Revista Brasileira de Farmacognosia 26 (2016) 50–55

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.journals.elsevier.com/revista-brasileira-de-farmacognosia

Original Article

Screening of ferulic acid related compounds as inhibitors of xanthine oxidase and cyclooxygenase-2 with anti-inflammatory activity

Shivraj Hariram Nile, Eun Young Ko, Doo Hwan Kim, Young-Soo Keum *

Department of Bioresources and Food Science, College of Life and Environmental Sciences, Konkuk University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 23 August 2015
Available online 29 November 2015

Keywords:
Ferulic acid
Gallic acid
Xanthine oxidase
Cyclooxygenase
SAR
Gout

ABSTRACT

The ferulic and gallic acid related compounds from natural origin were studied against xanthine oxidase and cyclooxygenase-2 along with their anti-inflammatory activity. The compounds gallic acid, ferulic acid, caffeic acid and *p*-coumaric acid revealed promising anti-inflammatory activity (30–40% TNF-α and 60–75% IL-6 inhibitory activity at 10 μM). Bioavailability of compounds were checked by *in vitro* cytotoxicity using CCK-8 cell lines and confirmed to be nontoxic, but found toxic at higher concentration (50 μM). Gallic, ferulic, caffeic acid was demonstrated potential dual inhibition toward xanthine oxidase and cyclooxygenase-2 as calculated by IC_{50}: 68, 70.2, and 65 μg/ml (xanthine oxidase) and 68.5, 65.2, and 62.5 μg/ml (cyclooxygenase-2), respectively. The structure activity relationship and *in silico* drug relevant properties (HBD, HBA, PSA, *c* Log P, ionization potential, molecular weight, E_{HOMO} and E_{LUMO}) further confirmed that the compounds were potential candidates for future drug discovery study, which was expected for further rational drug design against xanthine oxidase and cyclooxygenase.

Introduction

Gouty arthritis is a well known disease with an abrupt attack causing extreme pain in and around the joints due to activities occurred by xanthine oxidase (Mohapatra et al., 2015). Gout is nearly always associated with chronic hyperuricemia, a long-lasting abnormally high concentration of uric acid (hyperuricemia) in the blood. The higher levels of uric acid can reach a point where uric acid crystals (monosodium urate) are put differently and this hyperuricemia results in the deposition of crystals of sodium urate in tissues, especially in kidneys and joints (Bardin, 2004; Choi and Curhan, 2005). This process generates oxygen metabolites, which damage tissue, resulting in the release of lysosomal enzymes that induce an inflammatory response. This will lead to local decrease of pH which further causes more deposition of urate crystals (Nuki and Simkin, 2006). The diagnosis of gout is based on the presence of monosodium urate crystals in the synovial fluid (Martinon and Glimcher, 2006). This deposition will exacerbate leading to recurrent episodes of acute arthritis, the classic manifestation of gout. Meanwhile, controlling the uric acid level in the blood is still the main target particularly in the management of the chronic attacks (Mandell, 2002; Dincer et al., 2002). Xanthine oxidase (XO) has a major role in the uric acid production as XO is responsible for catalyzing the oxidation of hypoxanthine to form xanthine and finally to uric acid. Thus, this enzyme coordinates the reaction and produces uric acid from its precursors (Nile et al., 2013; Li et al., 2013). Similarly, certain enzymes such as cyclooxygenase-2 (COX-2) contributes its role in the gouty inflammation, though it heightened expressions in the presence of the accumulated MSU crystals, which in turn enhances the production of inflammatory prostaglandins leading to the increased production of IL-1β. Thus, COX-2 plays a major role in arousing the inflammatory responses and thus taking part in the advancement of the acute inflammation in the gouty arthritis patients (Pouliot et al., 1998; Mohapatra et al., 2015). There are certain measures taken in order to treat the disease by using non-steroidal anti-inflammatory drugs (NSAID), colchicine or gluco corticoids. NSAID are the class of drugs which causes COX-2 inhibition whereas colchicine is an antimycotic alkaloid that apart from disturbing the microtubule polymerization, it also inhibits the inflammation by preventing the IL-1β processing which was stimulated by the MSU crystals (Martinon et al., 2006; Ricciotti and FitzGerald, 2011). Examples of such drugs are allopurinol, probenecid and sulfinpyrazone (Mandell, 2002; Aggarwal et al., 2011). Modern medicines from natural products have little to offer for alleviation of gout, oxidative and inflammatory activity. There is an urgent need to discover compounds with xanthine oxidase inhibitory activities but devoid of the undesirable effects of allopurinol. One potential source of such compounds is medicinal

* Corresponding author.
E-mail: rational@konkuk.ac.kr (Y.-S. Keum).

