Antioxidant and antimicrobial activities of extract and essential oil of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen)

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The antioxidant potential of extracts and essential oils of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) were investigated, as well as total phenolics, total ascorbic acid (TAA), condensed tannins (CT) and antifungal potential. Antioxidant capacities of seeds and pods as assayed in methanol extracts, and of essential oils were at moderate levels. The antioxidant activity of seeds (29.59 µmol/g dry weight (DW)) was significantly higher than that of pods (14.23 µmol/g DW) by FRAP (Ferric Reducing Ability of Plasma) assay. Also the antiradical power of seeds (9.26 µmol/g DW) was higher than of pods (3.08 µmol/g DW) in the DPPH (1,1-diphenyl-2-picrylhydrazyl) test. Similarly, the antioxidant activity of seeds was higher than of pods in the β-carotene bleaching test. A lower 50% effective concentration (EC₅₀) was achieved with seeds (42.10 mg/mL) as compared to pods (120.50 mg/mL), while EC₅₀ of pod oils (11.9 mg/mL) was lower than seed oils (59.2 mg/mL). The total phenolic content in seeds (3.98 mg/g DW) was significantly higher than in pods (1.32 mg/g DW), while TAA content was significantly lower in seeds (3.49 mg/100 g DW) than in pods (6.25 mg/100 g DW). Differences in CT were non-significant. Antifungal tests exhibited that the extracts from seed were more effective than the control. Based on the present study the consumption of korarima as a spice may be used as source of antioxidants.

Key words: Zingiberaceae, *Aframomum corrorima*, korarima, extract, essential oil, antioxidant, antiradical and antifungal.

INTRODUCTION

The consumption of traditional diets prepared with spices, medicinal and aromatic herbs have gained increasing interest among consumers and the scientific community because they contain chemical compounds exhibiting antioxidant properties (Madsen and Bertelsen, 1995). These properties are attributed to a variety of active phytochemicals including phenolics, vitamins, carotenoids and terpenoids (Liu and Ng, 2000), compounds that are considered to have the ability to reduce oxidative damage associated with diseases like cancer, cardiovascular diseases, atherosclerosis, diabetes, asthma, hepatitis, liver injury, arthritis and ageing (Harman, 1995; Lee et al., 2000; Middleton et al., 2000). The ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been reported (Rice-Evans et al., 1996; Espin et al., 2000; Liu et al., 2003). As a potent antioxidant, ascorbic acid has the capacity to eliminate several different reactive oxygen species (Arrigoni and De Tullio, 2002). Tannins have been reported to have strong antioxidant activity (Cai et al., 2006). There is also growing interest, both in industry and in scientific research, for spices and medicinal herbs because of their antimicrobial activities (Soler-Rivas et al., 2000).
Korarima (Aframomum corrorima (Braun) P.C.M. Jansen) seeds, pods, leaves, rhizomes and flowers are used in traditional medicine and as spices in southern Ethiopia (Eyob et al., 2008). Despite the widespread uses of korarima, there is to our knowledge no literature containing reports on antioxidant and antimicrobial activities of korarima. There are, however, some reports on antimicrobial activities from related genus, Aframomum giganteum (Huguette et al., 2004), Aframomum melengueta (Adegoke et al., 2003) and Aframomum danielli (Adegoke and Gopalakrishna, 1998).

The objectives of the present investigations were to examine antioxidant activity of korarima seeds, pods and essential oils, as well as antifungal activity of extracts of seeds, pods, leaves and rhizomes of korarima.

MATERIALS AND METHODS

Plant material

Seeds, pods, leaves and rhizomes of korarima were collected from Chenchha highland of southern Ethiopia. They were dried in the shade at room temperature and stored at 4 - 5°C before being chopped in a food processor and ground in a mortar. All analyses were performed on a dry weight (DW) basis.

Isolation of oil

Fresh seed and pod korarima samples (100 g) were hydrodistilled in clevenger-type apparatus (Eyob et al., 2007). The oil was collected and dried over anhydrous sodium sulphate. The essential oil samples were stored in the dark at -20°C.

