Comparison of Antioxidant and Antimicrobial Activities of Essential Oils from *Hyptis suaveolens* and *Alpinia galanga* Growing in Northern Thailand

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ABSTRACT

The essential oils of Hyptis suaveolens (Labiatae) and Alpinia galanga (Zingiberaceae) obtained by hydrodistillation were compared for their antioxidant potentials and antimicrobial activities on the basis of chemical components of both oils. The antioxidant activity of the essential oils was determined by using two complementary methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical decolorization assay. A good correlation of % inhibition was observed between these two methods. The results obtained indicated that the essential oil of A. galanga possessed stronger antioxidant activity than that of H. suaveolens with the IC_{50} values of 550 and 3721 μg/ml, respectively. The antimicrobial activity of the essential oils was compared by the dilution method. The results showed that the essential oil of A. galanga was more active against the test microorganisms with the MID values of 1:320, 1:320, 1:160, 1:80, 1:80, 1:160 and 1:160 against Staphylococcus aureus, Streptococcus suis, Erysipelothrix rhusiopathiac, Pseudomonas aeruginosa, Escherichia coli, Pasteurella multocida and Actinomyces pyogenes, respectively. The higher potential in antioxidant and antimicrobial activities of A. galanga oil was supposed to be due to the composition of certain main constituents e.g., 1,8-cineole, 4-allyphenyl acetate and β-bisabolene within the essential oil.

Key words: Essential oil, Antioxidant activity, Antimicrobial activity

INTRODUCTION

It is well-known that free radicals and other reactive oxygen species formed in living cells play an important role in the origin of life and biological evolution (Lander, 1997; Mc Cord, 2000; Zheng and Storz, 2000). However, it has been found that those reactive species also play a cardinal role in oxidative damage to cellular compartment which leads to cell injury and death. This has been associated with pathogenesis of various chronic diseases, e.g., carcinogenesis, coronary heart disease and many other health problems related to advancing age (Cadenas and Davies, 2000; Marnett, 2000; Uchida, 2000). Thus, to increase the antioxidant intake in

human diet is an important way to minimize such oxidative damage. The synthetic antioxidants may exhibit most serious toxicity and require higher manufacturing costs than natural antioxidants. Therefore, there is a need to identify natural and possibly more economic and effective antioxidants with a potential to be incorporated into foods. A great number of plant species in different regions of the world have been screened for their antioxidant activity (Mantle et al., 2000; Auddy et al., 2003; Lee et al., 2003; Yingming et al., 2004). The majority of plant species investigated was traditionally used in herbal medicine. However, there are still many plant species which have not been examined on this matter or the knowledge about their antioxidative properties is very scarce. *Hyptis suaveolens* (Labiatae) and *Alpinia galanga* (Zingiberaceae) are among such plants. Both *H. suaveolens* and *A. galanga* are the edible plants which are usually applied in Thai food recipe as an appetizer because of their flavored essential oils. *H. suaveolens* is a medium aromatic annual shrub distributed in the tropical and subtropical regions. It was reported to be used for traditional medicine as an anticancer (Kingston et al., 1979). The ethanolic extract from its leaves exhibited healing property with supportive role of antioxidant enzyme (Annie wt al., 2003). *Alpinia galanga* is a herbaceous ground flora which is widely distributed throughout the tropics, particularly in Southeast Asia. The rhizome of *A. galanga* is widely used in dietary intake as well as in the traditional system of medicine. The essential oil of *A. galanga* rhizome has been found to have inhibitory activity against certain dermatophytes, filamentous fungi and yeast (Mohd et al., 2003). The rhizomal extract of *A. galanga* was found to have antiallergic activity (Matsuda et al., 2003a) whereas phenylpropanoid compounds isolated from this plant have shown the potent gastroprotective effect on ethanol-induced gastric lesions in rats (Matsuda et al., 2003b). As mentioned above, both plants are edible aromatic-flavored for food. The knowledge of biological potential, particularly about antioxidant activity of their essential oils, will provide essential data for the consumers to guide them to the plant to be chosen for their food appetizer with stronger biological activity. Since the biological activity of plant essential oils usually varies, depending on the place they are grown, therefore, the aim of this study was to investigate the antioxidant as well as the antibacterial activities of the essential oils from *H. suaveolens* and *A. galanga,* grown especially in northern part of Thailand. For this purpose, two complementary methods of free radical scavenging activity: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical decolorization assay were used. In addition, the antibacterial activity of these oils against certain pathogenic bacteria from human and animal sources was also investigated. The chemical composition of the oils was also determined in order to evaluate whether or not it could mainly affect such biological activities.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Hyptis suaveolens* and rhizomes of *Alpinia galanga* were harvested from the northern region of Thailand, Chiang Mai province. The voucher specimen of each sample is deposited in the Herbarium of Faculty of Pharmacy, Chiang Mai University, Thailand. The fresh sample of each plant was submitted to hydrodistillation (3 h), using a Clevenger apparatus.