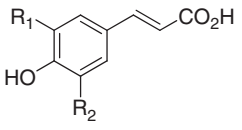
http://dx.doi.org/10.1016/j.bjp.2015.08.013
0102-695X/

materials of plant origin which is used to treat conditions similar to gouty arthritis (Nile and Park, 2014a,b). Phenolics and flavonoids are considered important components in the human diet because of their beneficial effects on human health. The phenolics and flavonoids are naturally occurring compounds that are constantly distributed in various foods, fruit juices and beverages from plant origin and display many bioactive and therapeutic properties, such as antioxidant, anticancer, antiviral, anti-inflammatory, and cardiac protective effects (Proestos et al., 2005; Nile and Park, 2014c). Most of the therapeutic properties of phenolics and flavonoids have been demonstrated to have potent antioxidant, anti-inflammatory and enzyme inhibition properties. Several phenolics and flavonoids have been described as inhibitors of the xanthine oxidase (XO) enzyme, similar to allopurinol in the treatment of gout (Bandgar et al., 2009; Nile and Park, 2013), for this reason, research into ferulic acid seems promising. Thus, the aim of this study is to investigate the potency of naturally occurring ferulic acid related compounds as xanthine oxidase and cyclooxygenase-2 inhibitors along with the properties like anti-inflammatory activity, structure activity relationship (SAR) and *in silico* drug relevant properties were studied.

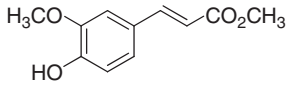
Materials and methods

Chemicals

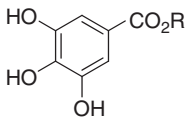
Xanthine oxidase, xanthine, allopurinol, myricetin, and isoquercetin were purchased from Sigma Chemical (Seoul, Korea). Ferulic acid (**2**), gallic acid (**6**), and their esters were supplied by Hi-Media Laboratories, Mumbai, India. Caffeic acid (**3**), sinapic acid (**4**), *p*-coumaric acid (**1**) procured from Sigma–Aldrich, Mumbai, India. Cyclooxygenase fluorescent inhibitor screening assay kit was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Bovine milk xanthine oxidase procured from (grade 1, ammonium sulfate suspension) Sigma–Aldrich, Mumbai, India.



1 R_1=R_2=H
2 R_1=OCH_3; R_2=H
3 R_1=OH; R_2=H
4 R_1=R_2=OCH_3



5



6 R=H
7 R=CH_3
8 R=$(CH_2)_2CH_3$
9 R=$(CH_2)_{11}CH_3$
10 R=$(CH_2)_{17}CH_3$

Anti-inflammatory and cytotoxicity assay

Pro-inflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Bandgar et al. (2009). During assay, THP-1 cells were cultured with penicillin and streptomycin (100 U/ml) and inoculated with 10% fetal bovine serum (FBS, JRH) in RPMI 1640 culture medium (Gibco BRL, Pasley, UK). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds (10 μM) in 0.5% DMSO was poured to each well and the plate were incubated for 30 min at 37 °C. Finally, LPS (*Escherichia coli* 0127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 μg/ml in each well. Plates were further incubated at 37 °C for 24 h in 5% CO_2. After incubation, supernatants were harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences, India). The cells were simultaneously evaluated for cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared to the control was calculated. In this the cytotoxicity was checked at lower, optimum and higher concentration (10, 25 and 50 μM), 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method (Bandgar and Gawande, 2010; Nile and Khobragade, 2011).

In silico *pharmacological property and SAR study*

The pharmacological properties of the compounds, such as molecular weight, *c* Log P and quantum chemical descriptors such as E_{HOMO} (Energy of highest occupied molecular orbital) and E_{LUMO} (energy of lowest unoccupied molecular orbital) of the synthesized compounds were calculated using a BioMed CaChe 6.1 (FujiSuit Ltd), a computer aided molecular design modeling tool for windows 98/20000/XP operating system. Other parameters such as HBA (hydrogen bond acceptor), HBD (hydrogen bond donor), molecular PSA (polar surface area), drug score and drug likeness of the compounds were also studied using online Osiris property explorer for drug bioavailability of chemical compounds. Since compounds are considered for oral delivery, they were also assessed for toxicity using *in silico* ADME prediction methods (Bandgar and Gawande, 2010; Nile and Khobragade, 2011).

