DOI: 10.5897/AJB08.265

ISSN 1684-5315 © 2008 Academic Journals

# Full Length Research Paper

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

S. Eyob<sup>1\*</sup>, B. K. Martinsen<sup>2</sup>, A. Tsegaye<sup>3</sup>, M. Appelgren<sup>1</sup> and G. Skrede<sup>2</sup>

<sup>1</sup>Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, P. O. Box 5003, NO-1432 Ås, Norway.

Accepted 23 June, 2008

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  $\beta$ -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 da-

mage 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).

<sup>&</sup>lt;sup>2</sup>Nofima Food, Matforsk AS, Food, Fisheries and Aquaculture Research, Norway, Osloveien 1, NO-1430, Ås, Norway.

<sup>3</sup>Hawassa University, P. O. Box 5, Awassa, Ethiopia.

<sup>\*</sup>Corresponding author. E-mail: solomon.eyob@umb.no. Tel: +4764966139. Fax: +47 64965615.

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 activeties of korarima. There are, however, some reports on antimicrobial activities from related genus, *Aframomum giganteum* (Huguette et al., 2004), *Aframomum melegueta* (Adegoke et al., 2003) and *Aframomum danielli* (Adegoke and Gopalakrishna, 1998).

Antioxidant ability of different plant extracts and oils can be measured using numerous assays. The various tests are based on specific feature of the antioxidant activity. Due to the complex composition of different plant products, more than one method is recommended for the evaluation of antioxidant activity (Chu et al., 2000). Methods currently used include the FRAP (ferric reducing antioxidant potential) (Benzie and Strain, 1996) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays (Brand-Williams et al., 1995), bleaching of  $\beta$ -carotene (Marco, 1968) and more generic assays for the determination of specific compounds or groups of antioxidants.

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 Chencha highland of southern Ethiopia. They were dried in the shade at room temperature and stored at  $4 - 5^{\circ}$ 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 karorima 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  $\text{-}20^{\circ}\text{C}$ .

# FRAP and DPPH assay

For preparing extracts, dried and ground korarima samples (0.2 g) were weighed into glass tubes and 1 mL of distilled water, followed by 9 mL methanol, was added. The samples were mixed and sonicated on a water bath at  $0\,^{\circ}\!\!\mathrm{C}$  for 15 min. The samples were centrifuged at 12,500 x g for 2 min at  $4\,^{\circ}\!\!\mathrm{C}$  and the supernatants were used for analysis.

The FRAP assay was performed following the method of Benzie and Strain (1996), modified for a Konelab 30i (Thermo Electron Corp. Vantaa, Finland) automatic analyzer (Volden et al., 2008). In the analyzer 8 µL sample was mixed with 200 µL FRAP reagent

(3.0 mM acetate buffer, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine), 20 mM FeCl $_3$ -6H $_2$ 0, ratio 10:1:1). The analyzer was set for 10 min incubation at 37  $^{\circ}$ C. Absorbance was measured at 595 nm. Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl chroman-2-carboxylic acid) was used as control. Results were expressed as  $\mu$ mol/g DW. All analyses were performed in triplicates.

Free radical scavenging activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the procedure described by Brand-Williams et al. (1995) with slight modification. Briefly, 0.1 mL sample was added to 3.9 mL DPPH methanolic solution (25 mg/L). The reaction mixture was covered and left in the dark at room temperature. After 2 h, the absorption was measured at 515 nm. Trolox was used as standard control. Remaining absorption (%) was calculated using the absorbance from a sample containing water instead of plant extract. The percentage remaining absorption was plotted against the sample concentration and a regression analysis was performed to calculate the effective concentration (EC50) i.e. the concentration required to decrease the initial absorption by 50%. Antioxidant capacity was calculated as the reciprocal of EC50 and given as  $\mu$ mol/g DW. All measurements were performed in duplicate.

The antiradical scavenging activity of seed and pod essential oils were evaluated in comparison with the reference Trolox as described above for extracts. The measurements were performed in duplicate. The antioxidant capacity to scavenge the DPPH radical for the oils was calculated by the following equation:

Scavenging effect (%) =

$$\left(1 - \frac{absorbance\ of\ sample\ at\ 515\ nm}{absorbance\ of\ control\ at\ 515\ nm}\right) x\ 100$$

# **Total phenolics**

Total phenolic content was determined by modification of the Folin-Denis method (Swain and Hillis, 1959). Ground, dry seeds and pods (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  $\mu$ L) was mixed with 100  $\mu$ L 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 ( $\mu$ g GAE/g DW).

