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XANTHINE OXIDASE INHIBITORY ACTIVITY OF NATURAL COMPOUNDS FROM FICUS MUCUSO WELW. (MORACEAE)

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Seventeen compounds were isolated from methanol soluble part of stem bark of F. mucuso, two of which were new compounds (mucusamide and mucusoside). Then fourteen isolated compounds, including two new, were subjected to the test of xanthine oxidase inhibitory activity. The two new compounds showed significant in vitro xanthine oxidase inhibitory activity.

Key words: mucusamide, mucusoside, xanthine oxidase inhibitor, ficus mucuso; moracea

Introduction

*Ficu*s, a genus of the family Moraceae, comprises about 150 species [1]. *Ficus* species have wide distribution and use worldwide traditionally as medicine, vegetable, food, fodder and fuel wood etc. They are widely used in traditional medicine in Azerbaijan too. Their uses, however, originated and are most widely found in the Middle East [2]. A lot of biologically active compounds are found in these species [3]. Medicinal plants play an important role in traditional medicine and are widely consumed as home remedies. The past decade has seen a significant increase in the use of herbal medicine due to their minimal side effects, availability and acceptability to the majority of the population.

Xanthine oxidase inhibitor is any substance that inhibits the activity of xanthine oxidase, an enzyme involved in urine metabolism. During the reoxidation of xanthine oxidase, molecular oxygen acts as electron acceptor, producing superoxide radical and hydrogen peroxide. In humans, inhibition of xanthine oxidase reduces the production of uric acid, and several medications that inhibit xanthine oxidase are indicated for treatment of hyperuricemia and

related medicals condition including gout. Gout is a disorder of urate metabolism characterized by the deposition of monosodium urate crystals in the joints and soft tissues. It is one of the most well-described diseases-descriptions of gout exist that date from the 5^{th} century BC [4]. More recent data indicate that xanthine oxidase also plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases and chronic heart failure [5].

Materials and Methods

The XO inhibitory activity of test compounds was determined by measuring the rate of hydroxylation of the substrate (xanthine) with the formation of uric acid, which is a colorless end product of the reaction and shows absorption at 295 nm [6]. Xanthine (X-0626) and xanthine oxidase (EC 1.1.3.22) (from butter milk) (Sigma Aldrich, Japan) were used. Briefly, the reaction mixture containing 10 μL of 1 mmol· L^{-1} pure sample was dissolved in DMSO, 150 μL of phosphate buffer (0.05 mol· L^{-1}, pH 7.4), 0.003 units of xanthine oxidase dissolved in buffer (20 μL), and 20 μL of 0.1 mmol· L^{-1} xanthine as substrate for enzyme. In the case of precipitation we reduced the concentration of pure sample until 0.25 mmol. After addition of xanthine oxidase, the mixture was incubated for 10 min at room temperature and pre-read in the UV region (λ max 295 nm). The substrate was added to reaction mixture, and final continuous reading for 15 min at an interval of 1 min was observed (Spectra MAX-340). The percentage inhibitory activity by the samples were determined against a DMSO blank and calculated using the following formula. Inhibition (%) = 100 - [(OD test compound /OD control) × 100]. The IC_{50} of the compounds as calculated using EZ-Fit windows-based software (Perrella Scientific Inc. Amherst, U.S.A.). To compare the inhibitory activities of the compounds, allopurinol was used as standard. The reaction for each compound was performed in triplicate.

Results and discussion

Xanthine oxidase is an enzyme which involve in defending the body against external agents during inflammatory responses. It is a form of xanthine oxidoreductase that generates reactive oxygen species is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. This enzyme plays an important role in the catabolism of purines in some species, including humans, and in diabetes and other metabolic disorders, therefore the discovery of its inhibitors may lead to tremendous efforts in drug development to treat such type of diseases in which xanthine oxidase is involved.

Two new sphingolipids mucusamide (1) and mucusoside (2) have been

isolated from methanol soluble part of the stem bark of *Ficus mucuso* WELW., together with fifteen known secondary metabolites including cellobiosylsterol (3), β-sitosterol (4), stigmasterol (5), β-sitosterol 3-*O*- -D-glucopyranoside (6), lupeol acetate (7), ursolic acid (8), procatechuic acid (9), 2-methyl-5,7-dihydroxychromone 8-*C*-β-D-glucoside (10), apigenin (11), (-)-epicatechin (12), (+)-catechin (13), *N*-benzoyl-L-phenylalanilol (14), α-acetylamino-phenylpropyl α–benzoylaminophenylpropionate (15), asperphenamate (16) and bejaminamide (17) [7]. The two new compounds together with known 12 compounds were subjected to the test of xanthine oxidase inhibitory activity. The result is shown in Table 1.

