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Original Article

Lipoxygenase inhibitors flavonoids from *Cyperus rotundus* aerial parts

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ABSTRACT

Cyperus rotundus L. (Suada, Sueda, family: Cyperaceae) is vastly spread in several world's subtropical and tropical regions. It had variable traditional uses and bioactivities. A new flavonol derivative: cyperaflavoside (myricetin 3,3′,5′-trimethyl ether 7-*O*-β-D-glucopyranoside) and five flavonoids: vitexin, orientin, cinaroside, quercetin 3-*O*-β-D-glucopyranoside, and myrcetin 3-*O*-β-D-glucopyranoside were separated from the methanolic extract of *C. rotundus* aerial parts. Their structures were verified based on UV, IR, NMR (1D and 2D), HRESIMS, and comparison with literature. All metabolites were assessed for their 5-lipoxygenase inhibitory potential. All compounds possessed 5-lipoxygenase inhibitory potentials with IC_{50}s 5.1, 4.5, 5.9, 4.0, 3.7, and 2.3 μM, respectively, in comparison to indomethacin (IC_{50} 0.98 μM). These results supported the traditional uses of *C. rotundus* in treating inflammation and its related symptoms.

Introduction

Inflammation a complex process is regulated by a precisely-modulated reaction between inflammatory mediators and cells (Sacca et al., 1997). The inflammatory mediators, including lipoxygenases (LOX) and cyclo-oxygenases (COX-1 and 2) enzymes, nitric oxide (NO), prostaglandin E2 (PGE2), cytokines such as tumor necrosis factor (TNF)-α and interleukins (IL), and transcription factor as nuclear factor (NF)-κB are released from the activated inflammatory cells (neutrophils, eosinophils, mononuclear phagocytes, and macrophages) (Al-Attas et al., 2015; Nguyen et al., 2015). TNF-α and IL intercellular signal proteins released by immune cells, have been identified to play a central role in the pathogenesis of many inflammation diseases, especially asthma and rheumatoid arthritis. The NF-κB a main regulator of the expression of several genes involved in activating the inflammation has been described to have a major role in pathogenesis of inflammatory bowel diseases and rheumatic diseases (Gautam and Jachak, 2009). Nitric oxide is a major inflammatory byproduct, and its production is controlled by nitric oxide synthases (NOS), which include endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). iNOS is highly expressed in macrophages, and its activation leads to organ destruction in some inflammatory and autoimmune diseases (Murakami and Ohigashi, 2007). The 5-lipoxygenase (5-LOX) enzyme, a non-haeme iron-containing dioxygenase, catalyzes the biosynthesis of leukotrienes (LT) from arachidonic acid (AA) (Steinhilber, 1999). Leukotrienes possess a significant role in numerous inflammatory diseases such as ulcerative colitis, atherosclerosis, asthma, rheumatoid arthritis, and several types of cancers (Nie and Honn, 2002; Radmark et al., 2007). Therefore, 5-LO inhibition has become the focal point of many therapeutic approaches for the treatment of many proliferative and inflammatory diseases (Mashima and Okuyama, 2015). Corticosteroids and non-steroidal anti-inflammatory drugs (NSAID) are the major groups of drugs used in treating inflammatory diseases but their uses associated with several serious side effects. Therefore, there is an urgent need to find safer anti-inflammatory agents. Alternatively, natural products represent a great prospect in the identification of bioactive lead metabolites and their development into drugs for the treatment of inflammatory diseases. In various traditional medicines, different plants extracts and/or their active constituents have been used for

