

BIOLOGICAL CHARACTERIZATION OF WATER EXTRACTS FROM THE CAPS AND STALKS OF WHITE *HYPsizYGUS TESSELLATUS* (BUNAPI SHIMEJI) AND *FLAMMULINA VELUTIPES* (ENOKI) MUSHROOMS

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Abstract

Context: Mushrooms have been consumed over the years for nutritional and medicinal values. The emergence of many disease conditions has necessitated studies into other sources of pharmacological agents other than the synthetic and chemical agents. The diversification of the sources of materials for combating human diseases is a better way of combating the issues of drug resistance currently experienced. The biological activities and composition of water extract from the caps and stalks of White *Hypsizygus tessellatus* (Bunapi shimeji), and *Flammulina velutipes* (Enoki mushroom) were evaluated.

Methods: The antioxidant and antibacterial activities were evaluated through their scavenging ability on 1, 1-diphenylpicrylhydrazyl (DPPH), and the inhibition of bacterial growth *in vitro*, respectively.

Results: From the results, the Enoki extract had the highest phenolic content compared to Bunapi shimeji. Enoki extracts, both caps, and stalks showed the highest scavenging activity against DPPH free radicals (>70 % and >60 %, respectively) at the tested concentrations. Bunapi shimeji extracts had the strongest activity against the tested G- organisms. Reduced activity was observed against the tested G+ organisms likely due to cell wall resistance. Gentamycin was used as the standard antibiotic. **Conclusion:** The outcome of the study showed that the studied mushrooms are good sources of natural antioxidants

Key word: *Hypsizygus tessellatus*, Bunapi shimeji, *Flammulina velutipes*, antibacterial, antioxidants.

INTRODUCTION

Many conditions arise today due to harmful effects of using synthetic compounds with antioxidant properties in food and other flavoring industries. This has necessitated the need for the development of natural substances which have antioxidant properties (Nevcihan and Cengiz, 2010; Ukaegbu et al., 2017). Mushrooms have been a source of food for many people and they are made up of dietary fibers, vitamins, low fat, minerals and minute calories of carbohydrates (Albouchi et al., 2013; Kharkwal et al., 2012; Jeyanthi et al., 2012). They are also endowed with polyphenols which have good antioxidant activities (Manzi and Aguzzi, 2001).

Many bioactive compounds like dietary fiber, lentinan, ergosterol, minerals, and vitamins have been sourced from the culture cilia and fruiting bodies of some mushroom over the years. The medicinal activities of these compounds including anti-cancer and anti-microbial have been studied and reported (Albouchi et al. 2013; Salem and Ibrahim, 2011; Omar et al., 2012; Sundaram et al., 2012; Kolanjinathan and Stella, 2011; Kim et al., 2011). The activity of the compounds portrays in their strong scavenging effect against reactive oxygen species (ROS). The ROS at a low concentration have no serious effect on the cells, but when the levels are raised, become harmful to the cells. The human body is equipped with a natural defense system against the ROS such as the superoxide dismutase, the catalase, and the glutathione peroxidase, but due to the rate at which the ROS are being generated, the body most times needs external help to completely scavenge the radicals (Nevcihan and Cengiz, 2010).

The addition of extra compounds like vitamins, proteins, and minerals which have antioxidant properties improves the body's scavenging ability against the free radicals (Ostrovidov et al., 2000). With the natural antioxidants, many diseases which are caused by the use of synthetic compounds such as cancer and DNA damages can be minimized (Halliwell, 1997; Nakayama et al., 1993). Although there are still arguments as to whether natural bioactive compounds can effectively act as radical scavengers when absorbed into the body, assay of the radical scavenging properties of these products have proven them to be capable of rapidly scavenging free radical both *in-vivo* and *in-vitro* (Nevcihan and Cengiz, 2010).

This study focused on the antioxidant and antibacterial activity of water extracts of two widely consumed edible mushrooms in Malaysia. The study evaluated the total phenolic and flavonoid contents, as well as the scavenging and antibacterial activities of the water extracts from the caps and stalks of White *Hypsizygus tessellatus* (Bunapi shimeji) and *Flammulina velutipes* (Enoki) mushrooms. The mushrooms were purchased from a popular store in Malaysia where they were kept refrigerated for sale. These mushrooms are largely consumed by Malaysians for assumed nutritional and medicinal benefits. This study, therefore, aims to evaluate the medicinal benefits of consuming these mushrooms through the investigation of the antioxidant and antibacterial activities of its water extracts.