The two new compounds showed significant *in vitro* xanthine oxidase inhibitory activity. Mucusoside showed more activity than mucusamide. To the best of our knowledge this is the first report of inhibitory activity of mucusoside and mucusamide.

Lupeol acetate, benjaminamide, *N*-benzoyl-*L*-phenylalanilol, procatechuic acid, (+)-catechin exhibited inhibitory effects on xanthine oxidase. Cellobiosylsterol, *β*-sitosterol, *β*-sitosterol-3-*O*-*β*-D-glucopyranoside showed no inhibitory effects on this enzyme.

According to Ali MS et al. ursolic acid was found to be an excellent inhibitor for the superoxides produced in the cellular system, while the same was inactive in the superoxide scavenging activity in cell-free system by using xanthine/xanthine oxidase system. In our experiments ursolic acid was also inactive [8].

The enormous amount of data published suggests that flavonoids are good inhibitor of xanthine oxidase. Cos et al. demonstrated strong inhibitory activity of apigenin towards to xanthine oxidase [9]. Aucamp et al. llustrated the xanthine oxidase inhibitory activity of catechins from tea [10]. In our study apigenin also showed strong inhibitory activity towards to XO.

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Table 1

In vitro inhibition of xanthine oxidase by natural compounds from ficus mucuso welw. (Moraceae).

Compound	Inhibition, %	IC_{50} ±SEM
lupeol acetate	21.4	-
β-sitosterol	-	-
ursolic acid (3-hydroxy-12-ursen-28-oic acid)	-	-
asperphenamate	17.1	-
N-benzoyl-*L*-phenylalanilol	16.6	-
apigenin	86.4	39.47 ± 0.47
3-*O*-methylquercetin	17.7	-
procatechuic acid	16	-
(+)-catechin	39	-
benjaminamide	10.6	-
β-sitosterol-3-*O*-*β*-D-glucopyranoside	-	-
mucusamide	26.7	-
mucusoside	57.7	525± 5.17
cellobiosylsterol	-	-
Allupurinol (standard)	95.7	13.70 ± 0.15

SEM: *standard error of mean; IC50: concentration of compound that gave 50 % inhibition*

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SELİKLİ FİKUSDA (FICUS MUCUSO WELW.) TƏBİİ BİRLƏŞMƏLƏRİN KSANTİNOKSİDAZANI İNHİBİRLƏŞDİRİCİ FƏALLIĞI

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XÜLASƏ

Selikli fikusun gövdə qabığının həll olan hissəsinin methanol ekstraktından on yeddi birləşmə ayrılmışdır ki, onlardan ikisi naməlum (mukuzamid və mukuzozid) idi. İki naməlum birləşmələr daxil olmaqla ayrılmış maddələrdən on dördünün ksantinoksidazaya görə inhibirə edici fəallığı öyrənilmişdir. Yeni birləşmələr *in vitro* xeyli ksantinoksidazanı inhibirləşdirir.

Açar sözlər: mukuzamid, mukuzozid, ksantinoksidaza inhibitoru, selikli fikus, tut.

ИНГИБИРУЮЩАЯ АКТИВНОСТЬ ПРИРОДНЫХ СОЕДИНЕНИЙ ИЗ ФИКУСА СЛИЗИСТОГО (FICUS MUCUSO WELW.) ПО ОТНОШЕНИЮ К КСАНТИНОКСИДАЗЕ

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РЕЗЮМЕ

Было выделено семнадцать соединений из метанольного экстракта растворимой части коры ствола Фикуса слизистого, два из которых были новыми соединениями (мукузамид и мукузозид). Четырнадцать выделенных соединений, в том числе два новых,были проверены на ингибирующую активность по отношению к ксантиноксидазе. Новые соединения показали *in vitro* значительное ингибиторное действие по отношению к ксантиноксидазе.

Ключевые слова: мукузамид, мукузозид, ингибитор ксантиноксидазы, фикус слизистый, тутовые.

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