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Antimutagenicity Screening of Extracts from Medicinal and Edible Plants against N-Methyl-N-nitrosourea by the Ames Assay

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N-Nitroso compounds are suspected to be causative agents for human cancer. N-Methyl-N-nitrosourea (MNU) is a typical direct acting mutagen with alkylation activity of DNA, and has been reported to be formed in vivo. Therefore, it is important for cancer chemoprevention to find some compounds to inhibit mutagenicity induced by MNU. The inhibitory effect of plant extracts against the mutagenicity of MNU was evaluated using the Ames assay with Salmonella typhimurium TA1535. Among 43 extracts derived from medicinal and edible plant assayed, Glycyrrhiza aspera ethanolic extract, Glycine max extract with 40% isoflavone aglycone (ISOMAX AG40) and Zingiber officinale ethanolic extract at the range 0.01–1.0 mg/plate inhibited MNU-induced mutagenicity in S. typhimurium TA1535. No cytotoxicity of the three extracts were observed in Salmonella typhimurium TA1535, indicating that inhibition of MNU-induced mutagenicity was apparently due to the antimutagenic potency of Glycyrrhiza aspera ethanolic extract, ISOMAX AG40 and Zingiber officinale ethanolic extract. Therefore, the results of relative mutagenicity showed that Glycyrrhiza aspera ethanolic extract and ISOMAX AG40 decreased the mutagenic effects to 5.4% and 2.6%, respectively, whereas Zingiber officinale ethanolic extract decreased it to 45%.

Key words: N-nitrosamide, Glycyrrhiza aspera, Glycine max, Zingiber officinale

Introduction

Humans are exposed to carcinogenic N-nitroso compounds exogenously and endogenously (1,2). Exogenous N-nitroso compounds exist in food (3), tabacco smoke (4), workplace conditions (5,6), and cosmetics (7,8). In addition, N-nitroso compounds formed endogenously mainly in the stomach and intestine (9,10) or infected site (11,12). Approximately, 45–75% of the total human exposure to N-nitroso compounds is estimated as a result of formation in vivo (2). Almost all N-nitroso compounds tested have been shown to have carcinogenic activity in experimental animals (13), and thus exposure to N-nitroso compounds is suspected to correlate with the induction of human cancer. Moreover, the endogenous formation of N-nitroso compounds is reported to relate with cancer induction in epidemiological study (10,14–17).

N-Methyl-N-nitrosourea (MNU) is formed by nitrosation of methylurea with nitrite in guinea-pig stomach and is absorbed from the stomach into the blood (18). Additionally, MNU is detected by nitrosation of creatinine at gastric pH (19) and of methylurea in intestinal conditions (20). MNU is a direct-acting carcinogen that induces cancer of various organs, mainly of the fore-stomach, brain and nervous system (13).

The intake of antimutagens and anticarcinogens in everyday life can prevent human from cancer (21–23). Studies on antimutagenesis against N-nitroso compounds can make significant contribute to cancer prevention (24), and it is important to discover naturally occurring or synthetic compounds which can inhibit the mutagenicity and carcinogenicity of N-nitrosamines (25–27).

Short-term bacterial mutation assays, such as the Ames assay, have been an effective screening tool for the identification of various mutagenic or antimutagenic compounds in complex materials (28,29). The assay has lots of advantages as an inexpensive and flexible screen-
ing which provides preliminary information on antimutagenesis. There are many reports about the antimutagenicity of herbal medicines (30–32), vegetables and fruits (33–39). Many studies have employed indirect acting mutagens (30–35,39) and direct acting mutagens; N-methyl-N′-nitro-N-nitrosoguanidine (MNNG), ethyl methanesulfonate (EMS) (35,37–39), while MNU was less studied (38,39). In this study, MNU was used as the mutagen in antimutagenic screening of extracts derived from medicinal and edible plants. Extracts derived from 43 medicinal and edible plants were evaluated for their antimutagenic effect against MNU using the Ames assay with *S. typhimurium* TA1535.

**Materials and Methods**

**Materials:** Sodium ammonium hydrogen phosphate tetrahydrate and iron(II) chloride tetrahydrate were purchased from Merck (Darmstadt, Germany). Ammonium hexafluorophosphate was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). Bacto agar and bacto nutrient broth were obtained from Becton Dickinson Microbiology Systems (Sparks, USA). β-Glucosidase was purchased from Nakal Pharmaceutical Industry Co., Ltd (Tokyo, Japan). MNU was purchased from Toshin Gousei (Tokyo, Japan). DIAION HP20 was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan). Other reagents used were obtained from Wako Pure Chemical Industries (Osaka, Japan).

