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Evaluation of the polyphenolic contents and some antioxidant properties of aqueous extracts of Garlic, Ginger, Cayenne Pepper and their mixture

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Summary

Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), and Cayenne Pepper (*Capsicum frutescens*) are common culinary spices that are used singly or combined in the diet of many populations of the world and there is a long-held belief of their health-enhancing properties. This study investigated the aqueous extracts each of garlic, ginger, cayenne pepper and a combination of the three for polyphenolic and antioxidant properties that might justify such claims. Antioxidant activities were studied using DPPH, ABTS, nitric oxide radical scavenging activities and reducing power assay. Each of the spice extracts showed high content of phenolics, flavonoids, flavonols and proanthocyanidins, with the pepper extract exhibiting the highest concentration of each polyphenol investigated. The antioxidant activities of the spices and their mixture were concentration dependent, though positively comparable with the standards used. Among the extracts, the mixture exhibited the highest antioxidant activity compared to the individual spices and standards probably due to a synergistic effect of combining the spices. The present study confirmed that the aqueous extracts of garlic, ginger and pepper exhibited significant polyphenolic content and antioxidant potentials.

Introduction

The formation of free radicals – reactive oxygen species (ROS) and reactive nitrogen species (RNS), as a by-product of cellular metabolism have been implicated in the generation of oxidative stress that leads to the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders, aging and certain types of cancer (Valko et al., 2004). The putative protective effects of antioxidants against these deleterious oxidative-induced reactions have received increasing attention lately, especially within biological, medical, nutritional, and agrochemical fields. In addition, the efficacy of plant derived phytochemicals on human ailments related to dietary habits has gained renewed interests in the recent past. Because these non-nutrient bioactive compounds are consumed in significant amounts through the diet, they may have long term physiological benefits without harmful side effects (Halliwell and Guteridge, 1989; Hazra et al., 2008; Rao et al., 2010).

Spices and herbs have been used to treat various diseases and ailments for thousands of years. Many herbs and spices, apart from their use as aroma additives in foods, have been reported to be excellent sources of phenolic compounds with good antioxidant activities. Food phenolics, especially from spices and their extracts, may therefore augment the body's source of natural antioxidants and also prevent or delay some chemical deterioration during storage of fat-containing food systems (Politeo et al., 2006).

Garlic, Ginger and Cayenne pepper have been used for thousands of years for culinary and medicinal purposes in many communities of the world. Garlic has been used around the world in cooking and to treat many conditions including hypertension, infections, snakebites, reducing cholesterol levels, as antineoplastic and antimicrobial (Koch and Lawson, 1996; Tattleman, 2005). Ginger is used in cooking and has been shown to exhibit antithrombotic, anti-rheumatic, anti-inflammatory and cholesterol reducing activities (Srivastava and Mustafa, 1992; Fuhrman et al., 2000). Medicinal applications of capsicum has a long history dating back to the Mayas and Aztecs, and is best known today as an ingredient in hot sauces, as a digestive aid, in the treatment of arthritis, cough, colds and to regulate blood pressure (Antonious et al., 2006; Beltran et al., 2007).

This study evaluated the antioxidant properties of the aqueous extracts of the three spices and their mixture comparing them with well known antioxidants in order to justify the much acclaimed medicinal potentials of these spices and to examine the effect of combining the three spices.

Materials and methods

Garlic, ginger and pepper were purchased from the Ipata market in Ilorin, Nigeria.

DPPH (Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium), Folin-Ciocalteus reagent, sodium carbonate, ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), sulfanilic acid, vanillin, ascorbic acid (Vitamin C), butylated hydroxyl toluene (BHT) and rutin were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). All other reagents used in this study were of analytical grade.

Preparation of spice extracts

Each of the spices – garlic, ginger and pepper – were individually sorted to remove grits and dirt, washed and thinly sliced. They were dried in the oven at 60 °C for 72 h. The dried spices were individually milled into fine powder, packed into airtight plastic bottles and stored at 4°C until needed. From the powdered samples, 50 g of each spice was mixed with 1000 ml of distilled water and boiled for 10 min at 100 °C. It was allowed to cool, filtered and then freeze-dried (Vir Tis bench top K, Vir Tis Co. Gardiner, NY). The freeze-dried sample was reconstituted with distilled water to give desired concentrations used in this study.