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treating a wide variety of inflammatory disorders (Gautam and Jachak, 2009; García-Lafuente et al., 2009). It has been reported that flavonoids possess anti-inflammatory activity in both proliferative and exudative phases of inflammation via inhibition of various enzymes such as xanthine oxidase, aldose reductase, phosphodiesterase, LOX, Ca(+2)-ATPase, and COX (García-Lafuente et al., 2009; Rathee et al., 2009). *Cyperus rotundus* L., Cyperaceae (Suada, Sueda) is vastly spread in several world's subtropical and tropical regions (Boulos and El-Hadidi, 1984). It is known as nut grass due to its tubers resemblance to nuts. The tubers are utilized as diuretic, anthelmintic, carminative, aphrodisiac, tonic, stomachic, sedative, and stimulant (Boulos and El-Hadidi, 1984). Also, tubers are used as a remedy for various ailments such as fever, dysentery, diarrhea, cholera, and renal colic (Boulos, 1983). Furthermore, the plant possessed varied bioactivities: cytotoxicity (Sayed et al., 2007, 2008), antioxidant (Nagulendran et al., 2007), anti-inflammatory, antipyretic, hypotensive, antiemetic (Sayed et al., 2001), anti-allergic (Meena et al., 2010; Jin et al., 2011), anticonvulsant (Mayur et al., 2011), anti-diarrheal (Daswani et al., 2011), anti-malarial, antimicrobial (Ahmad et al., 2012), hepatoprotective (Mohamed, 2015), insecticidal (Singh et al., 2012), and anti-diabetic (Bawden et al., 2002; Sayed et al., 2008). The former phytochemical researches on *C. rotundus* revealed the existence of sesquiterpenes (Bawden et al., 2002; Xu et al., 2008; Lawal and Oyedeji, 2009; Kim et al., 2013), saponins (Singh and Singh, 1980), alkaloids (Jeong et al., 2000), flavonoids (Sayed et al., 2001, 2007, 2008; Krishna and Renu, 2013), phenylpropanoids (Sayed et al., 2008; Zhou and Zhang, 2013), phenolic acids (Sayed et al., 2008), and iridoid glycosides (Zhou and Zhang, 2013; Mohamed, 2015). Resuming the phytochemical study on *C. rotundus*, a new flavanol glucoside: cyperaflavoside (**5**) and five known flavonoids (**1–4** and **6**) were separated and characterized. All isolated metabolites were examined for their 5-LOX inhibitory potential and their structural activity relationship was discussed.

Materials and methods

General experimental procedures

Hitachi-300 spectrophotometer was utilized to get UV spectra. IR spectra were performed on an Infrared-400 Shimadzu spectrophotometer. HRESIMS was acquired by LTQ Orbitrap. NMR was measured on a Bruker DRX600. A LCQ DECA mass spectrometer was used to get ESIMS. Chromatographic separations were carried out on SiO_2 60, sephadex LH-20, and RP_{18}. Pre-coated plates with silica gel 60 F_{254} (0.2 mm) was used for TLC. Purification of compounds was achieved using a 6 ml extraction tube LiChrolut EN/RP_{18} solid phase.

Plant material

Cyperus rotundus L., Cyperaceae, aerial parts were collected in March 2016 from King Abdulaziz University campus, Jeddah, Saudi Arabia. The plant was kindly identified based on the librarian database and morphological characters (Collenette, 1999) and proved by Dr. Nahed Morad, Faculty of Science, King Abdulaziz University. A voucher sample (2014-CR110) was kept in the Natural Products and Alternative Medicine Department herbarium, King Abdulaziz University.

Extraction and isolation

The powdered air-dried aerial parts (0.9 kg) were extracted with MeOH (4× 5 l). The total extract was evaporated to get 41.8 g residue. The residue was mingled with distilled water (150 ml) and successively partitioned among hexane (5× 500 ml), $CHCl_3$ (5× 500 ml), and EtOAc (5× 500 ml) to afford hexane (4.7 g), $CHCl_3$ (12.9 g), EtOAc (6.2 g), and aqueous (15.1 g) fractions. The EtOAc (6.2 g) fraction was submitted to sephadex LH-20 CC eluted with MeOH/$CHCl_3$ 90:10 to get seven subfractions: CRE-1-CRE-7. SiO_2 CC (70 g, 50× 2 cm) of CRE-2 (918 mg) using $CHCl_3$/MeOH (97:3 to 90:10) gave impure **5**. The purification was accomplished using RP_{18} CC, eluting with a gradient of H_2O/MeOH and LiChrolut RP_{18} extraction tube using a gradient of H_2O/acetonitrile to yield **5** (10.7 mg). CRE-3 (760 mg) was similarly handled as CRE-2 to afford **6** (13.6 mg). CRE-4 (1240 mg) was separated on RP_{18} CC (100 g, 50× 3 cm) using gradient of H_2O/MeOH to obtain **3** (31.5 mg) and **4** (57.2 mg). SiO_2 CC (30 g, 50× 2 cm) of CRE-5 (1725 mg) using $CHCl_3$/MeOH (94/6 to 85/15) afforded **1** and **2**. They were purified on RP_{18} CC (30 g, 50× 2 cm) using gradient of H_2O/MeOH to give **1** (37.2 mg) and **2** (62.6 mg).