FRAP and DPPH assay

For preparing extracts, dried and ground korarima samples (0.2 g) were weighed into test tubes. 10 mL 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonicated for 15 min on a water bath at 0°C and centrifuged at 12,500 x g for 2 min at 4°C. The supernatants were used for analysis on an automatic analyzer (Konelab 30i). Sample (20 µL) was mixed with 100 µL Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and incubated at 37°C (Volden et al., 2008). Absorption measurements at 765 nm were read after 15 min. Gallic acid was used as standard and results were calculated as gallic acid equivalent (µg GAE/g DW).

Total ascorbic acid (TAA)

The spectrophotometric method developed by Roe and Kuheter (1943) for estimation of ascorbic acid content of biological fluids was used for determination of TAA. In brief, 100 g dried samples were homogenized in a blender, mixed with 100 mL of 6% trichloroacetic acid (TCA) solution for 2 min, filtered using Whatman No.12 filter paper and centrifuged for 5 min at 1000 x g. From each test tube, 4 mL sample was added to 1 mL of 2,4-dinitrophenylhydrazine (2,4-DNPH) reagents. The samples were kept for 30 min at room temperature before reading the absorbance. The absorbance of samples and ascorbic acid standards were measured at 515 nm against distilled water as blank control. TAA was calculated as mg/100 g DW.
CT concentrations were determined by a modified version of the Condensed tannins (CT) method developed by Maxson and Rooney (1972). Dried, ground-pod and seed samples (100 mg) of korarima were extracted in 10 mL of 1% HCl in methanol for 24 h at room temperature using mechanical shaking. The mixture was centrifuged for 5 min at 1000 × g. 1 mL supernatant was mixed with 5 mL vanillin HCl reagent (8% concentrated HCl in methanol and 4% vanillin in methanol). The absorbance at 500 nm was read after 20 min. Catechin was used as the standard. Results were calculated as mg/g DW.

Crude extract percent recovery

Extraction yield of methanolic crude extracts were determined from obtained after removal of methanol using a rotary evaporator. The mass of the material prior to extraction and the mass of extract extraction yield was calculated as % after weighing.

β-Carotene bleaching test

The β-carotene bleaching method (Jayaprakash et al., 2001; Shahidi et al., 2001; Kaur and Kapoor, 2002) was used to determine the antioxidant activity. The β-carotene solution was prepared by dissolving 2 mg β-carotene in 10 mL chloroform. 1 mL β-carotene-chloroform solution was pipetted into a round-bottom rotary boiling flask containing 20 mg linoleic acid and 0.2 g Tween 40. Chloroform was removed by a rotary evaporator at 45°C for 5 min. Distilled water (50 mL) was slowly added with vigorous agitation to form an emulsion. Emulsion aliquots (5 mL) were transferred into tubes containing 0.2 mL of sample extracts. Control samples were prepared with 0.2 mL methanol instead of extracts. As soon as the emulsion was added to each tube, zero time absorbance was read at 470 nm against blank. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored by subsequent reading of absorbance at 15 min intervals until the color of the β-carotene in the control sample had disappeared (105 min). Butylated hydroxyl tolueine (BHT, 50 mg/L) was used as synthetic reference. Analyses were performed in duplicate. Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

\[ AA = \frac{1 - (A_t - A_s)}{(A'_{t} - A'_{s})} \times 100 \]

where \( A_t \) = measured absorbance value of control after incubation (105 min) at 50°C; \( A'_{t} \) = measured absorbance value of control at zero time; \( A'_{s} \) = measured absorbance value of sample at zero time; \( A_t \) = measured absorbance value of sample after incubation (105 min) at 50°C; \( A'_{s} \) = measured absorbance value of sample at zero time.

Extraction preparation and antifungal bioassay

Fungi were grown at 28°C and maintained on potato dextrose agar (PDA). Fungi were grown at 28°C and maintained on potato dextrose medium. Inoculum of each strain was prepared from an overnight culture. Fungal cell suspension (100 µL) was spread onto agar plates with a bent glass rod and 6 mm paper discs impregnated with extract were applied. The inhibition zones around the discs were measured after 72 h incubation. For negative controls, discs soaked in distilled water were used.