XO inhibitory activity

Xanthine oxidase (XO) activity was assayed spectrophotometrically by measuring the uric acid formation at 290 nm using a UV-visible spectrophotometer at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 75 μM xanthine and 0.08 units of XO. Inhibition of XO activity of individual isolated phenolics from maize (1.5 ml, 2 mM), was measured by following the decrease in the uric acid formation at 293 nM at 25 °C. The enzyme was pre incubated for 5 min, with test compound, dissolved in DMSO (1%, v/v), and the reaction was started by the addition of xanthine. Final concentration of DMSO (1%, v/v) did not interfere with the enzyme activity. The XO kinetic study was carried out using screening of ten ferulic acid related compounds (10, 25, 50 and 100 μg/ml) comparing with allopurinol (10, 25, 50 and 100 μg/ml) as positive control. All the experiments were performed in triplicates and IC_{50} values were expressed as means of three experiments (Nile et al., 2013; Nile and Park, 2014a).

COX-2 inhibitory activity

Cayman's COX fluorescent inhibitor screening assay provides a convenient fluorescent-based method for screening both ovine COX-1 and human recombinant COX-2 for isozyme-specific inhibitors. The assay utilizes the peroxidase component of COX. The reaction between prostaglandin- G2 and 10-acetyl-3, 7-dihydroxyphenoxazine produces the highly fluorescent compound resorufin. Resorufin fluorescence can be easily analyzed with an excitation wavelength of 540 nm and an emission wavelength of

Table 1
Anti-inflammatory activity against TNF-α and IL-6.

Samples	% Inhibition at 10 μM				
	TNF-α	IL-6	Toxicity		
			10 μM	25 μM	50 μM
p-Coumaric acid	30	60	0	1.3	3.2
Ferulic acid	40	72	0	1.5	4
Caffeic acid	35	68	0	1.6	3.5
Sinapic acid	23	56	0	2.1	4.5
Alkyl ferulates	20	40	0	2.4	5
Gallic acid	48	75	0	1.6	3.5
Methyl gallate	16	30	0.4	3	8
Propyl gallate	18	35	0	2	5
Lauryl gallate	20	25	0.5	4	8
Stearyl gallate	15	20	0.4	2	7
Dexamethasone	60	92	0	0	3

595 nm. The COX-2 assay consisted of a 200-μl reaction mixture containing 150 μl assay buffer, 10 μl heme, 10 μl COX-2, 10 μl fluorometric substrate, and 10 μl of ten ferulic acid related compounds (25, 50, 100 μg/ml) The reactions were initiated by quickly adding 10 μl AA, then incubating for 5 min at 37 °C temperature. Dup-697 was used as a positive control. All the experiments were performed in triplicates and IC_{50} values were expressed as means of three experiments (Li et al., 2013; Mohapatra et al., 2015).

Statistical analysis

All data are expressed as mean ± SD of triplicate experiments.

Results and discussion

Anti-inflammatory and cytotoxicity assay

All ten ferulic acid related compounds were evaluated for anti-inflammatory activity by TNF-α and IL-6 inhibition assays. Not all of the compounds showed a promising TNF-α inhibitory activity except gallic acid, ferulic acid, caffeic acid and *p*-coumaric acid up to 30–40% at 10 μM concentrations, also a promising IL-6 inhibitory activity was shown by same compounds that is by gallic acid, ferulic acid, caffeic acid and *p*-coumaric acid up to 60–75% inhibition at 10 μM concentration. As compared to the standard dexamethasone, the activity results are revealed to be comparable as summarized in Table 1. Most of the compounds did not show significant cytotoxicity at 10 μM concentration except lauryl gallate, methyl gallate and stearyl gallate at very negligible level but at higher concentrations many of the compounds revealed cell toxicity. The results presented for ferulic acid related compounds revealed excellent anti-inflammatory activities as compared to dexamethasone, a known anti-inflammatory agent. It is known that during inflammation and associated processes, there is an increased production of superoxide ions. It may be possible that the inhibition of superoxide generation in peritoneal macrophages is related to the anti-inflammatory activity of ferulic acid related compounds (Nile and Khobragade, 2011; Nile and Park, 2014d). Pro-inflammatory cytokines such as TNF-α, and IL-6 are produced and play critical roles in the inflammation processes. Among these pro-inflammatory cytokines, TNF-α has been highlighted recently as a main mediator in the inflammatory diseases mechanism (Maxiaad et al., 2011). High levels of pro-inflammatory cytokines, including TNF, have been detected in psoriatic skin lesions and joints of patients with the inflammatory disease (Mease, 2002). TNF-α may have a significant role in pathogenesis of several inflammatory diseases, such as psoriatic arthritis, juvenile rheumatoid arthritis, and Crohn's disease. Decrease in the TNF-α and an IL-6 level by these compounds suggests that it may be useful in a variety of inflammatory conditions (Ellerin et al., 2003; Kuek et al., 2007).