**Preparation of extracts derived from medicinal and food plants:** *Agaricus blazei* extract, *Carthamus tinctorius* extract, *Cinnamomum verum* aqueous extract, *Ganoderma lucidum* extract, *Glycyrrhiza aspera* extract, *Gardenia jasminoides* aqueous extract, *Zingiber officinale* aqueous extract; ground plants (100 g) were heated with water (1000 mL) at 85–95°C for 1 h, and then the mixture was filtered with suction. The residue was thereafter heated with water (1000 mL) at 85–95°C for 1 h, while the mixture was filtered with suction. The combined filtrates were concentrated under reduced pressure and vacuum dried to constant weight.

*Morus alba* extract and *Stevia rebaudiana* extract; ground plants (100 g) were heated with water (1000 mL) at 85–95°C for 1 h, and the mixture was then filtered with suction. The residue was re-refluxed with 30–70°C ethanol (1000 mL) for 1 h, and then the mixture was filtered with suction. The combined filtrates were concentrated under reduced pressure and vacuum dried to constant weight.

*Cimicifuga racemosa* extract, *Cinnamomum verum* ethanolic extract, *Cucurbita pepo* extract, *Echinacea purpurea* extract, *Eleutherococcus senticosus* extract, *Gynnema sylvestre* extract, *Hibiscus sabdariffa* extract, *Laurus nobilis* ethanolic extract, *Lepidium meyenii* extract, *Panax ginseng* extract, *Passiflora incarnata* extract, *Petroselinum crispum* extract, *Silybum marianum* extract, *Uncaria tomentosa* extract, *Valeriana officinalis* extract, *Zanthoxylum bungeanum* extract and *Zingiber officinale* ethanolic extract; ground plants (100 g) were refluxed with 30–70% ethanolic aqueous solution, (1000 mL) for 1 h, and then the mixture was filtered with suction. The residue was refluxed again with 30–70% ethanolic aqueous solution (1000 mL), and the mixture was filtered with suction. The combined filtrates were concentrated under reduced pressure and vacuum dried to constant weight.

*Glycyrrhiza aspera* ethanolic extract; ground plants (100 g) were refluxed with 95% ethanolic aqueous solution (1000 mL) for 1 h, and then the mixture was filtered with suction. The residue was refluxed again with 95% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was also filtered with suction. The combined filtrates were concentrated under reduced pressure and vacuum dried to constant weight.

*Apocynum venetum* extract (Venetron), *Arachis hypogaea* extract (PEXT), *Camellia sinensis* extract with 90% polyphenol (Teacalon 90S), *Gardenia jasminoides* ethanolic extract, *Ginkgo biloba* extract (Ginkgolon-24), *Glycine max* extract with 30% isoflavone (ISOMAX 30), *Glycine max* extract with 80% isoflavone (ISOMAX 80), *Glycine max* extract with 85% saponin, *Hypericum perforatum* extract, *Vaccinium myrtillus* extract (Bilberon-25) and *Vitis* extract (Vinofelen); ground plants (100 g) were refluxed with 30–70% ethanolic aqueous solution (1000 mL) for 1 h, and then the mixture was filtered with suction. The residue was refluxed again with 30–70% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was again filtered with suction. After the combined filtrates were concentrated under reduced pressure to remove ethanol, the residue was eluted on a DIONION HP20 (100 mL) with 30–80% ethanolic aqueous solution, and the eluent was concentrated under reduced pressure and vacuum dried to constant weight.

*Gardenia jasminoides* aqueous extract; ground plants (100 g) were heated with water (1000 mL) at 50°C for 1 h, and the mixture was then filtered with suction. The residue was heated again with water (1000 mL) at 50°C for 1 h, and the mixture was filtered with suction. β-Glucosidase (5 g) was added to the combined filtrate, and the mixture was heated at 50°C for 40 h, followed by heating 90°C for 10 min, and then the mixture was filtered with suction. The blue filtrate was concentrated under reduced pressure and vacuum dried to constant weight.