#### Total ascorbic acid (TAA)

The spectrophotometric method developed by Roe and Kuether (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.

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.

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

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.

#### Crude extract percent recovery

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.

## **β-Carotene bleaching test**

The β-carotene bleaching method (Jayaprakasha 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 βcarotene-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 toluene (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:

$$AA = [1 - (Ai - At) / (A'i - A't)] \times 100$$

Ai = measured absorbance value of sample at zero time; At = measured absorbance value of sample after incubation (105 min) at 50°C; A'i = measured absorbance value of control at zero time; A't =

measured absorbance value of control after incubation (105 min) at  $50^{\circ}\text{C}$ .

# 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 Penicillum 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  $\mu$ L) 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  $\mu$ mol/g DW) than the pods (14.23  $\mu$ mol/g DW) by FRAP assay. Similarly, the antiradical power of seeds (9.26  $\mu$ mol/g DW) was significantly higher than of pods (3.08  $\mu$ mol/g DW) in the DPPH test. The seeds showed nearly 2-fold of antioxidant potential

|        | Extr                  | act              | Essential oil         |                       |  |
|--------|-----------------------|------------------|-----------------------|-----------------------|--|
| Source | Concentration (mg/mL) | % DPPH remaining | Concentration (mg/mL) | Scavenging effect (%) |  |
| Pods   | 25.0                  | 93.00            | 7.8                   | 38.33                 |  |
|        | 62.5                  | 76.50            | 11.1                  | 47.04                 |  |
|        | 125                   | 47.00            | 15.6                  | 61.44                 |  |
|        | 25.0                  | 62.50            | 15.6                  | 6.87                  |  |
| Seeds  | 41.7                  | 48.50            | 26.0                  | 16.13                 |  |
|        | 62.5                  | 31.50            | 78.0                  | 69.01                 |  |
|        | 0.025                 | 85.50            | 0.1                   | 56.01                 |  |
| Trolox | 0.05                  | 72.50            | 0.125                 | 69.86                 |  |
|        | 0.125                 | 32.00            | 0.167                 | 91.02                 |  |

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

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 (EC50) of the seed extract demonstrated a higher antioxidant activity than the pod extract. From EC<sub>50</sub> 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

material will also lose vitamin C due to influence from oxygen.

# 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.

Both seed and pod oils were able to reduce the stable, purple-coloured radical DPPH into yellow-coloured DPPH reaching 50% of reduction with EC $_{50}$  values as follows: EC $_{50}$  (pod) =11.9 mg/mL; EC $_{50}$  (seed) = 59.2 mg/mL and EC $_{50}$  (Trolox) = 0.09 mg/mL. The EC $_{50}$  values showed

**Table 3.** Antioxidant activities (AA) of korarima seed and pod extracts and mean absorbance values of extracts on  $\beta$ -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.

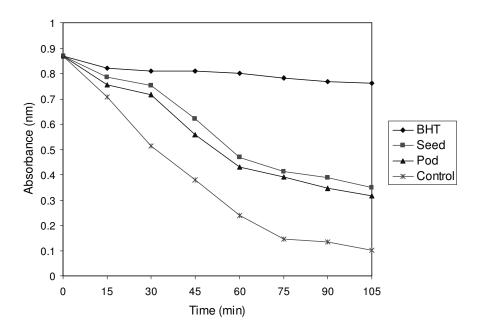
that the concentration of seed oil required was about 5-fold higher than the concentration of pod oil, thus indicating that the antioxidant activity of the pod oil was higher than that of the seed oil. The quantity of seed and pod oils required were about 658 and 132 fold, respectively, when compared with the commercial antioxidant Trolox. The antiradical scavenging activity of oils might be attributed to replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds as a result of their hydrogen donating ability (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



**Figure 1.** Antioxidant activity of korarima seed and pod extracts and BHT as measured by  $\beta$ -carotene bleaching method.