Chemicals

The following reagents obtained from Sigma-Aldrich, Inc., St. Louis (USA): 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were used. Potassium persulfate was obtained from Anala R®, BDH Chemicals Ltd. Poole (England). Ethanol was purified using rotary evaporator (EYELA®, N-100) (Japan).

Antioxidant activity study

1. DPPH free radical-scavenging assay

The scavenging effect on DPPH radical was determined by modifying the methods of Brand-Williams et al., (1995) and Gamez et al., (1998). The essential oils of *H. suaveolens* and *A. galanga* were separately mixed with ethanol to prepare an ethanolic test solution of 1 mg/ml samples. DPPH was dissolved in ethanol and mixed with the ethanolic test solution. The solution was adjusted to a final DPPH concentration of 100 µM. The mixture was shaken vigorously and left to stand for 5-60 min in the darkness at room temperature. The amount of DPPH remaining in each period of stand was determined spectrophotometrically at 540 nm, using a microtiter plate reader (Biorad 680, USA). The ethanolic test solution was diluted to different concentrations (0.1-1.0 mg/ml). After vigorous shake, the mixtures were left to stand for 30 min. All measurements were performed in triplicate. The radical scavenging activity was calculated as % inhibition from the following equation: % inhibition = $\{(OD_{blank} - OD_{sample}) / OD_{blank}\} \times 100$.

2. ABTS free radical decolorization assay

The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS.^{+}$) was generated according to the modified method of Re et al., (1999) by oxidation of ABTS solution (7mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 12 hours in the darkness at room temperature. The ABTS solution was diluted with ethanol to the absorbance of 0.7±0.2 at 750 nm. Series of essential oil solution in different concentrations were prepared by diluting with ethanol. An aliquot of 20 µl of the ethanolic dilution of each solution was added to 180 µl of ABTS. + radical cation solution. The absorbance, monitored for 60 min, was measured spectrophotometrically at 750 nm, using a microtiter plate reader (Biorad 680, USA). All measurements were performed in triplicate. The antioxidant activity of the essential oil was calculated as % inhibition from the following equation: % inhibition = $\{(OD_{blank} - OD_{sample}) / OD_{blank}\} \times 100$.

Microorganisms

The microorganisms used in this study were of clinical strains from human and animal sources as shown in Table 1. *Staphylococcus aureus* and *Pseudomonas aeruginosa*, representing gram-positive and gram-negative bacteria, respectively, were isolated from the patients reporting for wound dressing at Chiang Mai University Hospital, Thailand. The other gram-negative strains: *Escherichia coli, Pasteurella multocida* and *Actinomyces pyogenes* and gram-positive strains: *Erysipelothrix rhusiopathiac* and *Streptococcus suis* were isolated from the gastrointestinal tract of swine in Thailand. The isolated microorganisms were cultured overnight at 25°C in Muller Hinton agar (MHA) to reach the stationary phase of growth.

Table 1. Test microorganisms used in the study.