ISOMAX AG40; ground plants (100 g) were heated with water (1000 mL) at 70°C for 1 h, and the mixture was then filtered with suction. The residue was heated again with water (1000 mL) at 70°C for 1 h, and the
mixture was filtered with suction. The combined filtrates were eluted on DIAION HP20 (100 mL) with 50–95% ethanolic aqueous solution. After hydrochloric acid (20 wt%, 100 mL) was added to the solution, the mixture was refluxed for 4 h. The mixture was thereafter adjusted to pH 6–7 by addition of 20% NaOH, while the mixture was concentrated to 200 mL under reduced pressure. The formed precipitate was collected by suction filtration and then dried in vacuo to constant weight.

Glycine max extract with isoflavone aglycone; ground plants (100 g) were refluxed with 30–70% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was then filtered with suction. The residue was refluxed again with 30–70% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was filtered with suction. Hydrochloric acid (20 wt%, 100 mL) was added to the combined filtrates, while the mixture was refluxed for 4 h. The eluent was adjusted to pH 6–7 by addition of 20% NaOH, and the mixture was concentrated to 200 mL under reduced pressure, the formed precipitate was collected using suction filtration and then dried in vacuo to constant weight. Table 1 shows the extraction weight ratio, which is presented as a weight of crude plant used for the extraction versus a weight of the obtained extract.

Mutation assay: The antimutagenic effect of each plant extract was assayed according to the Ames method with a plate-incorporation protocol (28,29). Professor B. N. Ames (University of California, Berkeley, USA) kindly provided the S. typhimurium TA1535.

A solution of MNU (1.5 μmol/50 μL DMSO) was put into the test tube, and then added 0.1 M sodium phosphate buffer (pH 7.4, 0.5 mL), a solution with each concentration of extracts (50 μL in DMSO), a culture of the S. typhimurium TA1535 (0.1 mL), and then the solution was thoroughly mixed. Then top agar (2 mL) was added, and the mixture was poured onto a minimal-glucose agar plate. The revertant colonies were counted after incubating at 37°C for 44 h. Each sample was assayed using duplicate plates in three separate experiments. Results are expressed as the mean revertant colonies per plate ± standard error (SE). Plates with neither MNU nor plant extract were considered as negative controls. Although, MNU (1.5 μmol/50 μL) gave 1466 ± 70 colonies. All the tested plates were microscopically examined for thinning or absence of the background lawn and/or presence of micro colonies, which are considered indicators of toxicity induced by the test material. Neither MNU nor all plant extracts displayed toxicity to the S. typhimurium TA1535 under the conditions of the antimutagenicity test.

Mutagenic activity in the presence of extracts is expressed as percentage of mutagenicity (% = Rs/R × 100), where Rs is the number of his+ revertants/plate of plates exposed to MNU and plants extracts, and R is the numbers of his+ revertants/plate of the MNU. The number of spontaneous revertants were subtracted beforehand to give Rs and R. Thus the mutagenicity of the MNU in the absence of plant extracts is defined as 100% of MNU mutagenicity. Inhibitory effect was considered strong when the mutagenic effect was lower than approximately 40% and moderate when the mutagenicity in extracts were in the range of 40–75%. Mutagenic effect remaining 75% was considered weak, and was not recognized as a positive result.

Toxicity assays under the same conditions as used for the Ames test were performed to determine the maximum concentrations of plant extracts that could be added without exerting any toxic effects on the bacteria used in the Ames test. A solution of MNU (1.5 μmol/50 μL DMSO) was put into the test tube, and to the test tube was added 0.1 M sodium phosphate buffer (pH 7.4, 0.5 mL), each concentration of plant extract (50 μL), and a culture of the S. typhimurium TA1535 (0.1 mL). A portion of the mixture was diluted 10^4 times in 1/15 M PB. To a 200 μL of the diluted solution was added in a histidine-free top agar (2 mL), while the solution was poured on the NB agar plate. The colonies were counted after incubating at 37°C for 20 h. Each sample was assayed using duplicate plates in three separate experiments. A substance was considered cytotoxic when the bacterial survival was less than 80% of that observed in the negative control. The results establish the mutation frequency by calculating the number of mutants per 10^7 surviving bacterial cell (28,29).

**Results**

The antimutagenic effect of 43 medicinal and edible plants extracts on MNU was evaluated by means of the Ames assay. Table 1 shows the mutagenicity percentage of the extracts to MNU. The mutagenicity of MNU was markedly reduced by Glycyrrhiza aspera ethanolic extract (5.4%) and ISOMAX AG40 (2.6%), and moderately reduced by Zingiber officinale ethanolic extract (45%).