Statistical analysis

The results were statistically evaluated by one way analysis of variance (ANOVA) using SAS Version 9.1. The significance of the differences between means was determined by Tukey’s test (\( P < 0.05 \)).

RESULTS AND DISCUSSION

FRAP and DPPH assay of seeds and pods

The results of the antioxidant capacity of seeds and pods as determined by FRAP and DPPH assays were shown in Table 1. The antioxidant activities varied significantly (p<0.05) between seeds and pods by both the FRAP and DPPH procedures. The seeds showed significantly higher antioxidant activity (29.59 µmol/g DW) than the pods (14.23 µmol/g DW) by FRAP assay. Similarly, the antraradical power of seeds (9.26 µmol/g DW) was significantly higher than of pods (3.08 µmol/g DW) in the DPPH test. The seeds showed nearly 2-fold of antioxidant potential.

Table 1. FRAP and DPPH assay, total phenolics, TAA and CT content of korarima seeds and pods and % yield recovery of seed and pod methanol extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>FRAP (µmol/g DW)</th>
<th>DPPH (µmol/g DW)</th>
<th>Total phenolic Concentration (mg/g DW)</th>
<th>TAA (mg/100 g DW)</th>
<th>CT (mg/g DW)</th>
<th>Yield recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td>29.59 ± 1.84a</td>
<td>9.26 ± 0.10a</td>
<td>3.98 ± 0.27a</td>
<td>3.49±0.21b</td>
<td>1.18±0.10</td>
<td>4.13 ± 0.21 a</td>
</tr>
<tr>
<td>Pods</td>
<td>14.23 ± 0.53b</td>
<td>3.08 ± 0.06b</td>
<td>1.32 ± 0.07b</td>
<td>6.25±0.11a</td>
<td>1.31±0.09</td>
<td>2.23 ± 0.15 b</td>
</tr>
<tr>
<td>SE</td>
<td>1.35</td>
<td>0.08</td>
<td>0.19</td>
<td>0.17</td>
<td>NS</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

Means followed by different letters are significantly different (\( P < 0.05 \)).

SE = Standard error; NS = not significant.

### Condensed tannins (CT)

CT concentrations were determined by a modified version of the method developed by Maxson and Rooney (1972). Dried, ground-pod and seed samples (100 mg) of korarima were extracted in 10 mL of 1% HCl in methanol for 24 h at room temperature using mechanical shaking. The mixture was centrifuged for 5 min at 1000 x g. 1 mL supernatant was mixed with 5 mL vanillin HCl reagent (8% concentrated HCl in methanol and 4% vanillin in methanol). The absorbance at 500 nm was read after 20 min. Catechin was used as the standard. Results were calculated as mg/g DW.

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Extraction yield of methanolic crude extracts were determined from the mass of the material prior to extraction and the mass of extract obtained after removal of methanol using a rotary evaporator. The extraction yield was calculated as % after weighing.

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\[ AA = \frac{1 - (A_t - A_s)}{(A'_{t} - A'_{s})} \times 100 \]

where \( A_t \) = measured absorbance value of sample at zero time; \( A_t \) = measured absorbance value of sample after incubation (105 min) at 50°C; \( A'_{t} \) = measured absorbance value of sample at zero time; \( A'_{s} \) = measured absorbance value of sample at zero time.

### Extract preparation and antifungal bioassay

Seeds, pods, leaves and rhizomes of the korarima plant were well dried and then ground to powder. From each plant part about 100 g powder were separately soaked in 250 mL of 85% methanol in a bottle, vigorously shaken and filtered using Whatman No.12 filter paper. The methanol was removed in a rotary evaporator. Each crude extract was dissolved in methanol to give concentrations of 0.1, 0.2 and 0.4 mg/mL.

