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*Full Length Research Paper*

# Antimutagenic and co-mutagenic activities of some legume seeds and their seed coats

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**This study aimed to determine the phenolic content and antimutagenicity of some legume seeds and seed coats (black bean, mung bean, peanut, red kidney bean and soybean). The raw red kidney bean exhibited the highest phenolic content (103.2 ± 6.2 mg gallic acid equivalents (GAE)/g extract). The seed coats of black bean, peanut and red kidney bean showed high levels of phenolic content (> 200 mg GAE/g extract). This study also determined the antimutagenicity of legume seeds and seed coats against urethane induced somatic mutation and recombination in *Drosophila melanogaster*. As a result, legume seeds of red kidney bean showed the highest antimutagenicity (57.2%), followed by peanut (54.0%). Seed coats extracts at the lowest concentration exhibited weak antimutagenic activity (6.2 to 38.8%). The presence of phenolics in the extracts may be responsible for antimutagenicity against urethane. They may induce phase II detoxification enzymes, such as glutathione transferase. Moreover, they may also inhibit specific cytochrome P450s, which in turn leads to protection against mutagenesis by decreasing the metabolic activation of urethane. However, at the highest concentration, all seed coats extracts exhibited a synergistic effect on the mutagenicity of urethane. The finding from this study suggested that the antimutagenic/co-mutagenic activity depends upon the levels of phenolics.**

**Key words:** Legumes, phenolics, antimutagenicity, somatic mutation and recombination test (SMART), co-mutagenicity.

## INTRODUCTION

Common legume seeds, such as black bean, red kidney bean, mung bean, peanut and soybean have been used as foods and beverages. Several studies indicated that high consumption of legumes is associated with a decreased risk of various types of cancer, such as stomach, pancreas, colon, rectum and breast cancer (Messina et al., 1999). The protective effects of dry legumes observed in cancer may be due to phenolic components, other non-nutritive compounds and fiber (Oomah et al., 2006). Phenolics have been reported to possess antimutagenic activity against aflatoxin $B_1$ in the *Salmonella* assay (Aparicio et al., 2005). Moreover, phenolic compounds also have other molecular consequences, such as inhibitory effects on metabolic activation of carcinogens including alteration of the intercellular redox potential (Halliwell et al., 1995). However, plant phenolics have sometimes been reported to show pro-oxidant properties (Laughton et al., 1989).

Urethane is produced in fermented food products (bread, yogurt and cheese) and alcoholic beverages (white wine and beer) (IARC, 1974; Ough, 1976; Miller and Miller, 1983; Canas et al., 1989). This compound is used as an industrial chemical (Crout, 1976). It was found to induce point mutations, gene conversion, intra-chromosomal recombination, chromosomal aberrations and sister chromatid exchanges in yeast, plant systems and mammalian cells (Schlatter and Luitz, 1990). Furthermore, it was shown to induce genotoxicity in *Drosophila melanogaster* (Zimmerli and Schlatter, 1991). Therefore, urethane is used as a positive mutagen in evaluation mutagenicity and antimutagenicity in the somatic mutation and recombination test (SMART).

SMART in *D. melanogaster* has been designed to detect genetic damage in a rapid and inexpensive way. It is an *in vivo* system that uses a eukaryotic organism with

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metabolic machinery similar to that found in mammalian cells (Vogel and Zijlstra, 1987). This assay is based on the treatment of larvae during embryogenesis, the imaginal disc cells proliferate mitotically and many genetic events, such as point mutation, deletion, somatic recombination and non-disjunction can be determined on the wing of adult flies (Würgler and Vogel, 1986). If a genetic alteration occurs in one cell of the imaginal disc during mitotic proliferation, it will form a clone of mutant cells expressing the phenotype regulated by the specific genetic markers.

Several of the seed coats are low economic value by-products of the legume industry. However, the beneficial-health effect of these by-products can be attributed to micronutrients. Therefore, this study aimed to determine the phenolic content and antimutagenicity of some legume seeds and seed coats (black bean, mung bean, peanut, red kidney bean and soybean).