The revertant colonies decreased in a dose dependent manner by extract concentrations of 0.01–1.0 mg/plate (Fig. 1A). Cytotoxicity of the three extracts were also determined for each antimutagenesis experiment, and no cell toxic effect was observed in the range of concentration tested in the Ames assay (Fig. 1B). To assess the precise antimutagenic potency of the plant extracts, relative mutagenicity was calculated by dividing the number of mutants with the surviving fraction of bacteria (Fig. 1C).

Glycyrrhiza aspera ethanolic extract and ISOMAX AG40 showed a strong inhibitory effect against mutagenicity of MNU at a dosage of 1.0 mg/plate. Zingiber officinale ethanolic extract moderately inhibited muta-
Table 1. Mutagenicity of medicinal herbs and plants against MNU in *S. typhimurium* TA1535

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Original plant (production area)</th>
<th>Part for extraction</th>
<th>Extraction weight ratio (crude plant: extract)</th>
<th>Mutagenicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agaricus blazei</em> extract</td>
<td><em>Agaricus blazei</em> Murill (South America)</td>
<td>Fruiting body</td>
<td>2:1</td>
<td>111</td>
</tr>
<tr>
<td><em>Apoecium venetum</em> extract (Venetro)</td>
<td><em>Apoecium venetum</em> L. (China)</td>
<td>Leaf</td>
<td>25:1</td>
<td>91</td>
</tr>
<tr>
<td><em>Arachis hypogaea</em> extract (PEXT)</td>
<td><em>Arachis hypogaea</em> L. (Japan)</td>
<td>Seed skin</td>
<td>10:1</td>
<td>92</td>
</tr>
<tr>
<td><em>Camelina sinensis</em> extract with 90% polyphenol (Teaclone 90S)</td>
<td><em>Camelina sinensis</em> (China)</td>
<td>Leaf</td>
<td>10:1</td>
<td>76</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em> extract</td>
<td><em>Carthamus tinctorius</em> (China)</td>
<td>Flower</td>
<td>20:1</td>
<td>104</td>
</tr>
<tr>
<td><em>Cimicifuga racemosa</em> extract</td>
<td><em>Cimicifuga racemosa</em> (L.) Nutt. (North America)</td>
<td>Root</td>
<td>10:1</td>
<td>103</td>
</tr>
<tr>
<td><em>Cinnamomum verum</em> aqueous extract</td>
<td><em>Cinnamomum verum</em> (China)</td>
<td>Bark</td>
<td>10:1</td>
<td>96</td>
</tr>
<tr>
<td><em>Cinnamomum verum</em> Ethanolic extract</td>
<td><em>Cinnamomum verum</em> (China)</td>
<td>Bark</td>
<td>20:1</td>
<td>96</td>
</tr>
<tr>
<td><em>Cucurbita pepo</em> extract</td>
<td><em>Cucurbita pepo</em> L. (China)</td>
<td>Seed</td>
<td>20:1</td>
<td>119</td>
</tr>
<tr>
<td><em>Echinacea purpurea</em> extract</td>
<td><em>Echinacea purpurea</em> L. Moench (France)</td>
<td>Aerial part</td>
<td>5:1</td>
<td>101</td>
</tr>
<tr>
<td><em>Eleutherococcus senticosus</em> extract</td>
<td><em>Eleutherococcus senticosus</em> (Russia)</td>
<td>Root</td>
<td>5:1</td>
<td>105</td>
</tr>
<tr>
<td><em>Ganoderma lucidum</em> extract</td>
<td><em>Ganoderma lucidum</em> (Leys. ex. Fr.) Karst (China)</td>
<td>Fruit</td>
<td>15:1</td>
<td>123</td>
</tr>
<tr>
<td><em>Gardenia jasminoides</em> aqueous extract</td>
<td><em>Gardenia jasminoides</em> (China)</td>
<td>Fruit</td>
<td>20:1</td>
<td>102</td>
</tr>
<tr>
<td><em>Gardenia jasminoides</em> Ethanolic extract</td>
<td><em>Gardenia jasminoides</em> (China)</td>
<td>Fruit</td>
<td>20:1</td>
<td>92</td>
</tr>
<tr>
<td><em>Ginkgo biloba</em> extract (Ginkgolon-24)</td>
<td><em>Ginkgo biloba</em> L. (China)</td>
<td>Leaf</td>
<td>50:1</td>
<td>96</td>
</tr>
<tr>
<td><em>Glycine max</em> extract with 30% isoflavone (ISOMAX 30)</td>
<td><em>Glycine max</em> L. Kerr. (Korea)</td>
<td>Germ</td>
<td>20:1</td>
<td>92</td>
</tr>
<tr>
<td><em>Glycine max</em> extract with 80% isoflavone (ISOMAX 80)</td>
<td><em>Glycine max</em> L. Kerr. (Korea)</td>
<td>Germ</td>
<td>100:1</td>
<td>94</td>
</tr>
<tr>
<td><em>Glycine max</em> extract with 85% saponin</td>
<td><em>Glycine max</em> L. Kerr. (Korea)</td>
<td>Germ</td>
<td>20:1</td>
<td>105</td>
</tr>
<tr>