The bioassay was carried out using an agar diffusion method (Bauer et al., 1966). Potato dextrose agar (PDA) was used to culture the laboratory isolates of Aspergillus flavus and Penicillium expansum. Fungi were grown at 28°C and maintained on potato dextrose medium. Inoculum of each strain was prepared from an overnight culture. Fungal cell suspension (100 µL) was spread onto agar plates with a bent glass rod and 6 mm paper discs impregnated with extract were applied. The inhibition zones around the discs were measured after 72 h incubation. For negative controls, discs soaked in distilled water were used.

### Statistical analysis

The results were statistically evaluated by one way analysis of variance (ANOVA) using SAS Version 9.1. The significance of the differences between means was determined by Tukey’s test (\( P < 0.05 \)).

### RESULTS AND DISCUSSION

FRAP and DPPH assay of seeds and pods

The results of the antioxidant capacity of seeds and pods as determined by FRAP and DPPH assays were shown in Table 1. The antioxidant activities varied significantly (p<0.05) between seeds and pods by both the FRAP and DPPH procedures. The seeds showed significantly higher antioxidant activity (29.59 µmol/g DW) than the pods (14.23 µmol/g DW) by FRAP assay. Similarly, the antraradical power of seeds (9.26 µmol/g DW) was significantly higher than of pods (3.08 µmol/g DW) in the DPPH test. The seeds showed nearly 2-fold of antioxidant potential.
in FRAP assay and 3-fold in antioxidant potential in DPPH assay as compared to pods. These differences could be explained by different mechanisms of the analytical methods. Wangcharoen and Morasuk (2007) reported that some Thai culinary plants showed higher FRAP antioxidant activity than DPPH antioxidant potential, which is in agreement with our present findings. Because of the ease and convenience of reaction, DPPH now has widespread use in the free radical scavenging activity assessment (Brand-Williams et al., 1995). The effective concentration (EC$_{50}$) of the seed extract demonstrated a higher antioxidant activity than the pod extract. From EC$_{50}$ values it can be seen that the more efficient the antioxidant, the smaller the EC$_{50}$ value will be: EC$_{50}$ (pod) = 120.50 mg/mL; EC$_{50}$ (seed) = 42.10 mg/mL and EC$_{50}$ (Trolox) = 0.092 mg/mL. On average, seed extracts showed remarkable reduction in % remaining DPPH (31.5%) compared to pod extracts (47.0%) for concentrations of 62.5 mg/mL (seed) and 125 mg/mL (pod) when recorded after 120 min (Table 2), implying that seed extracts had stronger antioxidant property than pod extracts. The % remaining DPPH (32.0%) obtained for Trolox at very low concentration (0.125 mg/mL) was found to be corresponding to seed extract at 62.5 mg/mL. Thus, Trolox was more effective in scavenging DPPH compared to both seed and pod extracts. It is clear from Table 2 that the higher the percent remaining DPPH, the lower is the radical-scavenging activity of tested samples. The variability between antioxidant potentials of seeds and pods can be explained by the different degree of biosynthesis and accumulation of photochemical in different plant tissues, which could affect the level of antioxidant present in different plant parts.

### Total phenolic content of seeds and pods

Total content of phenolics in seed and pod samples of korarima obtained from extracts were shown in Table 1. The total phenolics were significantly higher (3.98 mg/g DW) in seeds than in pods (1.32 mg/g DW). Antioxidant activity and total phenolics in both plant parts exhibited a positive relationship, indicating that the phenolics are the main contributors to antioxidant activity. This is in agreement with the findings of Feryal et al. (2005) from different fruits and vegetables, and to findings of Pourmorad et al. (2006) studying Iranian medicinal plant species. A positive relationship between antioxidant activity and amount of total phenolic compounds was also reported for extracts of *A. indica* and *A. tagala* (Thirugnanasampandan et al., 2008). Phenolic compounds are known as high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals (Hall and Cuppett, 1997).