## MATERIALS AND METHODS

### Chemicals

Gallic acid and Folin-Ciocalteu reagent were purchased from Fluka Chemika (Buchs, Switzerland). Sodium carbonate anhydrous was purchased from Riedel-De Haen AG (Seelze, West Germany). Urethane was purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were of laboratory grade.

### Preparation of sample extracts

Seeds from black bean [*Bruguiera parviflora* (Roxb.) Wight & Arn. ex Griffith], red kidney bean (*Phaseolus vulgaris* L.), mung bean [*Vigna radiata* (L.) Wilzcek], peanut (*Arachis hypogaea* L.) and soybean [*Glycine max* (L.) Merr.] were divided into 2 portions. The first portion was used intact, whereas the second portion provided the seed coats. The legume seeds and seed coats were each divided into 2 groups; the first group was used as raw samples whereas the second group was processed by autoclaving at 121℃ for 20 min, and then dried at 60℃ for 24 h (processe d samples). All samples were ground to fine powder using a blender.

For extraction, 20 g of the legume seed powder (raw or processed samples) was extracted with 200 ml of 70% acetone for 24 h at room temperature, and then, was filtered. For seed coats, 2 g of the seed coat powder (raw or processed seed coats) were extracted with 40 ml of 70% acetone for 24 h and then filtered. The filtered extracts were concentrated in a vacuum evaporator at 40℃. The concentrated extracts were kept at -20℃ until use .

### Determination of total phenolic content

Total phenolic content of each extract was determined according to the method described by Amarowicz et al. (2004). Briefly, 10 µl of each extract was transferred into a 96-well microplate containing 160 µl of distilled water. After mixing, 10 µl of Folin–Ciocalteu reagent and 20 µl of a saturated sodium carbonate solution were added. The solution was mixed well and the absorbance was measured at 750 nm after 30 min incubation using a microplate reader (Sunrise, Tecan Co., Austria). The total phenolic content was calculated from a calibration curve of gallic acid solutions (ranging from 25 to 800 mg/L), and were expressed as milligrams of gallic acid equivalents (GAE) per gram of the extract. All measurements were done in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical significance of the difference of phenolic content between raw and processed samples was analyzed using student's *t*-test. Differences were considered as a significant value at $P< 0.05$.

### Evaluation of the mutagenic/antimutagenic activity (SMART)

Trans-heterozygous *Drosophila* larvae (72 h) for the two recessive wing cell markers, multiple wing hair (mwh) males and virgin *ORR; flr*$^3$ females, were transferred to a test tube (100 larvae/tube) containing each extract mixed with regular medium (mutagenic test) or regular medium containing 20 mM urethane (antimutagenic test) until they became adult flies. The wings were examined under a compound microscope at 400× magnification for the presence of mutant spots. Different types of spots, namely, single spots found either on the mwh or the *flr*$^3$ phenotypes (small single spots of 1 or 2 cells in size, large single spots of 3 or more cells), and twin spots found on adjacent mwh and *flr*$^3$ areas, were recorded. The estimation of spot frequencies and confidence limits due to mutation were performed with significance level of $\alpha = \beta = 0.05$ using the statistical procedure described by Frei and Wurgler (1988). The percentage of modification (inhibition or induction) was calculated as (Abraham, 1994):

$$\text{Percentage of modification} = \frac{a - b}{a} \times 100$$

Where *a* is the frequency of spots induced by urethane alone and *b* the frequency of spots induced by urethane in the presence of sample.

It is proposed that 20 to 40% inhibition represented weak antimutagenicity, while 40 to 60% inhibition and >60% inhibition are evidence of moderate and strong antimutagenicity, respectively.

## RESULTS AND DISCUSSION

The total phenolic content in the samples was determined by the Folin–Ciocalteu method. The chemistry behind the Folin–Ciocalteu assay relies on the transfer of electrons in alkaline medium from phenolic compounds (oxidizes phenolates) to molybdenum, forming blue complexes that can be detected spectrophotometrically at 750 nm (Singleton and Rossi, 1965). In this study, raw red kidney bean exhibited the highest phenolic content (103.2 ± 6.2 mg GAE/g extract) (Table 1). In the case of seed coats, the raw seed coats of peanut and red kidney bean displayed high content of total phenolics (331.8 ± 15.6 and 335.9 ± 19.8 mg GAE/g extract, respectively). This study found that the extracts of seed coats contained more phenolic content than those of legume seeds. According to Desphande et al. (1982) and Gonzalez de Mejia et al. (1999), phenolic compounds are mainly located in the seed coats. In the present study, the relationship between seed coats color and phenolic contents was observed. The darker colored seed coats extracts, such as black bean, peanut and red kidney bean, had greater phenolic contents (> 200 mg GAE/g dry extract) than the lighter colored seed coats extracts,