<td><em>Glycine max</em> extract with 40% isoflavone glycone (ISOMAX AG40)</td>
<td><em>Glycine max</em> L. Kerr. (Korea)</td>
<td>Seed</td>
<td>400:1</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Glycine max</em> extract with isoflavone glycone</td>
<td><em>Glycine max</em> L. Kerr. (Korea)</td>
<td>Germ</td>
<td>50:1</td>
<td>97</td>
</tr>
<tr>
<td><em>Glycyrrhiza aspera</em> aqueous extract</td>
<td><em>Glycyrrhiza aspera</em> Pallas (China)</td>
<td>Root</td>
<td>5:1</td>
<td>90</td>
</tr>
<tr>
<td><em>Glycyrrhiza aspera</em> Ethanolic extract</td>
<td><em>Glycyrrhiza aspera</em> Pallas (China)</td>
<td>Root</td>
<td>20:1</td>
<td>5.4</td>
</tr>
<tr>
<td><em>Gymnema sylvestre</em> extract</td>
<td><em>Gymnema sylvestre</em> R. Br. (India)</td>
<td>Leaf</td>
<td>5:1</td>
<td>106</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em> extract</td>
<td><em>Hibiscus sabdariffa</em> L. (Egypt)</td>
<td>Flower</td>
<td>6:1</td>
<td>98</td>
</tr>
<tr>
<td><em>Hypericum perforatum</em> extract</td>
<td><em>Hypericum perforatum</em> L. (Europe)</td>
<td>Aerial part</td>
<td>5:1</td>
<td>101</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> aqueous extract</td>
<td><em>Laurus nobilis</em> L. (Europe)</td>
<td>Leaf</td>
<td>20:1</td>
<td>107</td>
</tr>
<tr>
<td><em>Laurus nobilis</em> Ethanolic extract</td>
<td><em>Laurus nobilis</em> L. (Europe)</td>
<td>Leaf</td>
<td>10:1</td>
<td>100</td>
</tr>
<tr>
<td><em>Lepidium meyenii</em> extract</td>
<td><em>Lepidium meyenii</em> Walp (Peru)</td>
<td>Root</td>
<td>20:1</td>
<td>100</td>
</tr>
<tr>
<td><em>Morinda citrifolia</em> extract</td>
<td><em>Morinda citrifolia</em> (Southeast Asia)</td>
<td>Fruit</td>
<td>5:1</td>
<td>93</td>
</tr>
<tr>
<td><em>Morus alba</em> extract</td>
<td><em>Morus alba</em> L. (China)</td>
<td>Leaf</td>
<td>5:1</td>
<td>99</td>
</tr>
<tr>
<td><em>Panax ginseng</em> extract</td>
<td><em>Panax ginseng</em> C. A. Meyer (China)</td>
<td>Root</td>
<td>3:1</td>
<td>79</td>
</tr>
<tr>
<td><em>Passiflora incarnate</em> extract</td>
<td><em>Passiflora incarnate</em> (North America)</td>
<td>Aerial part</td>
<td>5:1</td>
<td>103</td>
</tr>
<tr>
<td><em>Petroselinum crispum</em> extract</td>
<td><em>Petroselinum crispum</em> (Europe)</td>
<td>Leaf</td>
<td>10:1</td>
<td>115</td>
</tr>
<tr>
<td><em>Salacia reticulata</em> extract</td>
<td><em>Salacia reticulata</em> (Southeast Asia)</td>
<td>Root</td>
<td>20:1</td>
<td>103</td>
</tr>
<tr>
<td><em>Silybum marianum</em> extract</td>
<td><em>Silybum marianum</em> (L.) Gaertn (Europe)</td>
<td>Seed</td>
<td>20:1</td>
<td>100</td>
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<tr>
<td><em>Stevia rebaudiana</em> extract</td>
<td><em>Stevia rebaudiana</em> Bert. (China)</td>
<td>Aerial part</td>
<td>20:1</td>
<td>106</td>
</tr>
<tr>
<td><em>Uncaria tomentosa</em> extract</td>
<td><em>Uncaria tomentosa</em> (Wild.) DC. (Peru)</td>
<td>Bark</td>
<td>7:1</td>
<td>116</td>
</tr>
<tr>
<td><em>Vaccinium myrtillus</em> extract (Bilberon-25)</td>
<td><em>Vaccinium myrtillus</em> L. (North Europe)</td>
<td>Fruit</td>
<td>100:1</td>
<td>98</td>
</tr>
<tr>
<td><em>Valeriana officinalis</em> extract</td>
<td><em>Valeriana officinalis</em> (Europe)</td>
<td>Root</td>
<td>5:1</td>
<td>91</td>
</tr>
<tr>
<td><em>Vitis</em> extract (Vinofelon)</td>
<td><em>Vitis</em> spp. (China)</td>
<td>Seed</td>
<td>100:1</td>
<td>97</td>
</tr>
<tr>
<td><em>Zanthoxylium bungeanum</em> extract</td>
<td>*Zanthoxylium bungeanum Maxim. (China)</td>
<td>Fruit skin</td>
<td>25:1</td>
<td>91</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> aqueous extract</td>
<td>*Zingiber officinale Rosc. (China)</td>
<td>Rhizome</td>
<td>10:1</td>
<td>96</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> Ethanolic extract</td>
<td>*Zingiber officinale Rosc. (China)</td>
<td>Rhizome</td>
<td>20:1</td>
<td>45</td>
</tr>
</tbody>
</table>