**Table 4.** Effect of various concentrations of korarima extracts of different plant parts on inhibition of *Aspergillus flavus* after 72 h of incubation period.

|            | Extract concentrations (mg/mL) |             |                 |                 |
|------------|--------------------------------|-------------|-----------------|-----------------|
| Plant part | 0                              | 0.1         | 0.2             | 0.4             |
| Seeds      | -                              | 3.10 ± 0.10 | $3.70 \pm 0.20$ | 4.40 ± 0.20     |
| Pods       | -                              | 1.73 ± 0.06 | $2.23 \pm 0.05$ | $3.03 \pm 0.06$ |
| Leaves     | -                              | -           | 1.63 ± 0.15     | $2.13 \pm 0.06$ |
| Rhizomes   | -                              | -           | 1.03 ± 0.06     | 1.43 ± 0.06     |

Values are in mm (mean ± SD).

**Table 5.** Effect of various concentrations of korarima extracts of different plant parts on inhibition of *Penicillium expansum* after 72 h of incubation period.

|            | Extract concentrations (mg/mL) |             |             |                 |  |
|------------|--------------------------------|-------------|-------------|-----------------|--|
| Plant part | 0                              | 0.1         | 0.2         | 0.4             |  |
| Seeds      | -                              | 2.37 ± 0.15 | 3.17 ± 0.21 | $4.00 \pm 0.20$ |  |
| Pods       | -                              | 1.57 ± 0.21 | 2.03 ± 0.06 | 2.90 ± 0.10     |  |
| Leaves     | -                              | -           | 1.47 ± 0.15 | 1.93 ± 0.06     |  |
| Rhizomes   | -                              | -           | -           | 1.23 ± 0.06     |  |

Values are in mm (mean ± SD).

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

<sup>-</sup> Refers to non measurable inhibition.

<sup>-</sup> Refers to non measurable inhibition.

2591

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 dill (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 aliments (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 phenolic 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.

#### **ACKNOWLEDGMENTS**

Authors gratefully acknowledge financial support from Norwegian State Educational Loan Fund (Lånekassen) and Norwegian Agency for Development Cooperation (NORAD) research project fund collaboration between Norwegian University of Life Sciences and Hawassa University, Ethiopia.

# **REFERENCES**

- Adegoke GO, Gopalakrishna AC (1998). Extraction and Identification of antioxidants from the spice *Aframomum danielli*, J. Am. Oil Chem. Soc. 75: 1047-1052.
- Adegoke GO, Makinde O, Kolowaole OF, Uzo-Peters PI (2003). Extraction and characterization of antioxidants from *Aframomum melegueta* and *Xylopia aethiopica*. Eur. Food Res. Technol. 216: 526-528.
- Akerele JO, Obasuyi O, Ebomoyi MI, Oboh IE, Uwumarongie OH (2008). Antimicrobial activity of the ethanol extract and fractions of the seeds of *Garcinia kola* Heckel (*Guttiferae*). Afr. J. Biotechnol. 7: 169-172.
- Arrigoni O, De Tullio MC (2002). Ascorbic acid: much more than just an antioxidant. Biochim. Biophys. Acta 1569: 1-9.
- Bauer AW, Kirby WMM, Sheriss JC, Turck M (1966). Antibiotic susceptibility testing by standarised single method. Am. J. Clin. Pathol. 45: 493-496.
- Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239: 70-76.
- Brand-Williams W, Cuvelier ME, Benset C (1995). Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28: 25-30
- Cai YZ, Sun M, Xing J, Luo Q, Corke H (2006). Structure radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci. 78: 2872-2888.
- Chu YH, Chang CL, Hsu HF (2000). Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 80: 561-566
- Dumas Y, Dadomo M, Lucca G, Grolier P, Di Lucca G (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J. Sci. Food Agric. 83: 369-382.
- Espin JC, Soler-Rivas C, Wichers HJ, Viguera-Garcia C (2000). Anthocyanin- based natural colorants: a new source of antiradical activity for foodstuff. J. Agric. Food Chem. 48: 1588-1592.
- Eyob S, Appelgren M, Röhloff J, Tsegaye A, Messele G (2008). Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) from southern Ethiopia. S. Afr. J. Bot. 74: 181-185.
- Eyob S, Appelgren M, Rohloff J, Tsegaye A, Messele G (2007). Chemical composition and physical properties of essential oils from fresh plant parts of korarima (*Aframomum corrorima*) cultivated in the highland of southern Ethiopia. J. Essent. Oil Res. 19: 372-375.
- Feryal K, Hande SB, Nuray K, Yeflim S (2005). Antioxidant Activity of Selected fruits and vegetables grown in Turkey, Turk. J. Agric. For. 9: 297-303.
- Harman D (1995). Role of antioxidant nutrients in aging: overview. Age. 18:51-62
- Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld P, Riechel TL (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46: 1887-1892
- Hall CA, Cuppett SL (1997). Structure-activities of natural antioxidants. In: Aruoma OI, Cuppett SL, eds., Antioxidant Methodology: *In vivo* and *in vitro* Concepts. AOCS Press Champaign, IL, pp. 141-172.
- Huguette A, Chantal M, Bessiere J-M (2004). Aromatic plants of tropical central Africa. Part XLIX†: Chemical composition of essential oils of the leaf and rhizome of *Aframomum giganteum* K. Schum from Gabon. Flav. Fragr. J. 19: 205-209.
- Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 73: 285-290.
- Kapoor A (1997). Antifungal activities of fresh juice and aqueous extracts of Turmeric and ginger (*Zingiber officinale*). J. Phytol. Res. 10: 59.
- Kaur C, Kapoor HC (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol. 37: 153-161
- Lee KJ, Mitchell, AE, Shibamoto T (2000). Determination of antioxidant