**Table 1.** Effect of heat treatment on total phenolic content of legume seeds and seed coats.

| Legume | Phenolic content (mg GAE/g dry extract) | |
|---|---|---|
| | Raw | Processed |
| **Legume seeds**[1] | | |
| Black bean | 53.4 ± 14.1[a] | 87.6 ± 2.1[b] |
| Mung bean | 87.1 ± 2.1[a] | 65.9 ± 3.3[b] |
| Peanut | 66.2 ± 2.5[a] | 97.1 ± 0.8[b] |
| Red kidney bean | 103.2 ± 6.2[a] | 58.5 ± 5.0[b] |
| Soybean | 28.8 ± 1.4[a] | 35.0 ± 2.1[a] |
| **Seed coats**[2] | | |
| Black bean | 212.9 ± 7.9[a] | 289.0 ± 13.6[b] |
| Mung bean | 128.4 ± 5.9[a] | 158.4 ± 9.8[b] |
| Peanut | 331.8 ± 15.6[a] | 355.0 ± 9.6[a] |
| Red kidney bean | 335.9 ± 19.8[a] | 339.0 ± 15.5[a] |
| Soybean | 32.6 ± 1.2[a] | 41.5 ± 1.0[b] |

Values are presented as mean ± SD of triplicate determinations. Values in the same rows followed by different letters (a, b) are significantly different at $P< 0.05$. [1]Total phenolic contents based a standard curve generated by 25 to 800 mg/L of gallic acid . [2]Total phenolic contents based a standard curve generated by 25 to 800 mg/L of gallic acid.

such as soybean.

After heat treatment, most of the legume seeds, except mung bean and red kidney bean, were shown to have high levels of total phenolic content (Table 1). The processed peanut showed the highest phenolic content (97.1 ± 0.8 mg GAE/g extract), whereas processed soybean showed the lowest phenolic content (35.0 ± 2.1 GAE/g extract). In addition, heat treatment showed an increase in phenolic content of all tested seed coats (Table 1). Previous studies have reported that a cooking process could change the physical characteristics and chemical composition of vegetables, thus the total phenolic content of different kinds of vegetables could be either higher or lower in comparison to the fresh samples. Turkmen et al. (2005) found out that cooking caused loss of phenolics in squash, peas and leek. On the other hand, cooking was found to give rise to an increase in phenolics in green beans, pepper and broccoli. Ismail et al. (2004) reported that thermal treatment on swamp cabbage lost the highest amount of phenolic content (26%), followed by cabbage (20%), spinach (14%), shallots (13%) and kale (12%) after a 1 min blanching in boiling water. In a study carried out, Lombard et al. (2005) indicated that baking increases concentrations of flavonols compared to raw onions.

Heat treatment increased the phenolic content of all tested seed coats. Bernhart and Schlich (2005) previously explained that heat treatment could lead to cellular disruption and disassociation of some phenolic compounds from cellular structures, such as lignin and polysaccharides. This finding indicated that the cooking of legume seeds before consumption or the use of seed coats by-products of the legume industry, which are commonly removed by roasting, could represent an inexpensive source of phenolic compounds (antioxidants).