Preparation of spice mixture extract

A mixture of the ground spices was prepared by weighing equal amounts of each spice [(50:50:50 g), (w/w/w)] which were thoroughly mixed together by passing through a coffee grinder of a home blender set. Fifty grams (50 g) of this mixture was mixed with 1000 ml of distilled water and boiled for 10 min at 100 °C. It was allowed to cool, filtered and then freeze-dried (Vir Tis bench top K, Vir Tis Co. Gardiner, NY) for 48 h. This mixture was stored in an airtight plastic bottle at 4 °C and reconstituted with distilled water to give desired concentrations as needed for the various analyses.

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Total phenolics

The Folin-Ciocalteu's reagent was used to determine the total phenolic content of the extracts (WOLFE et al., 2003). 2.5 ml of Folin-Ciocalteu's reagent diluted with distilled water 1:10 v/v was mixed with 0.5 ml of the spice extract and 2.0 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C to develop the color. The absorbance was read at 765 nm using a Hewlett Packard UV-VIS spectrophotometer. Samples were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the equation based on the calibration curve: $Y = 0.1216x$, $R^2 = 0.9365$; where x is the absorbance and y is the tannic acid equivalent (mg/g).

Total Flavonoids

Flavonoid content was determined as described by ORDOÑEZ et al. (2006) using quercetin as standard. 0.5 ml of 2 % $AlCl_3$ was added to 0.5 ml of the spice extract, incubated at room temperature for 1 h and the absorbance measured at 420 nm. The result was expressed as mg/ g using the equation: $Y = 0.0255x$, $R = 0.9812$; where x is the absorbance and Y the quercetin equivalent. All determinations were in triplicates.

Total Flavonols

Total flavonols was determined by the method described by KUMARAN and KARUNAKARAN (2007). To 2.0 ml of the spice extract was mixed 2.0 ml of $AlCl_3$ in ethanol, then 3.0 ml of sodium acetate solution (50 g/l) was added to the mixture. This was incubated at 20 °C for 150 min and the absorbance read at 440 nm. Total flavonol content was calculated as quercetin (mg/g) equivalent from the calibration curve using the equation: $Y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and Y the quercetin equivalent in mg/g.

Total Proanthocyanidin

The method described by SUN et al. (1998) was used to determine the total proanthocyanidin in the spice extracts. In a test tube, 3.0 ml of vanillin-methanol mixture (4 % v/v) was mixed with 0.5 ml of 1 mg/ml of the spice extract, 1.5 ml of HCl was added, vortexed and the solution allowed to stand at room temperature for 15 min. Absorbance was read at 500 nm and total proanthocyanidin was expressed as catechin equivalent using the equation derived from the calibration curve: $Y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and Y the catechin equivalent in mg/g.

Determination ferric ion reducing power

The reducing power of the spice extracts were determined according to the method described by OYAIZU (1986). 1.0 ml of the extract was prepared in cold distilled water. BHT and vitamin C (0.2-1.0 mg/ml) were mixed individually with a 0.5 ml of 0.2M phosphate buffer (pH 6.6) and 0.5 ml potassium ferricyanide (1% w/v). The resulting mixtures were incubated at 50 °C for 20 min and 2. 5 ml of 10 % trichloroacetic acid added to each of them. The entire mixture was centrifuged at 3000 rpm for 10 min and 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water, 0.5 ml of ferric chloride (0.1 %) was added, and the absorbance read at 700 nm. Ascorbic acid and BHT were used as standards for comparison.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the method of GARRAT (1964). In a test tube, 2.0 ml of a 10 mM sodium nitroprusside prepared in 0.5 ml saline phosphate buffer (pH 7.4), was mixed with 0.5 ml of the spice extracts, BHT and rutin individually at various concentrations (0.2-1.0) (Rutin and BHT were used as standards to compare the nitric oxide scavenging activities of the spice extracts). The mixture was incubated at 25 °C for 150 min after which 0.5 ml of the solution was mixed with 0.5 ml Griess solution [(1.0 ml sulphanilic acid reagent- 0.33 % in 20 % glacial acetic acid at room temperature for 5 min) with 1 ml naphthylethylenediamine dichloride (0.1 % w/v)], incubated for 30 min at room temperature and the absorbance read at 540 nm.