MATERIALS AND METHODS

Mushroom samples

The two mushrooms (White *Hypsizygus tessellatus*, henceforth referred to as Bunapi shimeji, and *Flammulina velutipes* referred to as Enoki) were bought from a popular market in Kuantan, Pahang, Malaysia. These mushrooms were morphologically identified with reference to literature.

Preparation of mushroom extracts

The mushrooms were separated into caps and stalks and extracted differently with water after pounding with mortar and pestle. The Bunapi shimeji stalks (193 g) and caps (83 g) were extracted with 500 and 200 mL of water, respectively at room temperature (RT) ($25 \pm 2^\circ\text{C}$) for 24 h at 150 revolutions per minute (rpm). The Enoki stalks (179 g) and the caps (17 g) were extracted with 500 mL and 150 mL respectively, of distilled water at RT for 24 h and 150 rpm. All the extracts were concentrated to 50 mL volume under reduced pressure in a rotary vapor (EYELA N-12001) before storing for 72 h in a freezer at -80°C (Thermo Scientific Forma 700 series). After the freezing period, the samples were freeze-dried for another 72 h in a freeze dryer (Labconco Freeze Dryer 79600311). The freeze-dried samples were homogenized in a mortar and stored at -20°C until then used for analysis. Before testing, the samples were re-dissolved in distilled water according to the desired concentrations.

Determination of total phenolic compound

The method of Slinkard and Singleton was used to determine the total soluble phenolic compounds in the water extracts of the mushrooms (Slinkard and Singleton, 1997). Briefly, 1 mL of the mushroom extracts at the concentration of 1 mg/mL was added into a 50 mL volumetric flask. The sample was diluted with 45 mL of distilled water followed by 1 mL of 2 N Folin-Ciocalteu reagent with vigorous shaking. After about 3 min, 3 mL of already prepared 2 % NaCO₃ was added to the flask and allowed for 2 h on a rotary shaker at 100 rpm. After 2 h, the absorbance of the green color was measured in a microplate reader (Infinite series M200) at 760 nm. Gallic acid was used as the standard phenolic compound. All the experiments were conducted in triplicates and the total phenolic concentration in the extracts determined as micrograms of Gallic acid equivalent (GAE) per milligram of water extracts from the standard Gallic acid standard graph:

$$\text{Absorbance (y)} = 0.0012 \times \text{total phenols } (\mu\text{gGAE mg}^{-1} \text{ of extracts})$$

$$(R^2 = 0.99)$$

Determination of total flavonoid content

The flavonoid content was determined using the method described by Meda et al (Meda et al., 2005). Briefly, 1 mL of 2 % AlCl₃ in absolute methanol was mixed with 1 mL extracts at the concentration of 1 mg/mL and incubated for 10 min. After 10 min at RT, the absorbance of the yellow color was measured using a microplate reader (Infinite series M200) at 415 nm. Quercetin was used as the standard. The total flavonoid content of the extracts was determined as micrograms of Quercetin equivalent (QE) per milligram from the standard Quercetin graph:

$$\text{Absorbance (y)} = 0.0032 \times \text{total flavonoid } (\mu\text{gQE mg}^{-1} \text{ of extracts})$$

$$R^2 = 0.96$$

DPPH radical scavenging

The 1,1-diphenyl-2-picryl-hydrazil (DPPH) reduction method described by Dorman et al and Li et al was used to determine the radical scavenging activity of the mushroom extracts (Dorman et al., 2004; Li, Yan, Xiaolin, Yi, Zheng, 2017). Briefly, DPPH solution was prepared in absolute methanol at the concentration of 126.5 μM. Then, 2 mL of the DPPH solution was mixed with 1 mL of varying concentrations (200, 400, 600, 800, and 1000 μg/mL) of the extracts in a glass tube. The mixture was shaken for about 30 sec and then incubated in the dark for 30 min at RT. The DPPH solution which was initially pink in color fades in color (pink to pale depending on the scavenger) when the antioxidants in the extracts donate hydrogen ions. The color change was monitored in a microplate reader (Infinite series M200) at 517 nm. Quercetin and ascorbic acid were used as the standard scavengers. All the experiments were performed in triplicates and the results presented as the mean of the readings. The percentage of DPPH radical scavenging was calculated from the equation below:

$$\text{DPPH scavenging effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A₀ is the absorbance of the negative control (2 mL of DPPH in methanol + 1 mL of methanol), and A₁ is the absorbance of the reaction mixture (2 mL of