The legume seeds and seed coats were not mutagenic since they did not significantly induce the frequencies of mutant spots, at any tested concentrations, to be higher than that of the negative control (Tables 2 and 3). Co-administration of the legume seeds extracts with urethane reduced the mutagenic effects of 20 mM urethane (Table 2). The antimutagenicity of legume seeds at the highest concentration displayed moderate activities (44.4 to 57.2%). Red kidney bean exhibited the highest antimutagenicity (57.2%), followed by peanut (54.0%). Soybean possessed the lowest antimutagenicity (44.4%). The result from the antimutagenic test indicated that the legume seeds had protective effects against *in vivo* induction of somatic mutation and mitotic recombination by urethane. As urethane was co-administered with the legume seeds extracts, it therefore possibly forms a complex with constituents of the extracts that may lead to detoxification. In addition, the presence of phenolics in the extracts may be responsible for mutagenicity of urethane. Prochaska and Talahay (1988) reported that polyphenols may induce phase II detoxification enzymes such as glutathione transferase (GST) that enhance the excretion of mutagens. Moreover, polyphenols may also inhibit specific cytochrome P450s (CYPs), which in turn leads to protection against mutagenesis by decreasing the metabolic activation of urethane (Abraham and Graf, 1996).

According to Huang et al. (1983), some polyphenols (tannins and catechins) extracted from legumes could inhibit activities of xenobiotic metabolizing enzymes, like the cytochrome P-450-dependent monooxygenase