Nitric oxide scavenging activity was calculated as $Abs_{(control)}$-$Abs_{(sample)}$/$Abs_{(control)}$ x100.

Where $Abs_{(control)}$ is the absorbance of NO radical + methanol, and $Abs_{(sample)}$ is the absorbance of NO radical +sample extract or standard.

DPPH scavenging activity

This was determined according to the method described by LIYANA-PATHIRANAN and SHAHIDI (2005). To 0.5 ml of the extract in methanol (0.2-1.0 ml) in a test tube was added 2.5 ml of 0.5 mM methanolic solution of DPPH, shaken vigorously and incubated for 30 min in the dark at room temperature. Absorbance was read at 517 nm and ascorbic acid was used as positive control. DPPH free-radical scavenging activity was calculated as

$$\% \text{ DPPH inhibition} = Abs_{(control)} - Abs_{(sample)} / \text{ x100}.$$

Where $Abs_{(control)}$ is the absorbance of DPPH radical + methanol, and $Abs_{(sample)}$ is the absorbance of DPPH radical +sample extract or standard.

ABTS radical scavenging activity

The method described by RE et al. (1999) was used to determine the ABTS scavenging activity of the spices. Two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate v/v were mixed together, allowed to react for 12 h at room temperature in the dark and used as the working solution. This was further diluted by mixing in 1 ml of freshly prepared ABTS solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Plant extracts of different concentrations (0.2-1.0)were allowed to react with 1 ml of the $ABTS^+$ and the absorbance was read at 734 nm after 7 min. Percentage $ABTS^+$ inhibition by the extract was calculated and compared with BHT and rutin using the equation:

$$\% \text{ } ABTS^+ \text{ scavenging activity} = Abs_{(control)} - Abs_{(sample)} / \text{ x 100}.$$

Where $Abs_{(control)}$ is the absorbance of ABTS radical + methanol, and $Abs_{(sample)}$ is the absorbance of ABTS radical + sample extract or standard.

Statistical analysis

The results were expressed as mean ± standard deviation, and were subjected to one way analysis of variance (ANOVA) and differences between means were separated by the Duncan's multiple range test (DUNCAN, 1955) using SAS, (2002) where $P < 0.05$ was considered significant.

Results

The total polyphenolic content of the spice extracts are shown in Tab. 1. While the flavonoids content in garlic and ginger were not significantly different ($P < 0.05$) from one another, both were significantly different from the values for pepper and the mixture extracts. Of the four extracts, pepper had the highest flavonoids content. The

Tab. 1: Total flavonoid, phenolic, proanthocyanidin and flavonol content of extracts of, garlic, gingers, pepper and their mixture (mg/ml)

Polyphenol	Garlic	Ginger	Pepper	Mixture
Flavonoid	4.08[a]	3.99[b]	7.05[c]	6.55[d]
Phenols	23.02[a]	22.09[b]	47.18[c]	18.97[d]
Proanthocyanidins	5.79[a]	3.74[b]	10.66[c]	9.4[d]
Flavonol	27.96[a]	32.62[b]	59.42[c]	55.34[d]

[*a-d] Values are means of 3 determinations ±SEM. [**] Values along the same row with different superscripts are significantly different (P<0.05).

total phenolics content in mg/g tannic acid equivalents of the four extracts was also highest in pepper, with the value for the mixture being significantly ($P < 0.05$) lower compared to the other extracts and the phenolic contents of garlic and ginger not being significantly ($P > 0.05$) different from each other. Total flavonol content was significantly high in pepper compared to the two other extracts and the mixture as recorded in quercetin equivalents, while the same trend was observed for the proanthocyanidin content of the four extracts. In effect, pepper exhibited the highest polyphenolic content among the three spices and their mixture.