DPPH in methanol + 1 mL of test samples) at varying concentrations/standard (Quercetin/ascorbic acid).

Determination of antibacterial activity

The antimicrobial activity of the extracts was tested against 4 bacteria (*Escherichia coli*, *Serratia marscescens*, *Bacillus subtilis*, and *Staphylococcus aureus*). Briefly, 5 mL of nutrient broth (Oxoid Basingstoke, Hampshire, England) sterilized at 121 °C for 15 min was added into sterile tubes with caps. Then, 1 mL of varying concentration of extracts (400, 800, 1200, 1600, and 2000 μg/mL) already prepared in sterile water was added to the test tubes. For the standard antibiotics, 1 mL of gentamycin at varying concentration (400, 800, 1200, 1600, and 2000 μg/mL) was added to the standard tubes. To all the tubes, 1 mL of broth containing about 1.0 X 10⁶ CFU/mL of the respective organism for each set of tubes was added. The control tubes contained 6 mL of the sterilized broth, 1 mL of sterile water. The experiments were carried out under Laminar flow in triplicates. All the tubes were incubated at 37 °C for 24 h. The absorbance of the broth cultures was measured at 600 nm in a microplate reader (Infinite series M200) and the percentage of inhibition calculated from the following equation:

$$\text{Bacterial inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A₀ is the absorbance of the control tubes (5 mL of sterile broth + 1 mL of sterile water + 1 mL of tested organisms), A₁ is the absorbance of tubes containing 5 mL of sterile broth, 1 mL of extracts/gentamycin at various concentrations, and 1 mL of tested organisms.

RESULTS AND DISCUSSIONS

The total phenolic content of the caps and stalks of the two mushrooms (Bunapi shimeji, and Enoki) is shown in Table 1. The total phenolic content in the water extracts of the caps and stalk of Enoki mushroom was (197.2 ± 4.8 μgGAE/mg and 105.6 ± 4.8 μgGAE/mg), respectively. Bunapi shimeji caps and stalk contained (125 ± 0.0 μgGAE/mg and 119.4 ± 4.8 μgGAE/mg), respectively of total phenolics. The antioxidant capability of mushrooms is due to the abundance of phenolic compounds such as flavonoids, phenolic acids, and tannins. These compounds exhibit various biological activities such as antimicrobial and anti-inflammatory (Nevcihan and Cengiz, 2010; Shanab, Shalaby, El-Fayoumy, 2011). The total phenolic content of the studied mushrooms was relatively high compared to the reports of other studies on the total phenolic compounds of edible mushroom (Marijana and Branislav, 2015). A high correlation between the biological activity of fungi and algae and their phenolic content have been reported (Sivakumar and Rajagopal, 2011; Demirel, Yilmaz-Koz, Karabay-Yavasoglu, & Sukatar, 2011), although some have reported no correlation (Heo Cha, 2005). From our study, we conclude that even though Enoki water extracts contained higher total phenolics, it did not reflect in their antibacterial activity against the tested organisms. Bunapi shimeji extracts with lesser phenolic compounds showed stronger antibacterial activity. This deviation could be attributed to the class of individual phenolic components that made up the estimated total phenolic compound.

Table 1. Total phenolic content of water extracts of Enoki and Bunapi shimeji mushrooms

Mushroom	Total phenolic content (μgGAE/mg of extract)	
	Caps	Stalks
Enoki	197.2 ± 4.8	105.6 ± 4.8
Bunapi	125.0 ± 0.0	119.4 ± 4.8

The values are given as the mean ± SD of three parallel measurements. GAE = Gallic acid equivalents.