**Table 2.** Percentage of modification (inhibition or induction) of the legume seed extracts.

| Treatment | | Survival (%) | Spots per wing (number of spots) statistical analysis[a] | | | | Percentage ihibition or induction (-) |
|---|---|---|---|---|---|---|---|
| Legumes seeds (mg) | Urethane (mM) | | Small single spots (1-2 cells) | Large single spots (>2 cells) | Twin spots | Total spots | |
| 0 | 0 | 98 | 0.075 (3) | 0.025 (1) | 0 | 0.100 (4) | |
| 0 | 20 | 96 | 10.125 (405)$^{+}$ | 6.925 (277)$^{+}$ | 2.525 (101)$^{+}$ | 19.575 (783)$^{+}$ | |
| **Black bean** | | | | | | | |
| 6.25 | 0 | 98 | 0.100 (4)$^{i}$ | 0 | 0 | 0.100 (4)$^{i}$ | - |
| 12.5 | 0 | 94 | 0.100 (4)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.125 (5)$^{i}$ | - |
| 25 | 0 | 89 | 0.175 (7)$^{i}$ | 0 | 0 | 0.175 (7)$^{i}$ | - |
| 6.25 | 20 | 89 | 7.475 (299)$^{+}$ | 3.05 (122)$^{+}$ | 1.6 (64)$^{+}$ | 12.125 (485)$^{+}$ | 38.1 |
| 12.5 | 20 | 85 | 6.25 (250)$^{+}$ | 2.775 (111)$^{+}$ | 1.3 (52)$^{+}$ | 10.325 (413)$^{+}$ | 47.3 |
| 25 | 20 | 84 | 4.525 (181)$^{+}$ | 3.625 (145)$^{+}$ | 1.675 (67)$^{+}$ | 9.825 (393)$^{+}$ | 49.8 |
| **Mung bean** | | | | | | | |
| 6.25 | 0 | 94 | 0.050 (2)$^{i}$ | 0.025 (1)$^{i}$ | 0.025 (1)$^{i}$ | 0.100 (4)$^{i}$ | - |
| 12.5 | 0 | 84 | 0.150 (6)$^{i}$ | 0.025 (1)$^{i}$ | 0.025 (1)$^{i}$ | 0.200 (8)$^{i}$ | - |
| 25 | 0 | 88 | 0.150 (6)$^{i}$ | 0 | 0.025 (1)$^{i}$ | 0.175 (7)$^{i}$ | - |
| 6.25 | 20 | 74 | 8 (320)$^{+}$ | 2.125 (85)$^{+}$ | 1.35 (54)$^{+}$ | 11.475 (459)$^{+}$ | 41.4 |
| 12.5 | 20 | 84 | 5.6 (224)$^{+}$ | 2.725 (109)$^{+}$ | 1.05 (42)$^{+}$ | 9.375 (375)$^{+}$ | 52.1 |
| 25 | 20 | 76 | 5.275 (211)$^{+}$ | 3 (120)$^{+}$ | 1.5 (60)$^{+}$ | 9.775 (391)$^{+}$ | 50.1 |
| **Peanut** | | | | | | | |
| 6.25 | 0 | 98 | 0.075 (3)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.100 (4)$^{i}$ | - |
| 12.5 | 0 | 99 | 0.125 (5)$^{i}$ | 0.075 (3)$^{i}$ | 0 | 0.200 (8)$^{i}$ | - |
| 25 | 0 | 98 | 0.125 (5)$^{i}$ | 0.025 (1)$^{i}$ | 0.050 (2)$^{i}$ | 0.200 (8)$^{i}$ | - |
| 6.25 | 20 | 80 | 6.05 (242)$^{+}$ | 2.75 (110)$^{+}$ | 1.375 (55)$^{+}$ | 10.175 (407)$^{+}$ | 48.0 |
| 12.5 | 20 | 83 | 6.675 (267)$^{+}$ | 2.25 (90)$^{+}$ | 0.85 (34)$^{+}$ | 9.775 (391)$^{+}$ | 50.1 |
| 25 | 20 | 75 | 5.025 (201)$^{+}$ | 2.575 (103)$^{+}$ | 1.4 (56)$^{+}$ | 9 (360)$^{+}$ | 54.0 |
| **Red kidney bean** | | | | | | | |
| 6.25 | 0 | 96 | 0.075 (3)$^{i}$ | 0 | 0 | 0.075 (3)$^{i}$ | - |
| 12.5 | 0 | 89 | 0.075 (3)$^{i}$ | 0 | 0 | 0.075 (3)$^{i}$ | - |
| 25 | 0 | 89 | 0.200 (8)$^{i}$ | 0 | 0.025 (1)$^{i}$ | 0.225 (9)$^{i}$ | - |
| 6.25 | 20 | 81 | 8.175 (327)$^{+}$ | 4.2 (168)$^{+}$ | 1.575 (63)$^{+}$ | 13.95 (558)$^{+}$ | 28.7 |
| 12.5 | 20 | 80 | 4.3 (172)$^{+}$ | 3.625 (145)$^{+}$ | 1.625 (65)$^{+}$ | 9.55 (382)$^{+}$ | 51.2 |
| 25 | 20 | 86 | 4.85 (194)$^{+}$ | 2.175 (87)$^{+}$ | 1.35 (54)$^{+}$ | 8.375 (335)$^{+}$ | 57.2 |
| **Soybean** | | | | | | | |
| 6.25 | 0 | 90 | 0.050 (2)$^{i}$ | 0 | 0.025 (1)$^{i}$ | 0.075 (3)$^{i}$ | - |
| 12.5 | 0 | 99 | 0.050 (2)$^{i}$ | 0.025 (1)$^{i}$ | 0.025 (1)$^{i}$ | 0.100 (4)$^{i}$ | - |
| 25 | 0 | 97 | 0.100 (4)$^{i}$ | 0 | 0.025 (1)$^{i}$ | 0.125 (5)$^{i}$ | - |
| 6.25 | 20 | 81 | 8.95 (358)$^{+}$ | 3.925 (157)$^{+}$ | 2.125 (85)$^{+}$ | 15 (600)$^{+}$ | 23.4 |
| 12.5 | 20 | 85 | 7.25 (290)$^{+}$ | 4.175 (167)$^{+}$ | 1.85 (74)$^{+}$ | 13.275 (531)$^{+}$ | 32.2 |
| 25 | 20 | 78 | 5.8 (232)$^{+}$ | 3.275 (131)$^{+}$ | 1.8 (72)$^{+}$ | 10.875 (435)$^{+}$ | 44.4 |

[a]Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control:+ = positive; - = negative; i = inconclusive; Propability level $\alpha = \beta = 0.05$. One side statistical tests.

system. Furthermore, approximately 0.1% of urethane was reported to be able to convert into hydroxylamine and exert its carcinogenic effect in multiple organs via generation of $O_2^-$ and $NO^{\cdot}$ resulting in oxidation and