Microorganism	Sources
gram-positive bacteria	
Staphylococcus aureus	human
Streptococcus suis	animal
Erysipelothrix rhusiopathiac	animal
gram-negative bacteria	
Pseudomonas aeruginosa	human
Escherichia coli	animal
Pasteurella multocida	animal
Actinomyces pyogenes	animal

Antimicrobial activity study

The antimicrobial activity of the essential oils was determined by the dilution method. In this technique, two-fold dilutions of the oil in dimethyl sulfoxide (DMSO; Sigma-Aldrich) aqueous solution, starting from 1:2 (v/v) were serially prepared. An aliquot of 2 ml of each dilution was mixed with prewarmed Muller Hinton agar. The mixture was then poured into a sterile petri dish. After the agar had congealed, a loop of each test microorganism suspension, concentration controlled by Mc Farland No 0.5, was inoculated. The plates were incubated in reversed position at 25°C. The maximum inhibitory dilution (MID), defined as the maximum dilution of the oil that was still able to inhibit the growth of the test microorganism, was determined. All experiments were done in three replications.

GC-MS analysis of essential oils

The GC-MS analysis of the oil samples was performed on a Hewlett-Packard model 6890 Series GC System equipped with a HP 5973 MS detector (EI mode, 70 eV). A column type, HP-5 (5% phenyl dimethylsiloxane) with a length of 30 m, an inside diameter of 0.25 mm and a film thickness of 0.25µm, was used. The temperature of the column was programmed to increase after 5 min from 70 to 150°C at the rate of 2°C/min and then after 5 min from 150 to 250°C at the rate of 1°C/min. Helium was used as a carrier gas at a flow rate of 1 ml/min. The injector and detector temperatures were 250 and 280°C, respectively. The components in essential oil were identified by comparing on the basis of gas chromatographic

retention indices, mass spectra from Wiley MS Chemstation Libraries (6th ed., G 1034, Rev.C.00.00, Hewlett-Packard, Palo Alto, CA) and the literature (Jennings and Shibamoto, 1981; Adams, 1996).

RESULTS AND DISCUSSION

Antioxidant activity

Several methods have been reported to be used for the evaluation of the antioxidant activity. However, there was always some difference among those test systems available and the results of a single-assay could give only a reductive suggestion of the antioxidant properties. Therefore, in the present study, two complementary methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and an improved 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical decolorization assay were used. These two methods are widely used to evaluate antioxidant activities in a relatively short time compared with other methods. The time-dependent activity in DPPH radical-scavenging of *A. galanga* and *H. suaveolens* oils was observed as shown in Figure 1. The pattern showed that the % inhibition of *H. suaveolens* oil increased rapidly during the first 5 min and reached the maximum inhibition of about 10% in 5 min. For *A. galanga* oil, the %inhibition also increased rapidly within the first 5 min but yet did not reach the maximum inhibition. After the first 5 min, the inhibition of *A. galanga* oil gradually increased consistently. It was shown that the longer the time, the stronger the inhibition of *A. galanga* oil. At 60 min, it was obviously seen that % inhibition of *A. galanga* oil was extremely higher than that of *H. suaveolens*.

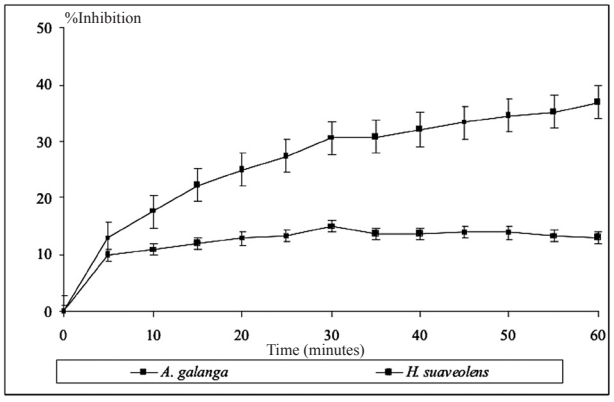

Figure 1. Time-dependent scavenging activity of essential oils by scavenging effect on DPPH radical. Each value is expressed as mean±SD (n=3).