Table 2. Flavonoids content of water extracts of Enoki and Bunapi shimeji mushrooms

Mushroom	Flavonoid content (μgQE/mg of extract)	
	Caps	Stalks
Enoki caps	113 ± .00	79.2 ± 3.6
Bunapi caps	143 ± 3.6	102.1 ± 4.0

The values are given as the mean ± SD of three parallel measurements. QE = Quercetin equivalents.

The flavonoids content of the caps and stalks of the mushrooms is shown in Table 2. The flavonoids content of the caps and stalk of Enoki mushroom was $(113.0 \pm 0.0 \mu\text{gQE}/\text{mg}$ and $79.2 \pm 3.6 \mu\text{gQE}/\text{mg}$), respectively while Bunapi shimeji caps and stalks contained $(143.0 \pm 0.0 \mu\text{gQE}/\text{mg}$ and $102.1 \pm 4.0 \mu\text{gQE}/\text{mg}$), respectively.

The radical scavenging activity of the water extracts is shown in Figure 1(A and B). The extracts from the caps of the Enoki mushroom showed better antioxidant activities than other tested caps, while Bunapi stalks extracts showed better antioxidant activities than the other tested stalks. Quercetin and ascorbic acid at the concentration of $300 \mu\text{g}/\text{mL}$ had DPPH radical scavenging activity of 68.8 ± 1.1 and $77.3 \pm 0.9 \%$, respectively.

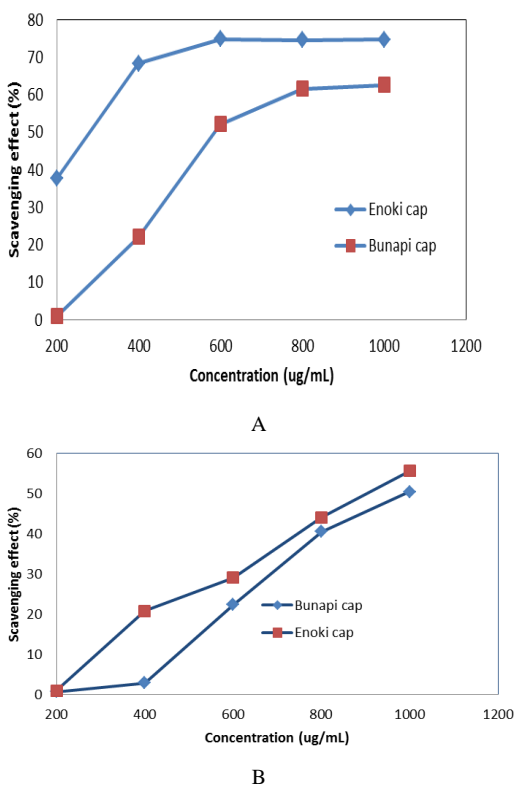


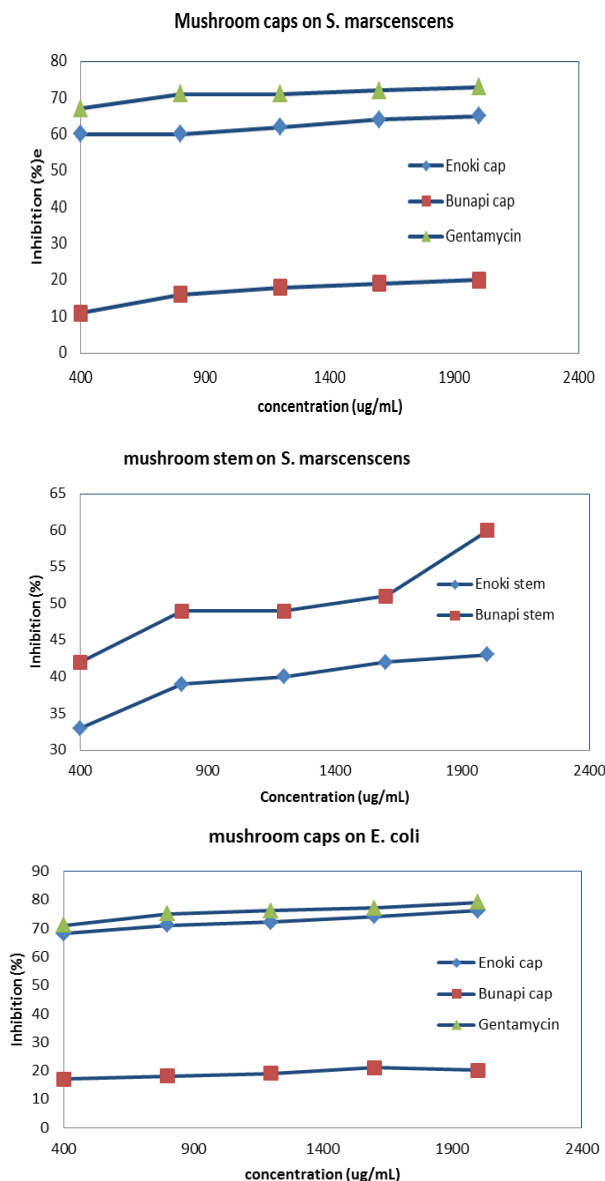
Figure 1: DPPH radical scavenging effect of A) caps, and B) stalks of Enoki and Bunapi mushrooms