The ferric reducing power is used to evaluate the antioxidant components in dietary polyphenols. Fig. 1 shows the reducing power of the spice extracts compared to vitamin C and BHT (which were used as standards). Vitamin C activity was significantly higher ($P < 0.05$) than that of BHT and the spice extracts. The extract of the mixture exhibited the highest reducing action of all the extracts and at all concentrations, followed by the pepper extract, with garlic showing the least activity.

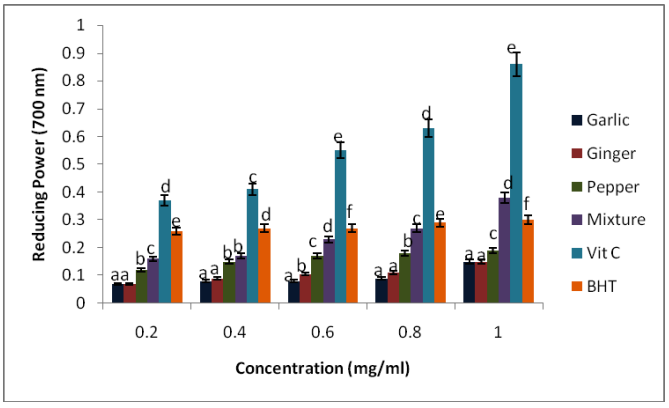

Fig. 1: Reducing power of aqueous extracts of garlic, ginger, pepper and their mixture.
[a-f] Values are means of 3 determinations ± SEM. Bars with different colours carrying different letters are significantly different (P<0.05). Vit. C = vitamin C, BHT = butylated hydroxyl anisole (standards).

There was a significant decrease in the concentration of the $DPPH^+$ radical due to the scavenging ability of the spice extracts (Fig. 2) which was dose-responsive. It is interesting to note again that the 1, 1-diphenyl-2-picrylhydrazyl ($DPPH^·$) scavenging activity of the mixture though not as high as those of the standards (vitamin C and BHT), was significantly higher ($P < 0.05$) than for the individual spices. The IC_{50} (concentration of sample needed to scavenge 50% of DPPH) was calculated by linear regression of plots. The mixture

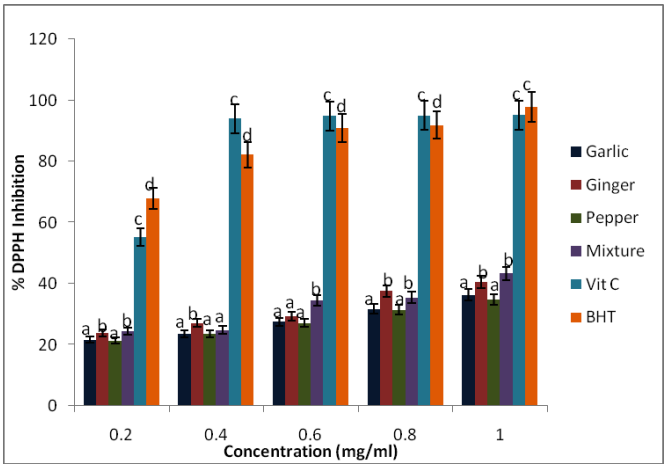

Fig. 2: DPPH radical scavenging activities of garlic, ginger, pepper and their mixture.
[a-d] Values are means of 3 determinations ± SEM. Bars with different colours carrying different letters are significantly different (P<0.05). Vit. C = vitamin C, BHT = butylated hydroxyl anisole (standards).

had an IC_{50} of 1.12 mg/ml, followed by ginger, garlic and pepper with IC_{50} of 1.21, 1.4 and 1.55 mg/ml, respectively. This could be an indication that the spices exhibited additive action in DPPH sca-venging. Of the two positive controls, BHT had the higher scavenging property than vitamin C at concentration of 1.0 mg/ml.