In silico *pharmacological property and SAR study*

To qualify the compound as a drug candidate, it is analyzed by the parameters set by Lipinski's rule of five using Osiris property explorer. The *c* Log P is the important physiochemical property indicating the lipophilicity and the ability of molecule to cross the various biological membranes. According to Lipinski's rule of five the *c* Log P value below 5 is feasible for a compound to be a future drug. The all ten ferulic acid related compounds showed a marginal lipophilicity within the range of 4.0–5.0. The molecular weight property of the compound is related to its *in vivo* administration. All the ferulic acid related compounds have the molecular weight within the acceptable range, that is, 400–500. The compounds showed the HBA below 10 and HBD below 5, which is also within the limit set by Lipinski's rule. The polar surface area (PSA) > 140 A^2 is thought to have low oral bioavailability, which is also revealed within the range for these compounds (Table 2). Interestingly the compounds also presented a better drug likeness values. Overall, the compounds ferulic acid, gallic acid, caffeic acid and sinapic acid showed a good drug score, calculated by combining all parameters. Drug toxicity is a factor of great importance for a potential commercial drug, since a significant number of drugs are disapproved in clinical trials based on their high toxicity profile (Bandgar et al., 2009; Bandgar and Gawande, 2010). The toxicity of the compounds is calculated in terms of mutagenicity, tumorigenic, reproductively effective and irritant. All the compounds were confirmed as non-mutagenic and therefore were biologically safe for intake.

XO inhibition by ferulic acid related compounds

The experimental evidence indicates that, all the ten ferulic acid related compounds under this study showed a good to excellent activity profile toward inhibition of xanthine oxidase inhibitory activity and formation of hydroperoxide. All of the ten ferulic acid related compounds demonstrated XO-inhibiting activity among which ferulic acid, gallic acid, caffeic acid, *p*-coumaric acid, alkyl gallate, and methyl gallate, showed an inhibition >50% at 100 μg/ml (Fig. 1). Flavonoids and phenolics are well known antioxidants and attract a significant curiosity and scientific demand by research scientist for utilization as possible therapeutic agents against various disorders and diseases mediated by active free radicals (Nile and Park, 2013, 2014b). Phenolics and flavonoids may acts as potent inhibitors against the metabolic enzymes such as cyclooxygenase, xanthine oxidase and lipooxygenase (Hoorn et al., 2002). Thus, the phenolic constituents may play an essential role in the inhibition of XO and these XO inhibitors and uricosuric agents are commonly used against the treatment and curing of inflammatory diseases and gouty arthritis. So the drug allopurinol is the drug of choice against XO activity; however it has serious side effects (Nile and Park, 2013, 2014b). Thus, new alternatives with increased therapeutic activity and lesser side effects are desired. This study suggested that a ferulic acid related compound imparts xanthine oxidase inhibition and significantly reduce formation of uric acid in human body, indirectly helping to reduce risk of gout by slowing the XO activity. Further *in vivo* experiments were needed to identify whether the active ferulic acid related compounds are potent inhibitors of XO or not, which are directly correlated to check the in progress and development of gout and related inflammatory disorders.

Table 2
In silico pharmacological parameters for bioavailability of ferulic acid related compounds.