Free radical scavenging is a mechanism for the inhibition of lipid oxidation by antioxidants, thereby preserving the body from the effects of ROS (Nevcihan and Cengiz, 2010). The extracts from the Enoki caps showed the strongest antioxidant activity at the tested concentrations. The antioxidant activities of the mushroom extracts increased with concentration increase. More than 70 % and 60 % of antioxidant activity were observed from the caps and stalks, respectively of the studied mushrooms. Quercetin and ascorbic acid at the concentration of $300 \mu\text{g}/\text{mL}$ had DPPH radical scavenging activity of 68.8 ± 1.1 and $77.3 \pm 0.9 \%$, respectively, which were relatively lower to the activity of the Enoki extracts. The strong antioxidant activity of the studied mushrooms could be attributed to their high total phenolic content as earlier reported (Sivakumar and Rajagopal, 2011).

The antimicrobial activity of the mushroom water extracts against tested bacteria is shown in Figure 2 (A-H). Enoki caps extracts and Bunapi stalks extracts showed good antibacterial activity against *Serratia marscescens* at a concentration range of $400 - 2000 \mu\text{g}/\text{mL}$. The extracts from the caps and stalks of Enoki showed good activities against *Escherichia coli*. Bunapi stalks extracts showed the greatest activity against *Escherichia coli* at the concentration range of $400 - 2000 \mu\text{g}/\text{mL}$. The activities of the extracts from the caps and stalks of all the studied mushrooms showed relatively lower activities against *Bacillus subtilis* and *Staphylococcus aureus* at the studied concentration range of $400 - 2000 \mu\text{g}/\text{mL}$. Gentamycin, the standard antibiotic used in the study at the same

concentration with the extracts ($400 - 2000 \mu\text{g}/\text{mL}$) showed a similar trend of activity against the tested organisms.

The permeability of antimicrobial agents depends on the cell wall composition of the organisms, can easily permeate through the poorly defended cell wall of G-bacteria, leading to increased sensitivity. Our findings are similar to other reports which suggested that the sensitivity of microorganisms to antimicrobial agents can be influenced by the cell wall composition of the organism (Albouchi, Hassen, Casabianca and Hosni, 2013; Kosanic and Rankovic, 2012). The water extracts from Bunapi shimeji showed stronger antibacterial activity than Gentamycin against *S. marscescens*, *S. aureus*, and *E. coli*, and lesser activity than Gentamycin against *B. subtilis*. These findings are the first reports on the antimicrobial activities of water extracts from both caps and stalks of Enoki and Bunapi shimeji mushrooms on *S. marscescens*, *S. aureus*, *E. coli* and *B. subtilis* although other mushrooms may have been studied and reported for microbial activities (Nevcihan and Cengiz, 2010; Farrera and Fadeel, 2015). The use of mushroom extracts as antimicrobial agents could eliminate the side effects of synthetic compounds in the human body. The activity of the crude extracts is promising and a possible fractionation of the extracts for possible identification of the major components responsible for the biological activities could shed more lights on the mechanism of action of these compounds.