**Table 3.** Percentage of modification (inhibition or induction) of the seed coat extracts.

| Treatment | | Survival (%) | Spots per wing (number of spots) statistical analysis[a] | | | | Percentage inhibition or induction (-) |
|---|---|---|---|---|---|---|---|
| Seed coats (mg) | Urethane (mM) | | Small single spots (1-2 cells) | Large single spots (>2 cells) | Twin spots | Total spots | |
| 0 | 0 | 98 | 0.075 (3) | 0.025 (1) | 0 | 0.100 (4) | |
| 0 | 20 | 83 | 6.55 (262)$^{+}$ | 2.275 (91)$^{+}$ | 0.9 (36)$^{+}$ | 9.725 (389)$^{+}$ | |
| **Black bean** | | | | | | | |
| 6.25 | 0 | 85 | 0.100 (4)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.125 (5)$^{i}$ | |
| 12.5 | 0 | 76 | 0.125 (5)$^{i}$ | 0.025 (1)$^{i}$ | 0.025 (1)$^{i}$ | 0.175 (7)$^{i}$ | |
| 25 | 0 | 73 | 0.175 (7)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.200 (8)$^{i}$ | |
| 6.25 | 20 | 87 | 5.05 (202)$^{+}$ | 2.125 (85)$^{+}$ | 0.725 (29)$^{+}$ | 7.9 (316)$^{+}$ | 18.8 |
| 12.5 | 20 | 87 | 5.025 (201)$^{+}$ | 1.55 (62)$^{+}$ | 0.825 (33)$^{+}$ | 7.4 (296)$^{+}$ | 23.9 |
| 25 | 20 | 85 | 7.85 (314)$^{+}$ | 2.6 (104)$^{+}$ | 1.225 (49)$^{+}$ | 11.675 (467)$^{+}$ | -20.1 |
| **Mung bean** | | | | | | | |
| 6.25 | 0 | 90 | 0.075 (3)$^{i}$ | 0 | 0.025 (1)$^{i}$ | 0.100 (4)$^{i}$ | |
| 12.5 | 0 | 96 | 0.125 (5)$^{i}$ | 0 | 0 | 0.200 (8)$^{i}$ | |
| 25 | 0 | 91 | 0.200 (8)$^{i}$ | 0.050 (2)$^{i}$ | 0 | 0.25 (10)$^{i}$ | |
| 6.25 | 20 | 83 | 4.95 (198)$^{+}$ | 2.325 (93)$^{+}$ | 1.15 (46)$^{+}$ | 8.425 (337)$^{+}$ | 13.4 |
| 12.5 | 20 | 81 | 6.475 (259)$^{+}$ | 1.95 (78)$^{+}$ | 0.925 (37)$^{+}$ | 9.35 (374)$^{+}$ | 3.9 |
| 25 | 20 | 89 | 7.35 (294)$^{+}$ | 2.525 (101)$^{+}$ | 1.45 (58)$^{+}$ | 11.325 (453)$^{+}$ | -16.5 |
| **Peanut** | | | | | | | |
| 6.25 | 0 | 97 | 0.050 (2)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.075 (3)$^{i}$ | |
| 12.5 | 0 | 93 | 0.075 (3)$^{i}$ | 0.050 (2)$^{i}$ | 0 | 0.125 (5)$^{i}$ | |
| 25 | 0 | 90 | 0.125 (5)$^{i}$ | 0.050 (2)$^{i}$ | 0 | 0.175 (7)$^{i}$ | |
| 6.25 | 20 | 96 | 5.9 (236)$^{+}$ | 2.025 (81)$^{+}$ | 1.2 (48)$^{+}$ | 9.125 (365)$^{+}$ | 6.2 |
| 12.5 | 20 | 83 | 8.3 (332)$^{+}$ | 2.3 (92)$^{+}$ | 1.3 (52)$^{+}$ | 11.9 (476)$^{+}$ | -22.4 |
| 25 | 20 | 84 | 9.025 (361)$^{+}$ | 2.575 (103)$^{+}$ | 1.25 (50)$^{+}$ | 12.85 (514)$^{+}$ | -32.1 |
| **Red kidney bean** | | | | | | | |
| 6.25 | 0 | 73 | 0.050 (2)$^{i}$ | 0.025 (1)$^{i}$ | 0.025 (1)$^{i}$ | 0.100 (4)$^{i}$ | |
| 12.5 | 0 | 78 | 0.075 (3)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.100 (4)$^{i}$ | |
| 25 | 0 | 87 | 0.125 (5)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.150 (6)$^{i}$ | |
| 6.25 | 20 | 86 | 4.225 (169)$^{+}$ | 1.075 (43)$^{+}$ | 0.65 (26)$^{+}$ | 5.95 (238)$^{+}$ | 38.8 |
| 12.5 | 20 | 87 | 5.775 (231)$^{+}$ | 2.85 (114)$^{+}$ | 1.9 (76)$^{+}$ | 10.525 (421)$^{+}$ | -8.2 |
| 25 | 20 | 72 | 6.475 (259)$^{+}$ | 3.5 (140)$^{+}$ | 2.05 (82)$^{+}$ | 12.025 (481)$^{+}$ | -23.7 |
| **Soybean** | | | | | | | |
| 6.25 | 0 | 91 | 0.075 (3)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.100 (4)$^{i}$ | |
| 12.5 | 0 | 90 | 0.125 (5)$^{i}$ | 0.050 (2)$^{i}$ | 0 | 0.175 (7)$^{i}$ | |
| 25 | 0 | 80 | 0.150 (6)$^{i}$ | 0.025 (1)$^{i}$ | 0 | 0.175 (7)$^{i}$ | |
| 6.25 | 20 | 80 | 3.2 (128)$^{+}$ | 1.95 (78)$^{+}$ | 0.95 (38)$^{+}$ | 6.1 (244)$^{+}$ | 37.3 |
| 12.5 | 20 | 69 | 5.45 (218)$^{+}$ | 2.7 (108)$^{+}$ | 2.425 (97)$^{+}$ | 10.575 (423)$^{+}$ | -8.7 |
| 25 | 20 | 63 | 5.6 (224)$^{+}$ | 3.975 (159)$^{+}$ | 1.95 (78)$^{+}$ | 11.525 (461)$^{+}$ | -18.5 |

