
Digalloyl glucose
Tagetes minuta
Asteraceae
Antioxidant
Anti-inflammatory

ABSTRACT

The three new metabolites: thiotagetin B (**2**) [(*Z*)-1″-([2,2′-bithiophen]-5-yl)-8″-chloro-6″,11″-dimethylundeca-6″,10″-dien-2″-yn-9″-one], tagetannin A (**9**) [3,4-bis-(galloyl-3,5-dimethyl ether)-(*α/β*)-D-glucopyranose], and tagetannin B (**10**) [2,3-bis-(galloyl-3,5-dimethyl ether)-(*α/β*)-D-glucopyranose], along with ecliptal (5-formyl-*α*-terthiophene) (**1**), 5-(4-hydroxybut-1-ynyl)-2,2′-bithiophene (**3**), scopoletin (**4**), *p*-hydroxybenzoic acid (**5**), protocatechuic acid methyl ester (**6**), gallic acid (**7**), and patuletin 7-O-*β*-D-glucoside (**8**) were isolated from the aerial parts of *Tagetes minuta* L. (Asteraceae). Their structures were verified by extensive spectroscopic analyses as well as by comparison with literature data. The isolated compounds were evaluated for their antioxidant and anti-inflammatory activities using DPPH and enzyme-linked immunosorbent assays, respectively. Compounds **5–10** possessed the highest antioxidant potential with a scavenging activity (SCA) ≈ 74 to 93% of DPPH radicals. Moreover, **5–10** displayed significant anti-inflammatory potential, while **4** showed moderate activity. Compounds **5–10** exhibited significant decreases in NFκB p65, TNF-α, and IL-6 levels at all tested concentrations.

1. Introduction

The genus *Tagetes* belongs to the family Asteraceae and includes approximately 56 species [1,2]. Several species of this genus have been investigated as possible sources of different chemical and biochemical compounds of high pharmaceutical and nutritional value [3–5]. The extracts and powders of *Tagetes* contain large amounts of orange-yellow carotenoids, which are utilized as colouring agents for foods such as vegetable oil, pasta, mayonnaises, margarine, salad dressing, confectionery, baked goods, dairy products, yogurt, ice cream, mustard, citrus juice, and in poultry feed [6]. *Tagetes minuta* L., commonly known as marigold, is an annual perennial herb. It is widely grown from temperate to tropical regions of the world in a wide range of climatic conditions [1]. It has been used as a remedy for several medical ailments such as colds, respiratory inflammations, intestinal and stomach problems, skin infections, coughs, catarrh, wounds, cuts, calluses, and bunions [6,7]. It is used to dilate the bronchi, facilitate the flow of mucus, and dislodge the chest congestion [8]. Moreover, *Tagetes minuta* has been utilized as a mosquito repellent in Kenya and possesses a strong larvicidal effect. Its flowers are used as an aperient, stomachic,

Abbreviations: A, absorbance; ANOVA, analysis of variance; COX-2, cyclooxygenase-2; COSY, Correlation Spectroscopy; DMEM, Dulbecco's modified eagle's medium; DMSO, dimethyl sulphoxide; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetate; ELISA, enzyme-linked immunosorbent assay; FBS, foetal bovine serum; HMBC, heteronuclear multiple bond correlation; HRESIMS, high resolution electrospray ionization mass spectroscopy; HRMS, high resolution mass; HRP, horseradish peroxidase; HSQC, heteronuclear single quantum coherence; IL-6, interleukin-6; IR, Infrared; MDA, malonaldehyde; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMR, nuclear magnetic resonance; NO, nitric oxide; NSAIDs, non-steroidal anti-inflammatory drugs; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PDA, photodiode array; PHA, phytohemagglutinin; PMNs, polymorphonuclear leukocytes; PYE, trideca-3,5,7,9,11-pentayn-l-ene; RBCs, red blood cells; RP_{18} CC, reversed phase-18 column chromatography; SCA, scavenging activity; S.D., standard deviation; SiO_2 CC, silica gel column chromatography; SOD, superoxide dismutase; TMB, 3,3′,5,5′-tetramethylbenzidine; TLC, thin layer chromatography; TNF-α, tumour necrosis factor-α; UV, ultraviolet