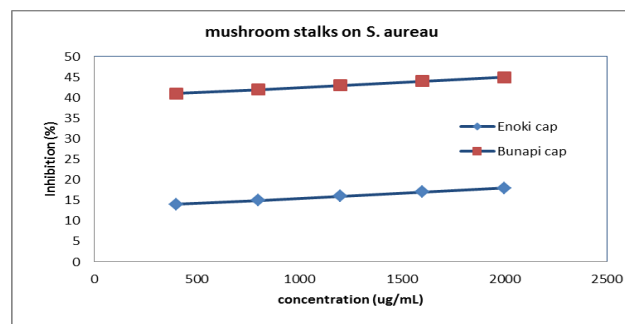
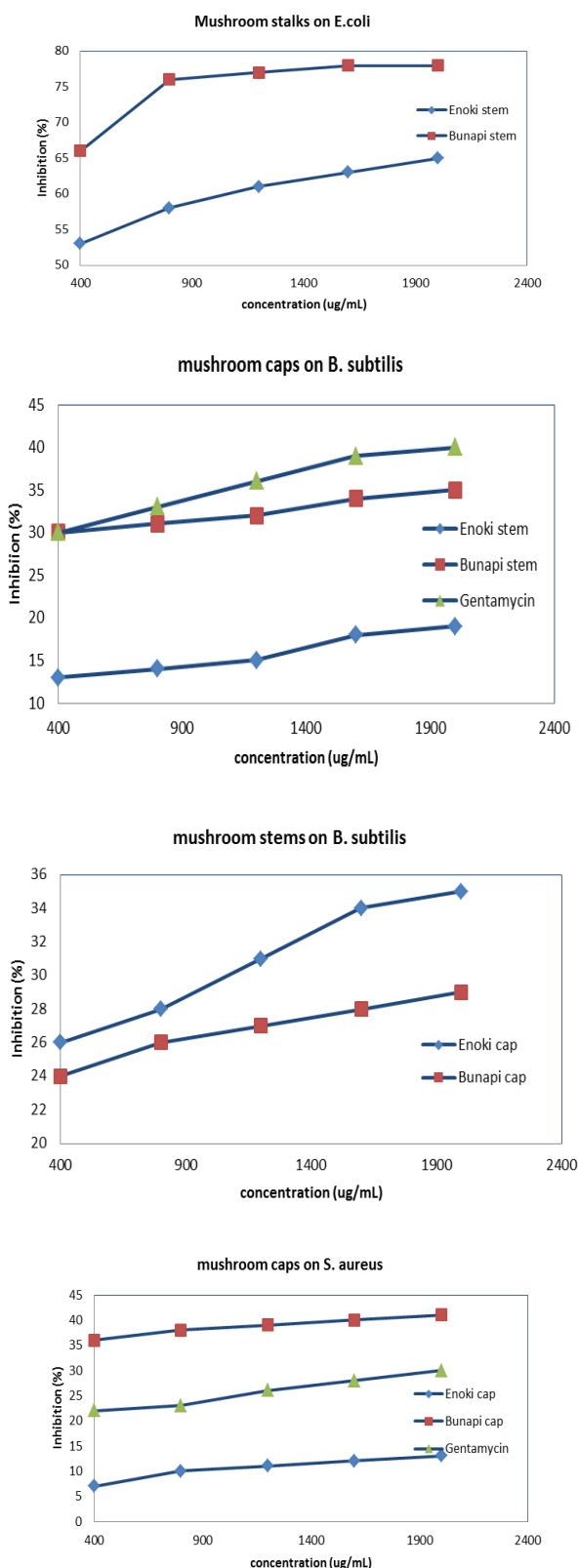


Figure 2: The antibacterial inhibition of (A) caps on *S. marscenscens*, (B) stalks on *S. marscenscens*, (C) caps on *E. coli*, (D) stalks on *E. coli*, (E) caps on *B. subtilis*, (F) stalks on *B. subtilis*, (G) caps on *S. aureus*, (H) stalks on *S. aureus*

Conclusively, the water extracts from the caps and stalks of the tested mushroom showed a considerable activity (especially from Bunapi shimeji) against the tested microorganisms. From the results, the mushrooms seem to be powerful natural antioxidants and antimicrobials with a reasonable amount of protein.

Conflict of interest

The authors declare no conflict of interest.

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