The extracts were also analyzed for free radical scavenging activity against $ABTS^+$. The extracts exhibited significant ($P < 0.05$) ABTS scavenging activities as their concentration increased getting to the peak at concentrations of 1.0 mg/ml (Fig. 3). The activities of the extracts from ginger, pepper and the mixture against $ABTS^+$ were quite high and not significantly different ($P < 0.05$) from those of the standards vitamin C and BHT at concentrations of 0.8 and 1.0 mg/ml, while that of garlic though equally high was significantly lower ($P < 0.05$) than the others. The IC_{50} was 0.04 mg/ml in ginger, 0.10 in the mixture, while for pepper and garlic were 0.45 and 0.66, respectively.

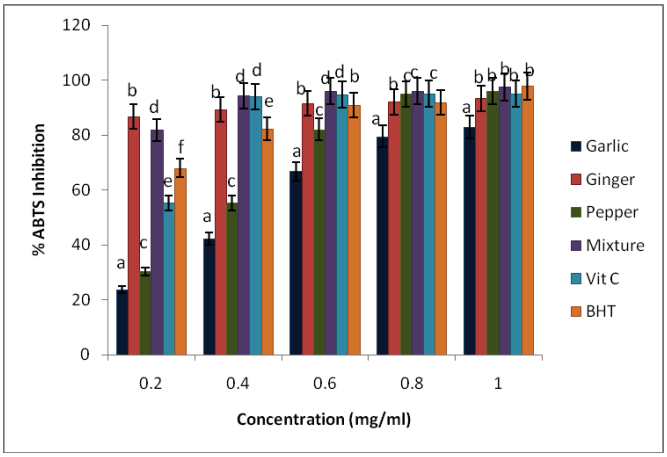

Fig. 3: ABTS activities of extracts of garlic, ginger, pepper and their mixture.
[a-f] Values are means of 3 determinations ± SEM. Bars with different colours carrying different letters are significantly different (P<0.05). Vit. C = vitamin C, BHT= butylated hydroxyl anisole (standards).

The scavenging activities of the spices on nitric oxide radical were high for all the extracts (Tab. 2). Nitric oxide scavenging activity increased in a concentration dependent manner, with maximum inhibition exhibited at 1.0 mg/ml of the extracts. The mixture showed the highest inhibition of 90.40, followed by pepper, garlic and ginger at 87.40, 87.00 and 82.67 %, respectively. Pepper and the mixture extract exhibited significantly high ($P < 0.05$) activity against the nitric oxide radicals at all concentrations, which were competitively comparable with the standards while garlic and ginger showed lower activities which were significantly different from one another.

Discussion

Polyphenolic compounds such as flavonoids, flavonols, proanthocyanidin and phenolics in plants have been reported to have strong antioxidant activities which help to protect cells against oxidative damage by free radicals (McCune and John, 2002; Liao et al., 2008). Flavonoids and phenols have been recognized to have antioxidant effects on human nutrition and health. Their mechanisms of action are through scavenging, chelating and termination of free radicals (Kessler et al., 2003). In this study, all the spices exhibited high phenolic and proanthocyanidin contents which have been reported to have high antioxidant activities (Luximon-Ramma et al., 2002). These activities probably account for some of the pharmacological effects of these spices on many diseases caused by free radicals. In addition, flavonoids and phenolic compounds are effective in preventing the formation of reactive oxygen species and protecting low density lipoprotein (LDL) from iron- and copper-mediated free radical production (Owen and John, 2002).

The reducing power of the extracts increased with increasing concentrations, with the extract from the mixture exhibiting the highest reducing power at 1.0 mg/ml among the extracts though vitamin C(the standard), showed the greatest activity. The trend observed was vitamin C>BHT>mixture>pepper>ginger>garlic. Deore et al. (2009) reported the reducing power of *Croton caudatum* ethanol extracts as a function of their concentration, a trend observed in this study.