* Corresponding author at: Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University, Al Madinah Al Munawarah 30078, Saudi Arabia.
E-mail address: sribrahim@taibahu.edu.sa (S.R.M. Ibrahim).

https://doi.org/10.1016/j.fitote.2017.12.024
Received 24 August 2017; Received in revised form 18 December 2017; Accepted 24 December 2017
Available online 27 December 2017
0367-326X/

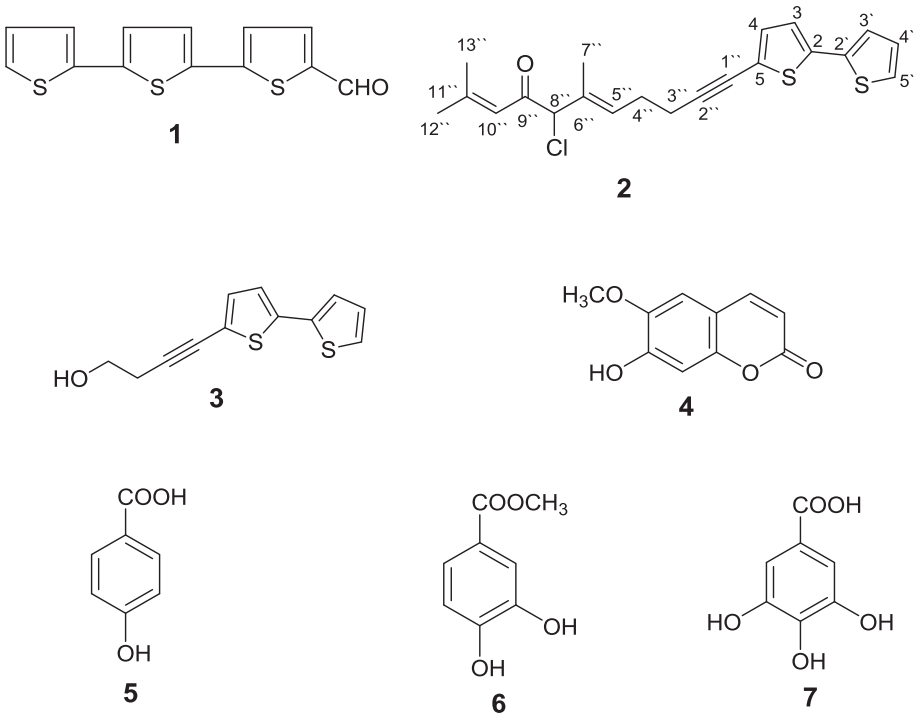

Fig. 1. Structures of isolated compounds **1–7**.

diaphoretic, and diuretic [9,10]. It has been shown to possess a variety of biological properties as *α*-amylase inhibitor, antimicrobial, antispasmodic, anti-parasitic, antiseptic, insecticide, sedative, anti-inflammatory, and an acaricide [2,8–10]. *Tagetes* oil is used as a flavour component in food products, including alcoholic and cola beverages, baked goods, frozen dairy desserts, gelatines, candy, puddings, relishes, and condiments [1,4]. Its essential oil possesses great aromatic potency in formulations, especially for modifying and embellishing the fruit-like initial notes in perfumes and colognes [10]. Previous chemical investigations of *Tagetes minuta* revealed the presence of thiophenes and flavonoids [1,2,4,5,11]. BBT (5-(3-buten-1-ynuyl)-2,2′-bithiophene), $BBT(OAc)_2$ [5-(3,4-diacetoxy-1-butynyl)-2,2′-bithienyl], BBTOAc (5-(4-acetoxy-1-butynyl)-2,2′-bithiophene), BBTOH (5-(4-hydroxy-1-butynyl)-2,2′-bithiophene), *α*-T (2,2′:5′,2″-terthienyl), 5-methyl-2,2′,5′,2″,5″,2‴,5‴,2‴-quinquethiophene, and thiotagetin A are the major thiophenes that have been isolated from *Tagetes minuta* [1,4,12]. In the present work three new compounds: thiotagetin B (**2**), tagetannin A (**9**), and tagetannin B (**10**), together with seven known metabolites (**1** and **3–8**) were identified from the aerial parts of *Tagetes minuta* (Figs. 1 & 2). The present study gives an account of the isolation and structural characterization of the new compounds using spectral tools, particularly 1D and 2D NMR and HRMS, as well as a comparison with evidences in the literature. Moreover, the antioxidant and anti-inflammatory potentials of the isolated metabolites were assessed in vitro using DPPH and enzyme-linked immunosorbent assays, respectively.

