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# Determination of the antigenotoxic potencies of some luteolin derivatives by using a eukaryotic cell system, *Saccharomyces cerevisiae*

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ABSTRACT

In this study, we aimed to examine the mutagenic and antimutagenic potencies of three luteolin derivatives (luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside and luteolin-7-*O*-glucuronide) by using a eukaryotic cell system, *Saccharomyces cerevisiae* (RS112).

In the antimutagenicity assays, these luteolin derivatives showed antimutagenic effects in deletion and intrachromosomal recombination events against ethyl methanesulfonate and acridine mutagen agents. In deletion events, the highest inhibition rates for luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside and luteolin-7-*O*-glucuronide against ethyl methanesulfonate were 57.6%, 58.3% and 62.5%, respectively. Likewise, the highest inhibition rates for luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside and luteolin-7-*O*-glucuronide against acridin were 21.8%, 22.4% and 23.6%, respectively. Our findings showed that these luteolin derivatives have stronger antimutagenic properties against ethyl methanesulfonate compared to the acridine mutagen agent.



## 1. Introduction

The genus *Mentha* is widely used in food, pharmaceutical, cosmetic and flavor industries (Bhat, Maheshwari, Kumar, & Kumar, 2002). This genus has been used as a folk remedy for treatment of flatulence, nausea, anorexia, liver illness, ulcerative colitis, and bronchitis which is attributed to its carminative, anti-inflammatory, antiemetic, diaphoretic, analgesic, antispasmodic, stimulant, anti-mutagenic emmenagogue, anticatharrhal, and antioxidant activities (Gulluce et al., 2007). Luteolin is one of the most common flavonoid and exists in many plant families, including *Mentha* subsp. (*Mentha piperita*) (Samejima, Kanazawa, Ashida, & Danno, 1995). This flavonoid is known to possess numerous biological activities. *In vivo* and *in vitro* studies have acknowledged that luteolin and its glycosides exert anti-oxidative and anti-inflammatory activities (Chen, Peng, Tsai, & Hsu, 2007). An *in vivo* study performed with a pathogenic bacterium inoculated in mice, showed that luteolin suppressed the presence of the pathogenic bacteria, inhibited the development of the pathogenic bacteria-specific antibody and reduced inflammation (Tormakangas et al., 2005). As luteolin can scavenge reactive oxygen and nitrogen species and induce antioxidant enzymes, this compound has been considered as a strong antioxidant and antioxidant inducer (Lemanska et al., 2004).

Recent studies have demonstrated that in certain concentrations (50 μM and higher) luteolin can exert toxic effects (inhibition of topoisomerase) (Cantero, Campanella, Mateos, & Cortes, 2006). According to these studies, luteolin can induce topoisomerase II-mediated DNA damage. It is claimed that the DNA damage caused by luteolin through topoisomerase II can be useful, as this can lead to death of cancer cells. There is no clear evidence whether the DNA damage caused by luteolin is selective to cancer cells however, it has been demonstrated that luteolin suppresses jun N-terminal kinase (JNK) in macrophages while luteolin activates this kinase in cancer cells (Karrasch, Kim, Jang, & Jobin, 2007). In addition, the induction of apoptosis in breast and prostate cancer cells has been linked to the inhibition of fatty acid synthesis caused by luteolin (Brusselmans, Vrolix, Verhoeven, & Swinnen, 2005). Another usage of luteolin in cancer cells is its sensitization activity, in which luteolin can be used as cancer cells sensitiser for the cytotoxic effects of some anticancer compounds (Shi, Ong, & Shen, 2004).

Pro-oxidant potency of luteolin has been demonstrated as well as anti-oxidant (Ju et al., 2007). The anti or pro-oxidant potencies of luteolin have been attributed to the concentrations of luteolin and the source of free radicals in cells. The antioxidant potency of free luteolin has been linked to certain elements such as Cd, Cu, V and Fe. For example, changes in the Fe concentration affect the activity of luteolin, that is, luteolin acts as an antioxidant when the concentration of Fe is less than 50 μM and acts as a pro-oxidant

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when the concentration of Fe is more than 100 μM (Sugihara, Arakawa, Ohnishi, & Furuno, 1999).