The oil solution was further diluted for the study of concentration effect on antioxidant power. This time, the period of mixing was fixed at 30 min. The results are shown in Figure 2. *A. galanga* oil exhibited lower inhibition when concentration was decreased. This was obviously observed when concentration range was lowered

from 1 mg/ml to 0.4 mg/ml. The concentration less than 0.4 mg/ml did not exhibit significant difference in activity. However, the lower activity as a result from lower concentration of *A. galanga* oil still showed higher activity than that of *H. suaveolens* whose activity was less affected by concentration. The antioxidant activity of the test samples, expressed as IC_{50} (the concentration of the oil required to inhibit the formation of DPPH radicals by 50%), is shown in Table 2. The result indicated a significant difference in power of scavenging ability of A.galanga oil, with 7-fold higher than that of *H. suaveolens.*

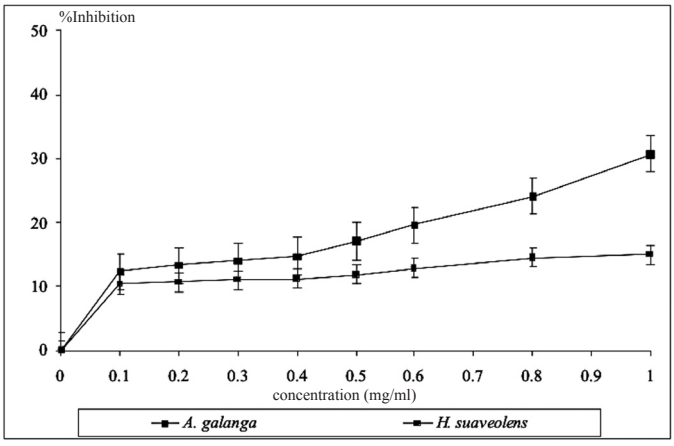

Figure 2. Concentration-dependent scavenging activity of essential oils by scavenging effect on DPPH radical. Each value is expressed as mean±SD (n=3).

Table 2. The values of IC_{50} of the essential oils.

Materials	**IC_{50} (μg/ml)***
H. suaveolens oil	3721±0.019
A. galanga oil	550±0.013

* mean±SD (n=3)

The results of ABTS experiment are presented in Figure 3. In this figure, the concentration-dependent patterns of scavenging free radical capacity from ABTS experiment is illustrated. The results were in good agreement with DPPH method that the scavenging activity of both oils was increased with the increasing concentration. The higher scavenging potential was found in the oil of *A. galanga*. Figures 4 and 5 show a linear correlation between % inhibition of ABTS and DPPH free radical after different concentrations of the essential oils of *H. suaveolens* and *A. galanga* were added, respectively. The linear correlation of *H. suaveolens* could be described as [% inhibition of ABTS] = [9.5471 (% inhibition of DPPH) –68.753], with $r^2 = 0.8054$, whereas that of *A. galanga* as [% inhibition of ABTS] = [0.4305 (% inhibition of DPPH) –51.284], with $r^2 = 0.8965$.

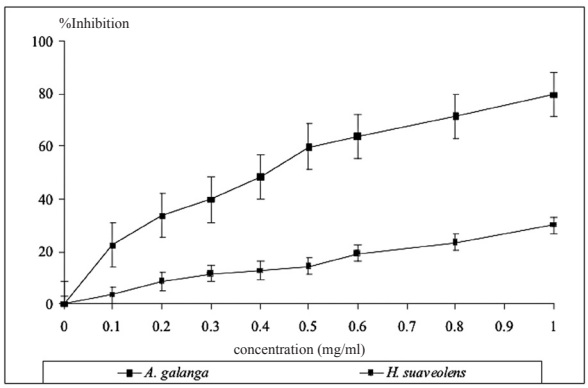

Figure 3. Concentration-dependent scavenging activity of essential oils by ABTS radical cation decolorization assay. Each value is expressed as mean±SD (n=3).