Sample	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	E_{HOMO}	E_{LUMO}	HBD	HBA	Mol. PSA	*c* Log P	Solubility	Drug likeness	Drug score
p-Coumaric acid	$C_9H_8O_3$	164	212	−4.28	−0.65	0	4	56.20	4.45	−4.20	0.53	0.32
Ferulic acid	$C_{10}H_{10}O_4$	194	170	−5.52	−0.61	0	5	56.20	4.12	−4.50	0.52	0.40
Caffeic acid	$C_9H_8O_4$	180	224	−4.25	−0.58	0	4	56.20	4.60	−4.20	0.41	0.38
Sinapic acid	$C_{11}H_{12}O_5$	224	204	−5.34	−0.70	0	4	65.5	5.40	−5.34	−0.22	0.35
Alkyl ferulates	$C_{40}H_{70}O_4$	614	96	−9.55	−0.85	0	6	85.5	6.86	−8.50	0.85	0.28
Gallic acid	$C_7H_6O_5$	170	260	−4.10	−0.60	0	4	55.40	4.11	−4.10	0.55	0.38
Methyl gallate	$C_8H_8O_5$	184	202	−4.61	−0.56	0	4	55.20	4.20	−4.22	−0.32	0.30
Propyl gallate	$C_{10}H_{12}O_5$	212	150	−5.45	−0.55	0	5	60.5	5.10	−4.40	−0.23	0.25
Lauryl gallate	$C_{19}H_{30}O_5$	338	98	−6.28	−0.68	0	5	72.5	6.85	−5.30	−0.42	0.22
Stearyl gallate	$C_{25}H_{42}O_5$	423	105	−6.85	−0.75	0	6	75.5	6.20	−6.10	−0.65	0.20

Inhibition of COX-2 by ferulic acid related compounds

Five ferulic acid related compounds demonstrated COX-2-inhibiting activity (Fig. 2), among which gallic acid, ferulic acid and caffeic acid showed an inhibitor >50% at 100 μg/ml. The mechanism of anti-inflammatory action is assumed to be mediated through the inhibition of COX-1 and COX-2. These enzymes catalyze the biosynthesis of prostaglandin H_2 from the arachidonic acid substrate. The inhibition of COX-1 results in some undesirable side-effects, whereas COX-2 inhibition provides therapeutic effects in pain, inflammation, cancer, glaucoma, Alzheimer's and Parkinson disease (Blobaum and Marnett, 2007). Therefore, we intended to examine the COX-1 and COX-2 inhibitory activity of ferulic acid related compounds demonstrated on purified enzymes as a mechanism of its topical anti-inflammatory action. Also it was found that many phenolic compounds such as chlorogenic acid, gallic acid,