Table 1
NMR spectral data of 5 (DMSO-d_6, 600 and 150 MHz).

Position	δH, m, (*J* in Hz)	δC, *m*	HMBC
2	–	164.1	–
3	–	140.4 C	–
4	–	178.1 C	–
5	–	165.2 C	–
6	6.90 brs	96.8 C	5, 7, 8, 10
7	–	165.4 C	
8	6.97 brs	92.3 CH	7, 6, 10
9	–	164.9 C	–
10	–	104.9 C	–
1′	–	119.4 C	–
2′	7.09 brs	106.9 CH	2, 3, 3′, 5′, 6′
3′	–	147.3 C	–
4′	–	145.2 C	–
5′	–	147.3 C	–
6′	7.09 brs	106.9 CH	2, 3, 3′, 5′, 6′
1″	4.21 d (7.8)	102.1 CH	7
2″		72.8 CH	–
3″	3.01–4.54	75.8 CH	–
4″		70.1 CH	–
5″		77.0 CH	–
6″		60.5 CH_2	–
3′,5′-OCH_3	3.76 s	56.0 CH_3	3′, 5′
3-OCH_3	3.72 s	59.3 CH_3	3

Spectral data

Cyperaflavoside (myricetin 3,3′,5′-trimethyl ether 7-*O*-β-D-glucopyranoside) (**5**): yellow amorphous powder; UV (MeOH) λ_{max}: 262, 354 nm; IR (KBr) ν_{max}: 3389, 2967, 1659, 1605 cm^{-1}; NMR data: see Table 1; HRESIMS *m*/*z* 523.1448 (calcd for 523.1452 $[M+H]^+$, $C_{24}H_{27}O_{13}$).

5-Lipoxygenase inhibitory assay

The 5-LOX activity of compounds **1–6** at four concentrations (0.1, 1, 10, and 100 μM) was evaluated as previously outlined (Yawer et al., 2007; Mohamed, 2016). A mixture of 10 μl of each compound (1 mM in MeOH), 20 μl lipoxygenase (70 units) in phosphate buffer (0.1 M aq, pH 8.0) to reach a 160 μl volume was incubated for 10 min at 25 °C. Then, the reaction was started by adding 10 μl linoleic acid solution (20 μM) as substrate, leading to (9*Z*,11*E*,13*S*)-13-hydroperoxyoctadeca-9,11-dienoate formation. The UV absorbance change at 234 nm was measured over a 6 min-period. All experiments were carried out in triplicate and the analysis took aplace using a 96-well microplate reader (Tecan Genios). The % inhibition was estimated as 100 × (E − S)/E, where S and E are the activities of enzyme in the presence and absence of the tested compound, respectively (Mohamed, 2016; Yawer et al., 2007). The positive control was

indomethacin. IC_{50} values were obtained by linear regression analysis (Noreen et al., 1998).

Results and discussion

Purification of metabolites

The dried aerial parts were extracted with MeOH. The concentrated MeOH extract was mixed with H_2O and partitioned among hexane, $CHCl_3$, and EtOAc. The EtOAc extract was submitted to SiO_2, sephadex LH-20, and RP_{18} CC to yield one new (**5**) and five known compounds (**1–4** and **6**).