### Total ascorbic acid of seeds and pods

TAA content was significantly higher in pods (6.25 mg/100 g DW) than in seeds (3.49 mg/100 g DW) as shown Table 1. The mean TAA content in the pods of korarima was 48% higher than in the seeds. This may be due to exposure of pods to light during the growth period as light has been reported to favour the accumulation of ascorbic acid (Dumas et al., 2003). In our experiments, the level of ascorbic acid was within the range of published data of common fresh fruit ranging from 0.5 to 226.8 mg/100 g found in different laboratories of different countries (Mélo et al., 2006; UHIS, 2005). A number of factors could have contributed to the relatively low accumulation of ascorbic acid in the korarima pods and seeds. Among these factors the cloudy weather and low light intensity year round in the production area may have contributed to the low content of ascorbic acid. Dried

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**Table 2.** Percent remaining DPPH of korarima seed and pod extracts and scavenging effect (%) of essential oils.

<table>
<thead>
<tr>
<th>Source</th>
<th>Concentration (mg/mL)</th>
<th>% DPPH remaining</th>
<th>Concentration (mg/mL)</th>
<th>Scavenging effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pods</td>
<td>25.0</td>
<td>93.00</td>
<td>7.8</td>
<td>38.33</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>76.50</td>
<td>11.1</td>
<td>47.04</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>47.00</td>
<td>15.6</td>
<td>61.44</td>
</tr>
<tr>
<td>Seeds</td>
<td>25.0</td>
<td>62.50</td>
<td>15.6</td>
<td>6.87</td>
</tr>
<tr>
<td></td>
<td>41.7</td>
<td>48.50</td>
<td>26.0</td>
<td>16.13</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>31.50</td>
<td>78.0</td>
<td>69.01</td>
</tr>
<tr>
<td>Trolox</td>
<td>0.025</td>
<td>85.50</td>
<td>0.1</td>
<td>56.01</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>72.50</td>
<td>0.125</td>
<td>69.86</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>32.00</td>
<td>0.167</td>
<td>91.02</td>
</tr>
</tbody>
</table>
Condensed tannin content of seeds and pods

Significant differences were not observed between pods and seeds in CT concentrations (Table 1). This is likely to be explained by no differences in biosynthesis of CT in the two types of tissue of the korarima plants at maturity. The data may suggest that more CT might have been trapped in the pods instead of translocation into the seeds. Relatively high content of CT in both pods and seeds may explain the astringent nature of korarima pods and seeds when tasted. The CT content of both pods (1.31 mg/g DW) and seeds (1.18 mg/g DW) were in the range of previous studies with other plants, with CT ranging from 0.0196 mg/g in mango cv. "Espada" to 2.5264 mg/g in banana cv. "Pacovan" (Mélo et al., 2006). Condensed tannins are widely distributed in vegetables and fruits. They are biologically active compounds that have received attention in relation to their antioxidant properties, which may correspond to a protective health action (Hagerman et al., 1998).

Extract percent recovery

The % recovery yield of crude methanol extract of seeds and pods were significantly different (Table 1). The highest yield was obtained from seed extracts (4.13%) compared to pod extracts (2.23%). This might be due to different levels of chemical and physical constituents in seeds and pods.

DPPH assay of essential oils

The DPPH free radical scavenging activities of the korarima oils at various concentrations were determined and compared with that of the commercial antioxidant Trolox (Table 2). All the tested samples showed lower DPPH radical scavenging activity when compared with the standard. The highest antioxidant scavenging effect (%) was obtained with Trolox (91.02%) for concentration of 0.167 mg/mL, while it was 69.01% for 78.00 mg/mL concentration of seed oil, and 61.44% for 15.6 mg/mL concentration of pod oil when recorded after 120 min. The essential oils of both plant tissues reduced the concentration of DPPH free radical with an efficacy far less than that of the synthetic reference antioxidant. The essential oils of both plant tissues reduced the concentration of DPPH free radical with an efficacy far less than that of the synthetic reference antioxidant (Brand-Williams et al., 1995).