2. Experimental

2.1. General experimental procedures

UV spectra were obtained with a Hitachi-300 spectrophotometer (Kyoto, Japan). Optical rotation was measured on a PerkinElmer Model 341 LC polarimeter (PerkinElmer, Waltham, MA, USA). The IR spectrum was recorded on a Shimadzu Infrared-400 spectrophotometer (Shimadzu, Kyoto, Japan). ESIMS spectra were obtained with an LCQ DECA mass spectrometer coupled to an Agilent 1100HPLC system equipped with a photodiode array detector (ThermoFinnigan, Bremen, Germany). HRESIMS was obtained with an LTQ Orbitrap mass spectrometer (ThermoFinnigan, Bremen, Germany). NMR spectra were recorded on a Bruker Avance DRX 600 MHz spectrometer (Bruker BioSpin, Billerica, MA, USA). Semi-preparative HPLC was performed on an Agilent 1100 system, including a G1311A QuatPump, a G1315B UV–vis photodiode array detector, a G1332A degasser, and Agilent 1100 HPLC workstation (Agilent Technologies, Santa Clara, USA). The separation was carried out on an Agilent Zorbax SB-C18 column (9.4 × 250 mm) (Agilent Technologies, Santa Clara, USA). The effluents were monitored with a PAD detector at 320 nm. For column chromatography, silica gel (0.063–0.200 mm, Merck, Darmstadt, Germany), sephadex LH-20 (0.25–0.1 mm, Sigma-Aldrich, St. Louis, MO, USA), and RP_{18} (0.04–0.063 mm Merck, Darmstadt, Germany) were used. Pre-coated SiO_2 60 F_{254} plates (0.2 mm, Merck, Darmstadt, Germany) were used for thin-layer chromatography. Six millilitre LiChrolut EN/RP_{18} solid phase extraction tubes (RP_{18}, 40–63 μm, Merck, Darmstadt, Germany) were used for compound purification. The compounds were detected by UV absorption at λ_{max} 255 and 366 nm followed by spraying with a *p*-anisaldehyde:H_2SO_4 or isatin:H_2SO_4 spray reagent, then heating at 110 °C for 1–2 min. All authentic samples were obtained from the Natural Products and Alternative Medicine Department, Faculty of Pharmacy, King Abdulaziz University.

2.2. Plant material

Aerial parts of *Tagetes minuta* L. were collected in March 2015 from Al-Baha city, Saudi Arabia. The plant was identified on the basis of its morphological features and the library database [13] and confirmed by Dr. Nahed Morad (Faculty of Science, King Abdulaziz University, Saudi Arabia). A voucher specimen (TM-3-2015) was archived at the Department of Natural Products and Alternative Medicine herbarium, King Abdulaziz University, Saudi Arabia.

2.3. Extraction and isolation

The air-dried, powdered aerial parts (0.7 kg) were macerated with MeOH (2 × 3 L). The total MeOH extract was concentrated under a vacuum to give 16.5 g of a dark green residue, which was mixed with 150 mL of distilled water and fractionated successively between *n*-