In this study, we aimed to examine the mutagenic and antimutagenic potencies of these luteolin derivatives (L7G, L7R and L7Gl) by using a eukaryotic cell system, *Saccharomyces cerevisiae* (RS112). The test system used in this study, the yeast deletion (DEL) assay, has been used for cancer and mutation research as well as for the investigation of the genotoxicity of environmental contaminants and agricultural chemicals (Brennan & Schiestl, 2004). Performing the yeast DEL assay has some advantages compared with other short term tests in detecting the genotoxicity of environmental contaminants and potential pharmaceuticals. Firstly, the evaluation of compounds using the DEL assay can provide a mechanistic insight about a compound's genotoxicity, because yeast DEL assay detects genomic rearrangement, which is a DNA deletion formed by DNA double-strand breaks. Secondly, the yeast DEL assay measures the deletion recombination [deletion events (DEL events)] and interchromosomal recombination (ICR events). Both of these events are induced with a wide range of mutagenic factors such as oxidative stress, ionising and nonionising radiation, intercalation and alkylating agents. Furthermore, the yeast DEL assay responds to many compounds, which are poorly detected by other short-term mutagenicity assay such as *Salmonella* reverse mutation (Ames) test. While Ames is one of the most widely used short term mutagenicity test, the Ames test detects only about 50% of carcinogens compared to the yeast DEL assay (Brennan & Schiestl, 2004). Thirdly, yeast cells are simple eukaryotic organisms that have a significant homology to mammalian systems (Rodriguez et al., 2004). Briefly, the yeast DEL assay is considered to be safer, more sensitive and specific for detecting carcinogen agents compared to other short term tests. Yeast DEL might show the same properties for detecting antimutagenic compounds versus the mutagen/carcinogen agents used. Considering all these mentioned reasons, we aimed to perform the yeast DEL assay as a predictive screen for detecting the antimutagenic activity of these three luteolin derivatives by using two mutagenic agents which have different mode actions. Ethylmethan sulphonate (EMS) acts as an alkylation agent while acridin (AC) acts as intercalation agent.

## 2. Materials and methods

### 2.1. General experimental procedures

$^1$H and $^{13}$C NMR spectra were recorded on Varian Mercury plus 400 MHz for proton and 100 MHz for carbon by using tetramethylsilane (TMS) as internal standard. The solvent was DMSO-$d_6$. Electron impact mass spectra (EIMS) were performed on Finnigan MAT 95 spectrometer. Silica gel 60 (0.063–0.200 mm, Merck) and Sephadex LH-20 (Fluka) were used for column chromatographic separations. Lichroprep RP-18 (25–40 μm, Merck) reversed phase material was used for vacuum liquid chromatography (VLC). TLC analyses were carried out on pre-coated Kieselgel 60 F254 aluminum sheets (Merck). The compounds were detected by UV fluorescence and spraying 1% vanillin–$H_2SO_4$ reagent, followed by heating at 105 °C for 1–2 min.

### 2.2. Plant samples

The plant samples of *M. longifolia* (L.) Hudson subsp. *longifolia* were collected at the flowering stage from different locations in the vicinity of Erzurum located in the Eastern Anatolia in Turkey. The taxonomic identification of plant material was confirmed by Dr. Meryem Sengul Koseoglu, a senior plant taxonomist in Biology Department of Ataturk University, Erzurum – Turkey. The voucher specimen has been deposited at the Herbarium of the Department of Biology (ATA Herbarium 9732), Ataturk University. The collected aerial parts (leaves and stems) of plants were dried in the shade, then; both the leaves and the stems were ground in a grinder with a 2 mm in diameter mesh.