The model of scavenging the stable DPPH radical is a widely used method in the evaluation of the free radical scavenging ability of various compounds (Ghaseni et al., 2009). Fig. 3 depicts that the scavenging activities of all the extracts and the standards for DPPH are dose responsive, that is, the higher the concentration, the greater the scavenging activity. High antioxidant activity of *A. sativum* has been reported by Benkeblia (2005), but activity depends on both phenolics and sulphur compounds of the alliums. Nuutila et al. (2003) and Capasso (2013) in studies comparing the antioxidant properties of different *allium* species and antioxidant action of garlic respectively reported that the lowest antioxidant activity was in garlic (*A. sativum*), this is similar to the trend observed in this study between the activities of garlic, ginger, pepper and the mixture of the three spices, as garlic showed the least free radical scavenging activity with DPPH, ABTS and the least reducing power.

Ginger showed almost similar activity against free radicals like garlic though in some instances it showed higher values. According to Ghasemzadeh et al. (2010), ginger showed high antioxidant activity with DPPH though less than that of the standard used. This is similar to our observation in the present study as ginger showed high scavenging activities with both DPPH and ABTS.

Pepper (chili) on the other hand showed consistently high reactivity for reducing power, DPPH, ABTS and nitric oxide scavenging. This reactivity was highly correlated with total flavonoids, phenolics, proanthocyanidin and other bioactive compounds (capsaicinoids, carotenoids) of the extract which was similar to the trend observed by Benkeblia (2005) and Bae et al. (2012), though Rahiman et al. (2013) reported that *Capsicum frutescens* seed oil exhibited the least antioxidant activity among some common home remedies with storage time.

The consumption of these spices therefore, could offer protection against chronic diseases caused by free radicals and could also augment cellular defenses against oxidative damage.

We report here for the first time the antioxidant activities of a mixture of garlic, ginger and pepper. Interestingly, when these spices were combined, the total antioxidant activity increased which did not show any correlation with the phenolic content. The extract of the mixture exhibited the highest free radical scavenging activity with DPPH, ABTS and nitric oxide as well as the greatest reducing power. This may be as a result of synergistic activity of the antioxidant powers of the three spices. This trend is similar to the findings of Shobana and Naidu (2000) who reported that a spice mix of (ginger, onion and garlic; onion and ginger; ginger and garlic) showed a cumulative inhibition of lipid peroxidation by exhibiting a synergistic property when compared with the individual spices. Seah et al. (2010) also reported a similar trend with keanghleung paste (a mixture of turmeric rhizome, garlic and chili). These attributes implies that a mixture of these spices could be more effective in combating diseases than each spice used in isolation.

Conclusion

This study has revealed that aqueous extracts of garlic, ginger and pepper singly or as a mixture, possess high polyphenolic content and high antioxidant activities in different systems providing support for their acclaimed health benefits. Therefore, further study of their effectiveness in animal models of disease and oxidative stress would be undertaken to provide a useful basis for nutritional advice.

Tab. 2: Percentage (%) Nitric oxide scavenging activity of the aqueous extracts of garlic, ginger, pepper and their mixture

Concentration (mg/ml)	Garlic	Ginger	Pepper	Mixture	Rutin**	BHT**
0.2	80.79±0.02^{a}	74.61±0.03^{b}	83.58±1.05^{c}	85.30±0.33^{d}	82.20±1.27^{c}	94.50±1.27^{e*}
0.4	81.10±0.01^{a}	75.84±0.09^{b}	84.40±1.20^{c}	85.40±0.10^{c}	86.06±0.67^{c}	96.50±1.27^{d}
0.6	85.20±0.00^{a}	77.24±0.04^{b}	86.51±0.00^{c}	86.50±0.17^{c}	94.95±00^{d}	97.41±1.83^{e}
0.8	86.25±0.14^{a}	79.84±0.04^{b}	87.00±0.04^{c}	87.41±0.06^{c}	95.12±1.75^{d}	98.22±2.42^{e}
1.0	87.00±0.02^{a}	82.67±0.03^{b}	87.40±0.15^{a}	90.40±0.10^{c}	98.53±0.67^{d}	98.73±2.92^{d}

*$^{a-e}$ Values are mean of 3 determinations ± SEM. **Rutin and BHT are standards. ***values along the same row with different superscripts are significantly ($P<0.05$) different.

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