[a]Statistical diagnoses using estimation of spot frequencies and confidence limits according to Frei and Würgler (1988) for comparison with negative control: + = positive; - = negative; i= inconclusive; Propability level $\alpha = \beta = 0.05$. One side statistical tests.

depurination of DNA (Sakano et al., 2002). Thus, it is possible that phenolics in legume seeds extracts might scavenge $O_2^-$ and $NO^{\cdot}$ in urethane metabolism.

In the case of seed coats, the lowest concentration (6.25 mg) exhibited weak antimutagenicity (6.2 to 38.8%). Red kidney bean showed the highest antimutagenicity

(38.8%), followed by soybean (37.3%). At the highest concentration (25 mg), the extracts of all seed coats exhibited synergistic effect on the mutagenicity of urethane (Table 3). It is quite possible that the seed coats extracts which have an abundance of phenolic compounds (flavonoids) exhibit prooxidant activity. The phenolic compounds can both behave as antioxidants and prooxidants depending on their concentration. Several classes of plant derived polyphenols exhibit oxidative DNA damage particularly in the presence of transition metal ions. Ahmad et al. (2005) found out that DNA damage by resveratrol–Cu(II) occurs by both Haber Weiss reaction ($O2^{\bullet -}$ + $H_2O_2$ = $O_2$ + $OH^{\bullet}$ +$OH^-$) and Fenton reaction ($H_2O_2$ + Cu (I) = $^{\bullet}OH$ + $OH^-$ + Cu (II)). $H_2O_2$ can be generated from oxygen by polyphenolics. Thus, $H_2O_2$ can take part in both Haber Weiss and Fenton-type OH• formation and DNA cleavage reactions. Furthermore, Cu(II) can be reduced to Cu(I) by resveratrol and it is the re-oxidation of Cu(I) to Cu(II) which gives rise to OH• (Rahman et al., 1990). Moreover, a number of polyphenols, including quercetin, can bind to DNA (Alvi et al., 1986) and this direct interaction may be an important mechanism of mutagenicity.

The results suggested that the anti-mutagenic/co-mutagenic activity largely depends upon the amount of phenolics. The extracts of legume seeds contained lower content of phenolics than the extracts of seed coats, which showed appropriate phenolics content for inhibiting mutagenicity of urethane in the SMART assay. The results indicated that intake of legume-derived phenolics and other phytochemicals in our daily foods may protect against mutagenicity of urethane.

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