### 2.3. Conditions, extraction, fractionation and isolation

Aerial parts of the plant (1000 g) were air-dried in laboratory at room temperature and extracted four times with MeOH at 40 °C (4 × 2 L). After evaporation of the combined extracts *in vacuo*, 140 g of MeOH extract was obtained. The crude extract was dissolved in water and submitted to liquid–liquid partitions successively with petroleum ether (25 × 0.5 L), $CHCl_3$ (8 × 0.5 L), EtOAc (10 × 0.5 L) and *n*-butanol (15 × 0.5 L). Thereafter, the solvents were evaporated under reduced pressure in a rotary evaporator oven at 45 °C. The residues obtained were 33.4, 14.7, 8.4 and 36.3 g, respectively. Fractionation of *M. longifolia* (L.) Hudson subsp. *longifolia* was done by using the Ames test system as a guide to obtain the mutagenic or antimutagenic compounds. According to the results, *n*-butanol extract showed significant antimutagenic activity (Orhan et al., 2012). Then, the *n*-butanol extract (36.3 g) was first submitted to a silica gel column and eluted with a solvent gradient of $CHCl_3$–MeOH (100:0→0:100, v/v) to afford five main fractions (Frs. A–E, 200 ml each) by TLC profile, performed with $CHCl_3$–MeOH–$H_2O$ (61:32:7, v/v) as mobile phase. The mutagenic and antimutagenic potentials of them were reevaluated by using the same test systems and two of them (C and E fractions) were determined as antimutagenic. The fraction C (11.8 g) was rechromatographed by Sephadex LH-20 CC; eluting with MeOH to give a subfraction (Fr. $C_1$). Subfraction $C_1$ was further fractionated by vacuum liquid chromatography using reversed phase material with $H_2O$–MeOH (100:0→50:50, v/v) to afford three subfractions (Frs. $C_{1.1}$, $C_{1.2}$, and $C_{1.3}$). Fr. $C_{1.3}$ gave pure L7G (7 mg). Fraction $C_{1.1}$ was further fractionated by vacuum liquid chromatography using reversed phase material with $H_2O$–MeOH (100:0→50:50, v/v) to afford Fr. $C_{1.1a}$, followed by silica gel column chromatography eluted with a gradient of $CHCl_3$–MeOH (85:15→80:20, v/v) to yield L7R (18.3 mg). The fraction E (8.2) was re-chromatographed by Sephadex LH-20 CC, eluting with MeOH to give a subraction (Fr. $E_1$). The subraction E1.1 (272 mg) was rechromatographed by Sephadex LH-20 CC, eluting with MeOH to give a subraction (Fr. $E_{1.1a}$). Purification of subraction $E_{1.1a}$ (235 mg) by Sephadex LH-20 CC using $H_2O$–MeOH (100:0→50:50, v/v) gave L7Gl (4 mg).

### 2.4. The DEL assay

#### 2.4.1. Media

YPAD media was prepared as follows: one percent yeast extract, 2% peptone, 2% glucose, 40 μg/ml adenine sulfate, solidified with 2% agar in distilled water. The synthetic complete (SC) medium was preperad as follow: yeast nitrogen base 0.67%, glucose 2%, agar 2% plus the following amino acids and bases per 600 ml of distilled water: 40 mg each of adenine sulphate, L-isoleucine, L-leucine, L-lysine–HCl, L-tyrosine, 30 mg of L-arginine–HCl, L-histidine–HCl, L-methionine, uracil, 60 mg of L-tryptohpan. The drop-out medium (SC-HIS) was prepared as SC medium but lacking histidine (SC-HIS). The SC-Ade medium (SC-Ade) was prepared as SC medium but lacking adenin (SC-Ade). The inoculation (–LEU) medium was prepared as SC medium but lacking leucine. Top agar was prepared as follow: 0.6% agar and 0.6% NaCl.

#### 2.4.2. Yeast strains used

The diploid *S. cerevisiae* strain RS112 was used to determine the frequency of DEL recombination: MATa/MATα ura3-52/ura3-52