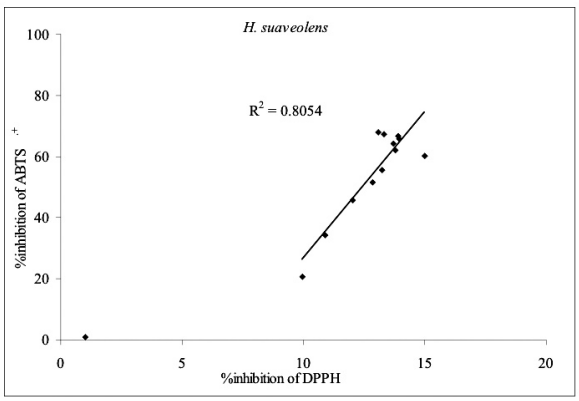

Figure 4. Correlation curve between %inhibition of *H. suaveolens* oil in DPPH and ABTS method.

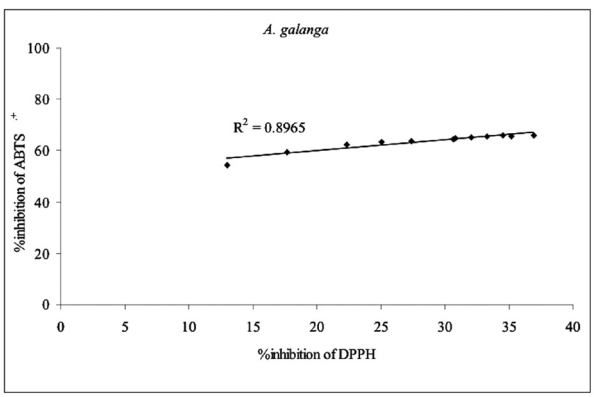

Figure 5. Correlation curve between %inhibition of *A. galanga* oil in DPPH and ABTS method.

Antimicrobial activity

The well-known conventional method used for study of antimicrobial activity is disc diffusion agar. In this technique, several discs soaked with the test samples are serially put on the top surface of the agar, containing certain amount of the test microorganism. After incubation under a suitable condition, the inhibition zone for each disc is determined. This technique can not be used for quantitative comparison of essential oils of *H. suaveolens* and *A. galanga* because the oils possess the unique property of high volatility as fumigants. To achieve our main theme, the oil dilution was firstly mixed with the agar, and then a certain amount of the test microorganisms was inoculated. By this technique, all test microorganisms were exposed to the same condition of oil concentration and vapor activity. The absence of any inoculated microorganisms in the test plate after incubation indicated the inhibition of such strain by the dilution of the oil. The maximum inhibitory dilutions (MID) of the essential oils of *H. suaveolens* and *A. galanga* against the test microorganisms are shown in Table 3. The results showed that the essential oils of *H. suaveolens* and *A. galanga* could inhibit the growth of all test microorganisms. For comparison, the essential oils of both plants were less active against most of gram-negative bacteria, particularly *P. aeruginosa* and *E. coli*, than gram-positive bacteria. This might be due to the protection by a hydrophilic outer membrane of the gram-negative bacteria which suppressed the passage of the lipophilic essential oil (Mann, 2000). The essential oil of *A. galanga* was more active against all test microorganisms than that of H. suaveolens. Its activity was highest against *S. aureus* and *S. suis* with the MID value of 1:320. This was considered to be due to the different composition of major active components in each oil. Therefore, the study of chemical composition of both oils was deemed necessary.

Table 3. MID values of *H. suaveolens* and *A. galanga* oils.

Microorganisms	**MID Values**	
	H. suaveolens	***A. galanga***
Staphylococcus aureus	1:160	1:320
Streptococcus suis	1:160	1:320
Erysipelothrix husiopathiac	1:80	1:160
Pseudomonas aeruginosa	1:20	1:80
Escherichia coli	1:20	1:80
Pasteurella multocida	1:80	1:160
Actinomyces pyogenes	1:80	1:160