*a* The extraction weight ratio is presented as a weight of crude plant used for the extraction versus a weight of the obtained extract.

*b* The percentages show the mutagenicity of MNU at the presence of 1.0 mg extract/plate.

dicogenicity of MNU in a range of concentration 0.01–1.0 mg/plate. The antimutagenic activity of *Zingiber officinale* Ethanolic extract did not reach a maximum at 1.0 mg/plate. However, a higher concentration of *Zingiber officinale* Ethanolic extract was not used for this preliminary first screening.

**Discussion**

Many fruits, vegetables and plant components, as well as medicinal plants have been reported to inhibit
chemically induced mutagenicity in the Ames assay (30–39). Many studies have employed indirect acting mutagens (30–35) and direct acting mutagens; MNNG or EMS (35,38,39), while MNU was less studied (38,39). In the present study, MNU was used to evaluate the antimutagenicity of extracts from medicinal and edible plants in the absence of metabolic activation system. An extract of medicinal and edible plant was obtained under different conditions for each plant to obtain the best yield for a biological effect. Among 43 extracts from medicinal and edible plants assayed, Glycyrrhiza aspera ethanolic extract, ISOMAX AG40 and Zingiber officinale ethanolic extract reduced revertants colonies induced by MNU in S. typhimurium TA1535 without cytotoxicity. Thus, the three extracts were found to possess antimutagenic activity against MNU.

The roots of Glycyrrhiza species have long been used worldwide as a herbal medicine and natural sweetener (40,41). The genus Glycyrrhiza (Leguminosae) consists about 30 species including Glycyrrhiza glabra, Glycyrrhiza uralensis, Glycyrrhiza inflata, Glycyrrhiza aspera, etc. Glycyrrhiza glabra and Glycyrrhiza uralensis have been used as a Chinese herbal medicine in Japan, and have also been studied on the biological activity and their components. The antimutagenic activity extracted from Glycyrrhiza glabra has been evaluated in several investigations (33,34,42–45). In antimutagenic studies toward MNU, an aqueous extract from Glycyrrhiza glabra is reported to reduce the frequency of chromosome aberrations in cells of bulb onion (45). On this note, the present data showed that Glycyrrhiza aspera ethanolic extract inhibited MNU-induced mutagenicity in S. typhimurium TA1535.