Bleaching test on seed and pod extracts

The antioxidant ability and mean absorbance values of extracts from seeds, pods and BHT in the β-carotene bleaching test were significantly different (P < 0.05), as shown in the Table 3. Both seed and pod extracts gave relatively higher inhibition and exhibited varying degrees of antioxidant capacity when compared with blank control. The mean antioxidant activities of pods, seeds and synthetic reference (BHT) were 28.18, 32.04 and 85.69%, respectively. The bleaching of β-carotene in the presence of seed and pod extracts, synthetic reference and blank control as a function of time, was shown in Figure 1. Less bleaching of β-carotene emulsion was observed in samples containing seed and pod extracts as compared to blank. The difference in antioxidant activities of the seeds and pods might be attributed to a difference in total phenolic content. The results of this assay were in consistence with the data obtained from the FRAP and DPPH assay, with seeds demonstrating higher antioxidant activity than pods.

Antifungal activities

The antifungal activity of the tested plant parts against A. flavus and P. expansum are shown in Tables 4 and 5, respectively. All samples tested gave different inhibition

| Table 3. Antioxidant activities (AA) of korarima seed and pod extracts and mean absorbance values of extracts on β-carotene bleaching test. |
|-----------------|------------------|------------------|
| Extract         | AA (%)           | Absorbance value |
| Control         | -                | 0.3865 b         |
| Pods            | 28.18 c          | 0.5479 ab        |
| Seeds           | 32.04 b          | 0.5803 ab        |
| BHT             | 85.69 a          | 0.8021 a         |
| SE              | 0.88             | 0.21             |

Means followed by different letters are significantly different (P < 0.05).
SE = Standard error; NS = not significant.
activities towards tested organisms when compared with the negative control. Among the four tested plant extracts, seed extracts showed the highest anti-fungal activity, followed by pod extracts. The inhibitory effects of leaf and rhizome were lower and had no activity towards tested organisms at the lowest concentrations. In the light of these results, we can conclude that increments in concentrations of all plant parts may improve the inhibitory activities towards tested organisms when compared with the negative control. Among the four tested plant extracts, seed extracts showed the highest anti-fungal activity, followed by pod extracts. The inhibitory effects of leaf and rhizome were lower and had no activity towards tested organisms at the lowest concentrations. In the light of these results, we can conclude that increments in concentrations of all plant parts may improve the inhibitory activities towards tested organisms when compared with the negative control. 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effects of extracts.

The crude seed extract was the most active with the largest inhibition zone (4.40 mm) and (4.00 mm) against A. flavus and P. expansum at concentration of 0.4 mg/mL, respectively. In other studies, ginger inhibited growth of Aspergillus, a fungus known for the production of aflatoxin, a carcinogen (Kapoor, 1997). The crude ethanol extract of the seeds of Garcinia kola Heckel (Guttiferae) showed significant inhibitory activity against fungi like P. notatum, A. niger and Candida albicans (Akerele et al., 2008). In the study of Singh et al. (2005) it was found that dial (Anethum graveolens L.) extracts and oils were effective in controlling the growth of Aspergillus and Penicillium species. The essential oil of ginger, turmeric and cardamom have shown antibacterial effects towards Escherichia coli, Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes when tested by a disc diffusion assay (Norajit et al., 2007). The results of our present study on antifungal properties in korarima can partly support the use of this medicinal plant as traditional remedies for different ailments (Eyob et al., 2008).

Conclusion

The present study shows that extracts and essential oils of korarima may be potentially used as good sources of antioxidants. The overall results obtained from seed extracts were better than those obtained from pod extracts while essential oils from pods were better than seed oils in terms of antioxidant activities. Consumption of foods prepared with korarima spice may have significant health benefits. The observed antifungal properties of the korarima extracts may have useful implications for detailed studies of their natural antimicrobial agents. Further, the study can be continued to determine specific pharmacological constituents and other compounds associated with korarima plant parts, particularly seeds as health supplements in functional food ingredients. The FRAP, DPPH and β-carotene bleaching assays gave comparable and consistent results for the antioxidant activity measured in methanolic extracts of korarima seeds and pods. However, as the FRAP technique showed the highest values, it would be an appropriate technique for determining antioxidants in korarima seed and pod extracts.

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