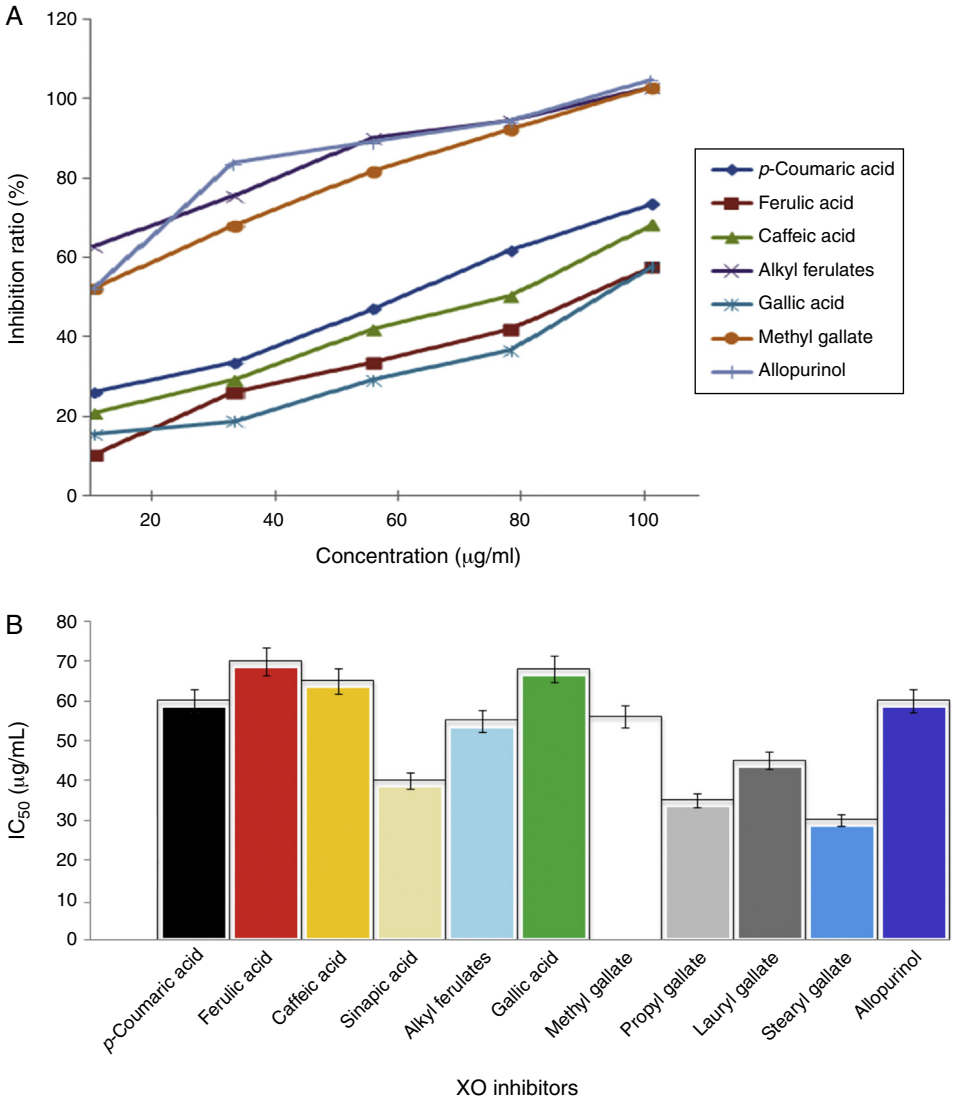


Fig. 1. (A) Inhibition ratio (%) and (B) the calculated IC_{50} of ferulic and gallic acid related compounds for inhibition of XO. Six compounds *viz.*; *p*-coumaric acid, ferulic acid, caffeic acid, alkyl ferulates, gallic acid and methyl gallate showed an inhibition >50% at 100 μg/ml. Allopurinol is a positive control.