- properties of aroma extracts from various beans. J. Agric. Food Chem. 48: 4817-4820.
- Liu F, Ng TB (2000). Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci. 66: 725-735.
- Liu YW, Han CH, Lee MH, Hsu FL, Hou WC (2003). Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity *in vitro*. J. Agric. Food Chem. 51: 4389-4393.
- Madsen HL, Bertelsen G (1995). Spices as antioxidants, Trends Food Sci. Tech. 6: 271-277.
- Marco GJ (1968). A rapid determination method for evaluation of antioxidants. J. Am. Oil Chem. Soc. 45: 594-598.
- Maxson ED, Rooney LW (1972). Evaluation of methods for tannin analysis in sorghum grain. Cereal Chem. 49: 719-729.
- Mélo ÉA, Galvão de Lima VLA, Maciel MIS, Caetano ACS, Leal FLL (2006). Polyphenol, Ascorbic Acid and Total Carotenoid Contents in Common Fruits and Vegetables. Braz. J. Food Technol. 9: 89-94.
- Middleton E, Kandaswamy C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol. Rev., 52: 673-751.
- Norajit K, Laohakunjit N, Kerdchoechuen O (2007). Antibacterial Effect of Five Zingiberaceae Essential Oils. Molecules, 12: 2047-2060.
- Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 5: 1142-1145.
- Rice-Evans CA, Miller NJ, Paganda G (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med. 20: 933-956.
- Roe JH, Kuether CA (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. 147: 399-407.
- Shahidi F, Chavan UD, Naczk M, Amarowicz R (2001). Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). J. Agric. Food Chem. 49: 926-933.

- Singh G, Maurya S, De Lampasona MP, Catalan C (2005). Chemical constituents, antimicrobial investigations and antioxidant potentials of *Anethum graveolens* L. essential oil and acetone extract: Part 52. J. Food Sci. 70: 208-215.
- Soler-Rivas C, Espin JC, Wichers HJ (2000). An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochem. Anal. 11: 330-338.
- Swain T, Hillis WE (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agr. 10: 63-68.
- Thirugnanasampandan R, Mahendran G, Bai VN (2008). Antioxidant properties of some medicinal *Aristolochiaceae* species. Afr. J. Biotechnol. 7: 357-361.
- UHIS (2005). Vitamin C content of fruit of the world. http://www.naturalhub.com/natural\_food\_guide\_fruit\_vitamin\_c.htm.
- Volden J, Borge GIA, Bengtsson GB, Hansen M, Thygesen IE, Wicklund T (2008). Effect of thermal treatment on glucosinolates and antioxidant related parameters in red cabbage (*Brassica oleracea* L. ssp. *capitata* f. *rubra*). Food Chem. 109: 595-605.
- Wangcharoen W, Morasuk W (2007). Antioxidant capacity and phenolic content of some Thai culinary plants. Mj. Int. J. Sci. Tech. 1: 100-106.