*Structural characterization of **5***

Compound **5** was separated as yellow amorphous powder and had positive reactions for flavonoids (Mabry et al., 1970; Ibrahim et al., 2012). It possessed a pseudo-molecular ion peak at *m/z* 523.1448 ($[M+H]^+$, calcd for 523.1452, $C_{24}H_{27}O_{13}$) in HRESIMS, corresponding to a formula $C_{24}H_{26}O_{13}$. The ESIMS showed a prominent fragment at *m/z* 360 [M+H−(Glu)]+, indicating **5** was a flavonoid with hexose unit. Its UV exhibited distinctive absorptions for flavonol at 262 and 354 nm (Mabry et al., 1970). The IR displayed distinguishable bands at 3389 (OH group), 2967 (C—H aliphatic), 1659 (α,β-unsaturated CO), and 1605 (C—H aromatic) cm^{-1}. The ^{13}C and HSQC displayed 24 carbons resonances, including one methylene, one carbonyl (δ_C 178.1), three OCH_3, nine CH, and 8 oxygen-linked quaternary carbons. The ^1H NMR displayed two *meta*-coupled protons resonances at δ_H 6.90/H-6 and 6.97/H-8 (Mohamed et al., 2015). They correlated to the carbons at δ_C 92.3 (C-6) and 96.8 (C-8) in the HSQC, indicating a *tetra*-substituted A-ring (Mohamed et al., 2013; Agrawal, 1992) (Table 1). This was assured by the HMBC cross peaks of H-6/C-10 and C-8 and H-8/C-10and C-6 (Fig. 1). Also, the ^1H NMR displayed a broad signal at δ_H 7.09/H-2′, 6′, correlating to the carbon at δ_C 106.9 (C-2′, 6′) characteristic for a *tetra*-substituted B-ring (Mohamed et al., 2014). The two singlet signals at δ_H 3.76 and 3.72 exhibited HSQC correlations to the carbons at δ_C 56.0 and 59.3, assignable to C-3′, C-5′, and C-3-OCH_3 groups, respectively. This was assured by HMBC cross peaks of the signals at δ_H 3.76/C-3′ and C-5′ and 3.72/C-3. Thus, the aglycone part of **5** was assigned as myricetin 3,3′,5′-trimethyl ether and ascertained by the ESIMS fragment peak at *m/z* 360 $[M+H-(Glu)]^+$ (Mabry et al., 1970). Moreover, anomeric signals at δ_H 4.21 (H-1″)/δ_C 102.1 (C-1″) and other carbon signals at 60.5–77.0 ppm were observed, suggesting the existence of β-glucose moiety in **5** (Agrawal, 1992). In the HMBC, the cross peak from H-1″/C-7 (δ_C 165.4) established the connectivity of the glucose moiety at C-7 (Fig. 1). Therefore, **5** was identified as myricetin 3,3′,5′-trimethyl ether 7-*O*-β-D-glucopyranoside and named cyperaflavoside.

The other compounds were specified as vitexin (**1**) (Harborne, 1994), orientin (**2**) (Leitão and Monache, 1998), cinaroside (**3**) (Malikov and Yuldashev, 2002; Yuldashev and Karimov, 2001),

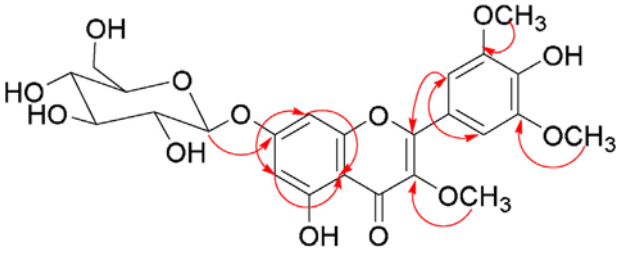


Fig. 1. Some key HMBC correlations of **5**.

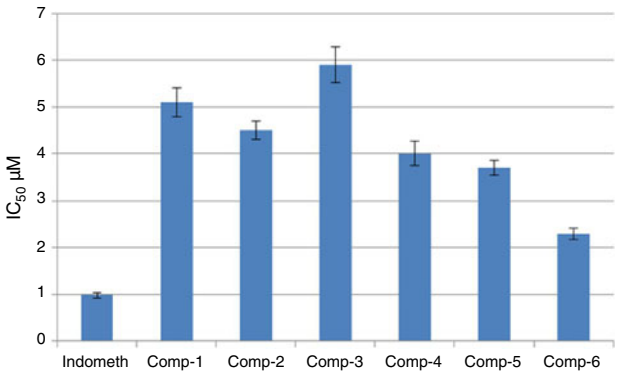


Fig. 2. 5-Lipoxygenase inhibitory activity of compounds **1–6**.