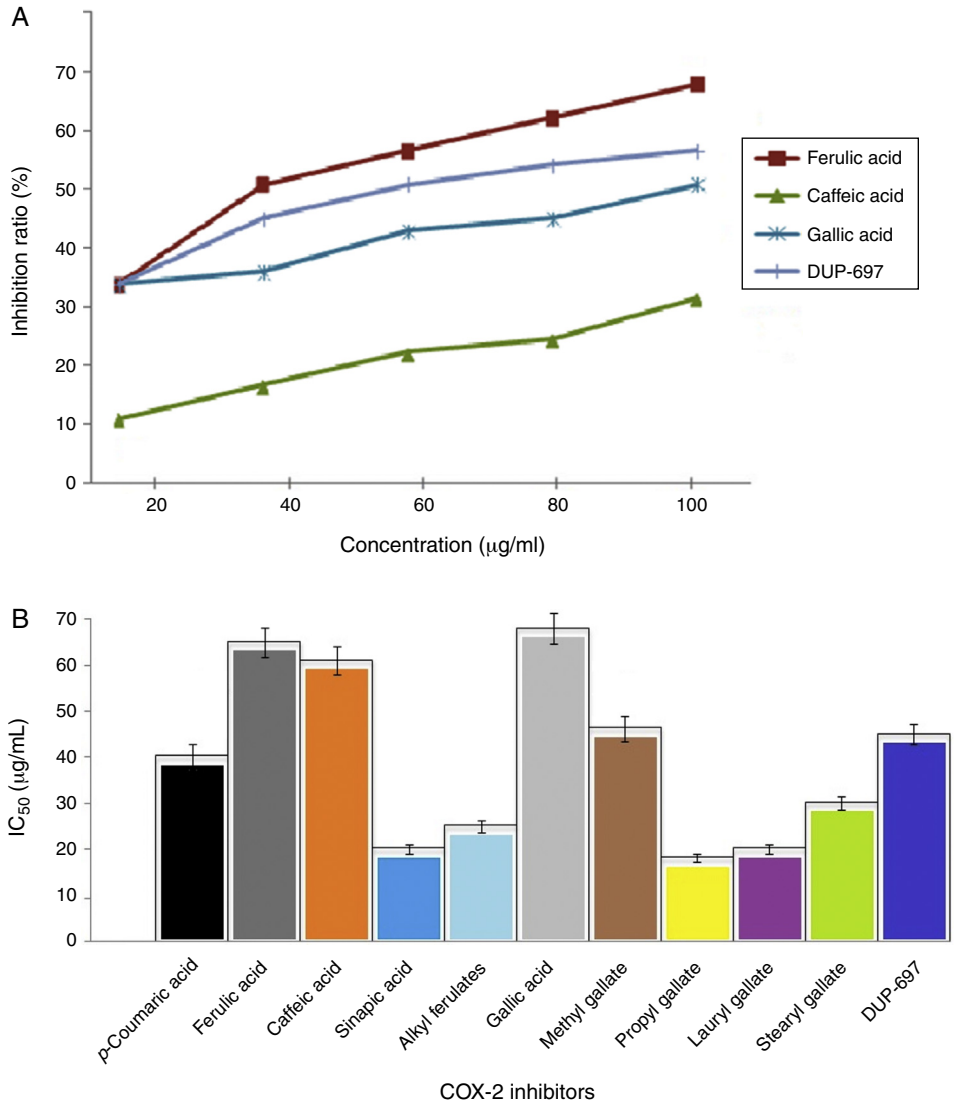


Fig. 2. (A) Inhibition ratio (%) and (B) the calculated IC_{50} of ferulic and gallic acid related compounds for inhibition of COX-2. The five compounds demonstrated COX-2-inhibiting activity among which ferulic acid, caffeic acid and gallic acid showed an inhibition >50% at 100 μg/ml. DUP-697 is a positive control.

rosmarinic acid, catechin, hesperidin, hesperetin, lutenolin, naringenin, naringin have been demonstrated possessing the inhibitory effect for COX-2 expression (Raso et al., 2001; Shen et al., 2008). Thus the result indicates that the ferulic acid related compounds may reduce the inflammatory activity by virtue of inhibition of COX-2 enzyme which provides pro-inflammatory activity.

The result indicates that the ferulic acid related compounds may reduce the inflammatory activity by virtue of inhibition of xanthine oxidase and COX-2 enzyme which provide pro-inflammatory activity. These compounds might be acts as good source of xanthine oxidase and COX-2 inhibitory agents, but further *in vivo* and clinical study should be required for their characterization as a drug against various diseases and disorders related to activity of xanthine oxidase and COX-2 enzymes. Furthermore the individual the ferulic acid related compounds can be subjected to optimization so as to design and develop a lead anti-inflammatory and antigout drug with an improved potency. For development of rational drug development against XO and cyclooxygenase-2 it is important to check the structure–activity relationships and other factors of related to ferulic acid compounds such as binding affinity, kinetic parameters and changes at active site of these studied enzymes were discussed, which is expected for further drug development and design.

Authors' contributions

SHN performed the laboratory work including *in vitro* xanthine oxidase and cyclooxygenase-2 activity, also *in silico* study and data analysis. VB assisted in anti-inflammatory assays. DHK provided idea and project plan with subsequent editing and checking of manuscript data and SHN contributed to critical reading and writing of the manuscript.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

This study was supported by KU-Research professor program-2015, Konkuk University, Seoul, South Korea.

References

Aggarwal, B.B., Prasad, S., Reuter, S., Kannappan, R., Yadev, V.R., Park, B., Sung, B., 2011. Identification of novel anti-inflammatory agents from ayurvedic medicine for prevention of chronic diseases: "reverse pharmacology" and "bedside to bench" approach. Curr. Drug Targets 12, 1595–1653.

Bandgar, B.P., Gawande, S.S., Bodade, R.G., Gawande, N.M., Khobragade, C.N., 2009. Synthesis and biological evaluation of a novel series of pyrazole chalcones as an anti-inflammatory, antioxidant and antimicrobial agents. Bioorg. Med. Chem. 17, 8168–8173.

Bandgar, B.P., Gawande, S.S., 2010. Synthesis and biological screening of a combinatorial library of β-chlorovinyl chalcones as anticancer, anti-inflammatory and antimicrobial agents. Bioorg. Med. Chem. 18, 2060–2065.

Bardin, T., 2004. Current management of gout in patients unresponsive or allergic to allopurinol. Joint Bone Spine 71, 481–485.

Blobaum, A.L., Marnett, L.J., 2007. Structural and functional basis of cyclooxygenase inhibition. J. Med. Chem. 50, 1425–1441.

Choi, H.K., Curhan, G., 2005. Gout: epidemiology and lifestyle choices. Curr. Opin. Rheumatol. 17, 341–345.

Dincer, H.E., Dincer, A.P., Levinson, D.J., 2002. Asymptomatic hyperuricemia: to treat or not to treat. Cleve Clin. J. Med. 69, 594–600.

Ellerin, T., Rubin, R.H., Weinblatt, M.E., 2003. Infections and anti-tumor necrosis factor α therapy. Arthritis Rheumatol. 48, 3013–3022.

Hoorn, D.E.C.V., Nijiveldt, R.J., Leeuwen, P.A.M.V., Hofman, Z., M'Rabet, L., Bont, D.B.A.D., Norren, K.V., 2002. Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. Eur. J. Pharmacol. 451, 111–118.