Glycine max is a Liguminosae family as well as the genus glycyrrhiza (46,47). Their components are also similar with the genus glycyrrhiza. Many flavonoids and isoflavones have already been identified from genus glycyrrhiza (40,41,48–52) or Glycine max. Among the components, glabrene was reported to be attributable to antimutagenic using Glycyrrhiza extract against EMS (53), and genistein showed antimutagenicity on MNU only at its high concentration (43% inhibition using 300 µg/plate of genistein toward 10 µg/plate of MNU) (54). In this study, antimutagenicity of Glycyrrhiza aspera ethanolic extract or ISOMAX AG40 against MNU may be involved in isoflavones such as glabrene and genistein.

Furthermore, Zingiber officinale was used in traditional medicine for common cold, digestive disorders, and rheumatism (55). While ginger essential oil was assayed for antimutagenic potential against direct acting mutagens such as MNNG and sodium azide, etc (56). In addition, components of Zingiber officinale include zingiberene, 6-, 8- and 10-gingerol, 6-, 8- and 10-shogaol and 6-paradol, etc (57,58). 6-Gingerol and 6-shogaol have been reported to be mutagenic in the presence of S9 mix, whereas zingerone suppresses mutagenic activity in 6-gingerol and 6-shogaol (59,60). Phenolic compounds are well known for their antimutagenicity (61), thus the phenolic compounds in Zingiber officinale could be involved in the antimutagenicity against MNU.

N-Nitrosamine formation is known to be inhibited by ascorbic acid, thiols, metals, phenolic compounds, etc. in vitro, and consequently decreases the mutagenicity induced by N-nitrosamines (38,62,63). There are several ways in which mutagenesis by direct acting mutagens can be reduced or prevented; MNU is decomposed to non-mutagenic products, or the decomposition of MNU to reactive electrophile is accelerated while the electrophile reacts to other nucleophile before reaching DNA extra- or intra cellullarly (38). Another possibility is to induce the repair enzyme in Salmonella strain (37). Glycyrrhiza Root ethanolic extract has been suggested not just to be desmutagenic in their antimutagenicity on EMS (44). Although ginger essential oil inhibits microsomal cytochrome P450 enzyme and increases the level of phase II enzymes for indirect acting mutagens (56), the effectiveness against direct acting MNU indicates not to relate with the metabolizing enzymes. Apparently, some flavonoids have shown antimutagenicity against direct acting mutagens (64,65), however the antimutagenic mechanism is still unclear as well as Glycyrrhiza Root ethanolic extract and Zingiber officinale ethanolic extract. Indeed, the inhibitory effect towards the mutagenicity of direct acting mutagens is probably caused by a chemical reaction between the mutagens and the inhibitor, which prevents the reactive mutagenic products from reaching the DNA.

In the present study, aqueous extracts derived from Glycyrrhiza aspera and Zingiber officinale did not inhibit MNU-induced mutagenicity. Glycine max extract with 30% isoflavone (ISOMAX 30) and Glycine max ex-
tract with 80% isoflavone (ISOMAX 80) did not show antimutagenicity. ISOMAX AG40, which contains a high amount of aglycone among the soybean extracts used in this study, inhibited MNU-induced mutagenicity. These data indicated that the antimutagenic effect against the N-nitrosamides can be attributed to ethanol-soluble constituents. Moreover, our data agreed with the data by Ikken, et al. where the inhibitory activities of Glycyrrhiza glabra ethanolic extract and aglycons against direct acting mutagens are more effective than those of Glycyrrhiza glabra aqueous extract (32,33) and the corresponding glycoside (66). The glycosides can be difficult to pass through the bacterial membrane due to the higher water-solubility, then their inhibitory effect on the aqueous extract was lower than that of ethanolic extract. As the glycosides are hydrolyzed to the corresponding aglycons in vivo (67), the glycosides can inhibit MNU-induced mutagenicity after the absorption.

Conclusion
Among 43 extracts from medicinal and food plants assayed Glycyrrhiza aspera ethanolic extract, Glycine max extract with 40% isoflavone aglycone (ISOMAX AG40) and Zingiber officinale ethanolic extract were found to inhibit the mutagenicity of MNU in S. typhimurium TA1535. Certain constituents may be useful for chemoprevention against genotoxic environment agents. Further investigation is required to identify the antimutagenic compounds from the extracts and understand the antimutagenic mechanism by which they may provide protection against mutagenic N-nitrosamides.

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