Chemical composition of *H. suaveolens* and *A. galanga* oils

Water-distillation of aerial parts of *H. suaveolens* yielded 0.03% (v/w) of the pale yellow oil, whereas that of *A. galanga* was yellow with a yield of 0.06% (v/w). About 16 constituents (90.16% of the total oil) and 20 constituents (94.25% of the total oil) were identified by means of GC-MS analysis of the essential oil from *H. suaveolens* and *A. galanga*, respectively, as shown in Table 4. The major com-

pounds in the essential oil of *H. suaveolens* with percentage higher than 10% were sabinene (21.69%), α-terpinolene (12.99%), 1, 8-cineole (12.56%) and β-caryophyllene (11.70%). Regarding the previously-reported content of *H. suaveolens* essential oil (Azevedo et al., 2001), it is interesting to point out that there are important quantitative differences. For example, α-bergamotene (Asekun et al., 1999) was found to be one of the major constituents in *H. suaveolens* oil from Nigeria, but it was present at very low concentration in our sample. Also, caryophyllene oxide which could not be detected in our sample was detected as the main component in *H. suaveolens* oil from Cuba (Pino et al., 2003). These results suggest that the environmental factors strongly influence the essential oil composition. The essential oil of *A. galanga* was found to be composed mainly of 1, 8-cineole (46.22%). The other components with lower concentration but still higher than 5% were 4-allyphenyl acetate (9.38%), β-bisabolene (6.04%) and β-pinene (5.21%). From this result, incorporated with the literature reviewed, we found that the composition of *A. galanga* oil from our present study also demonstrated quantitative differences according to geographical factor. The major contents of *A. galanga* oil from India were reported as 1, 8-cineole (18.40%) and camphor (7.70%) (Jirovetz et al., 2003). In our research, the major component with highest concentration was the same compound as 1, 8-cineole but much higher in quantity of 46.22%. Moreover, the other main compounds were obviously different as 4-allyphenyl acetate (9.38%) and β-bisabolene (6.04%).

However, by comparing between the essential oils of *H. suaveolens* and *A. galanga*, it was clearly found that there were significant differences in composition of the main constituents in each oil even about 11 constituents identified were common for both oils. This difference was considered to be the major effect in biological potency of the oil.

Table 4. Chemical compositions of essential oils from *H. suaveolens* and *A. galanga*. (%).

Compounds[a]	**Rt**[b]	***H. suaveolens***	***A. galanga***
1. α-pinene	5.235	2.00	4.34
2. sabinene	6.560	21.69	0.48
3. β-pinene	6.657	6.26	5.21
4. 1-octen-3-ol	6.896	1.92	-
5. β-myrcene	7.098	0.50	0.77
6. α-terpinene	8.160	0.96	0.26
7. limonene	8.699	4.36	-
8. 1, 8 – cineole	8.859	12.56	46.22
9. γ-terpinene	10.070	1.47	0.42
10. α-terpinolene	11.597	12.99	0.15
11. terpinen-4-ol	16.795	3.15	3.67
12. (-)-α-terpineol	17.798	-	1.72
13. (-)-bornyl acetate	23.194	-	0.21

Compounds[a]	Rt[b]	*H. suaveolens*	*A. galanga*
14. 4-allyphenyl acetate	27.418	-	9.38
15. geranyl acetate	29.557	-	0.52
16. methyl-eugenol	31.145	-	3.22
17. β-caryophyllene	31.225	11.70	-
18. α-bergamotene	32.309	2.14	4.05
19. α-humulene	33.225	0.80	0.98
20. β-farnesene	33.746	-	1.83
21. bicyclogermacrene	35.824	3.02	-
22. β-bisabolene	36.796	-	6.04
23. β-sesquiphellandrene	37.644	-	3.22
24. γ-bisabolene	38.038	-	1.56
25. phenanthrene	71.252	4.67	-
Total		**90.16**	**94.25**

[a]Compounds listed in order of elution from HP-5 column
[b]Retention time (as minutes)

CONCLUSION

Several studies are going on throughout the world to search for compounds that are biologically active with low profile of side effect. In this experiment, the essential oils obtained by hydrodistillation of *A. galanga* and *H. suaveolens*, grown in northern part of Thailand and used as Thai food additives, were investigated for their antimicrobial as well as antioxidant activities. The results showed that the essential oil of *A. galanga* possessed stronger antimicrobial and antioxidant activities than that of *H. suaveolens*. This stronger activity was most likely due to the composition of main constituents in *A. galanga* oil. The essential oil of *A. galanga* is thus suitable to be promoted for food additive because of its high potential biological activity besides its good-flavored property.

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