Kuek, A., Hazleman, B.L., Östör, A.J.K., 2007. Immune-mediated inflammatory diseases (IMIDs) and biologic therapy: a medical revolution. Postgrad. Med. J. 83, 251–260.

Li, Y., Frenz, C.M., Li, Z., Chen, M., Wang, Y., Li, F., Luo, C., Sun, J., Bohlin, L., Li, Z., Yang, H., Wang, C., 2013. Virtual and *in vitro* bioassay screening of phytochemical inhibitors from flavonoids and isoflavones against xanthine oxidase and cyclooxygenase-2 for gout treatment. Chem. Biol. Drug Des. 81, 537–544.

Mandell, B.F., 2002. Hyperuricemia and gout: a reign of complacency. Cleve Clin. J. Med. 69, 583–592.

Martinon, F., Glimcher, L.H., 2006. Gout: new insights into an old disease. J. Clin. Invest. 116, 2073–2075.

Martinon, F., Pétrilli, V., Mayor, A., Tardivel, A., Tschopp, J., 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440, 237–241.

Maxiaad, A., Sannaad, C., Frauad, M.A., Pirasbd, A., Karchulicd, M.S., Kasture, V., 2011. Anti-inflammatory activity of *Pistacia lentiscus* essential oil: involvement of IL-6 and TNF-α. Nat. Prod. Commun. 6, 1543–1544.

Mease, P.J., 2002. Tumour necrosis factor (TNF) in psoriatic arthritis: pathophysiology and treatment with TNF inhibitors. Ann. Rheum. Dis. 61, 298–304.

Mohapatra, S., Kabiraj, P., Agarwal, T., Asthana, S., Annamalai, N., Arsad, H., Siddiqui, M.H., Khursheed, A., 2015. Targeting *jatropha* derived phytochemicals to inhibit the xanthine oxidase & cyclooxygenase-2: *in silico* analysis towards gout treatment. Int. J. Pharm. Pharm. Sci. 7, 360–363.

Nile, S.H., Kumar, B., Park, S.W., 2013. In vitro evaluation of selected benzimidazole derivatives as an antioxidant and xanthine oxidase inhibitors. Chem. Biol. Drug. Des. 82, 290–295.

Nile, S.H., Park, S.W., 2014a. Antioxidant α-glucosidase and xanthine oxidase inhibitory activity of bioactive compounds from maize (*Zea mays* L.). Chem. Biol. Drug. Des. 83, 119–125.

Nile, S.H., Park, S.W., 2014b. Edible berries: bioactive components and their effect on human health. Nutrition 30, 134–144.

Nile, S.H., Park, S.W., 2014c. HPTLC analysis, antioxidant and antigout activity of Indian plants. Iran. J. Pharm. Res. 12, 531–539.

Nile, S.H., Park, S.W., 2014d. HPTLC analysis, antioxidant, anti-inflammatory and antiproliferative activities of *Arisaema tortuosum* tuber extract. Pharm. Biol. 52, 221–227.

Nile, S.H., Park, S.W., 2013. Total phenolics, antioxidant and xanthine oxidase inhibitory activity of three colored onions (*Allium cepa* L.). Front. Life Sci. 7, 224–228.

Nile, S.H., Khobragade, C.N., 2011. In vitro anti-inflammatory and xanthine oxidase inhibitory activity of *Tephrosia purpurea* shoot extract. Nat. Prod. Commun. 6, 1437–1440.

Nuki, G., Simkin, P.A., 2006. A concise history of gout and hyperuricemia and their treatment. Arthritis Res. Ther. 8, 1–6.

Pouliot, M., James, M.J., McColl, S.R., Naccache, P.H., Cleland, L.G., 1998. Monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes. Blood 91, 1769–1776.

Proestos, C., Boziaris, I.S., Nychas, J.E., 2005. Analysis of flavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 93, 1998–2004.

Raso, G.M., Meli, R., DiCarlo, G., Pacilio, M., DiCarlo, R., 2001. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. Life Sci. 68, 921–931.

Ricciotti, E., FitzGerald, G.A., 2011. Prostaglandins and inflammation. Arterioscler. Thromb. Vasc. Biol. 31, 986–1000.

Shen, Y.C., Chen, S.L., Zhuang, S.R., Wang, C.K., 2008. Contribution of tomato phenolics to suppression of COX-2 expression in KB cells. J. Food Sci. 73, C1–C10.