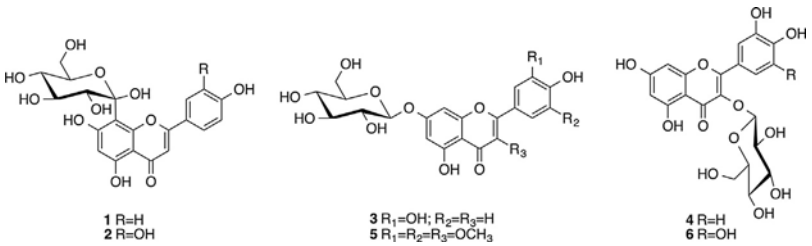
quercetin 3-*O*-β-D-glucopyranoside (**4**) (Al-Musayeib et al., 2013; Harborne, 1994), and myrcetin 3-*O*-β-D-glucopyranoside (**6**) (Braca et al., 2001).

5-LOX inhibitory activity of the test compounds

Inflammation is a defense reaction of the body and a local response of living tissues to injury aimed at eliminating or limiting the spread of an injurious agent (Al-Attas et al., 2015). The medicinal plants utilization or their active metabolites is becoming a progressively attractive aspect for treating diverse inflammatory disorders. The anti-inflammatory capacities of various medicinal plants can be referred to the existence of various substances: triterpenoids, flavonoids, tannins, alkaloids, saponins, and anthraquinones, which act as inhibitors of pro-inflammatory mediators and molecular targets in inflammatory responses (Mohamed et al., 2014; Al-Attas et al., 2015; Khedr et al., 2016). Thus, we investigated the isolated flavonoids **1–6** from *C. rotundus* aerial parts, in an attempt to explore their inhibitory activity against 5-LOX and highlight their structure-activity relationships. It is noteworthy that **2** and **4–6** displayed prominent 5-LOX inhibitory activities (Fig. 2). Their IC_{50} values were found to be 4.5, 4.0, 3.7, and 2.3 μM, respectively compared to indomethacin (IC_{50} 0.98 μM). While **1** and **3** had moderate activity with IC_{50}s 5.1 and 5.9 μM, respectively.

Structure–activity relationship

The important moieties in flavonoids as anti-inflammatory are the 5,7-OH (A-ring), C_2 and C_3 double bond, and 4′- or 3′,4′-OH (B-ring). The 3-OH group is significant for anti-inflammatory and LOX inhibitory activity (Kim et al., 2004, 1998). So, flavonols are more potent than flavone as in **4–6** versus **1–3**. Increasing number of OH-groups in ring B leads to increase in activity as in **6**. Introducing a sugar moiety at position C-3, C-7, or C-8 significantly lessens the anti-inflammatory effect, indicating the importance of the bioavailability and lipophilicity of the scaffold (Lago et al., 2014) as in **3** and **4**. Also, OH groups at C-4′, C-5, or C-7 have been sipposed to be substantial for activity as in **1**, **2**, **4**, and **6**. C-5 OH (A-ring) is significant for activity due to its interaction with the C-4 carbonyl, forming an intramolecular H-bond and increasing activity and any substitution of it leads to a decrease in activity. Similarly, C-3 and C-7 OH groups are important for activity and their substitution decreases the activity as in **3** and **5** comapred to **4** and **6**, respectively. Introducing any substituent at C-8 leads to a slightly decease in the activity, which may be due to steric clashes in the binding crevice (Lättig et al., 2007) as in **1** and **2**. The presence of methoxy groups increase LOX inhibitory activity, because they change the pharmacokinetic behavior and increase lipophilicity and bioavailability of scaffold as in **5** (Kim et al., 2004).



Conclusion

A new flavonol glycoside, cyperaflavoside (**5**) and five known flavonoids (**1–4** and **6**) were separated from *C. rotundus* aerial parts. Their structural elucidation was achieved with the aid of extensive spectroscopic techniques. Compounds **2** and **4–6** showed strong 5-LOX inhibitory potential.

Authors' contributions

SRMI: manuscript preparation and submission, data acquisition, analysis, and interpretation of NMR data. GAM: plant collection, concept and design of the study, and supervision of the study. RAA and KZA: shared in writing and revising the manuscript. AAE and MFZ: interpretation of biological data and sharing in writing the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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