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PP-343 [Apitherapy]

Identification of Cough Suppressant Components in Honey: Flazin and a New Compound, Melpyrrole

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Objectives To evaluate the cough suppressant effect of honey in a controlled experimental setting and identify its active components

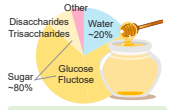
Background

Honey as cough suppressant

Honey has long been used as a folk remedy for cough, and numerous studies have demonstrated its ability to improve both the frequency and severity of cough compared to usual care.

2007	The first report: honey can soothe nighttime cough in children [1]
2010	Cochrane review: Honey probably relieves cough symptoms in children better than no treatment (updated: 2012, 2014, 2018; headquarters: London)
2011	A study shows honey or honey with coffee is effective to adult cough [2]
2015	Codeine was contraindicated in children under 12 in EU (US: 2017, Japan: 2019)

Some of the earliest records of using honey as a remedy for cough can be traced back to ancient Egypt.



○ Other proteins, lipids, minerals, vitamins, other organic compounds (phenolic acids, flavonoids, melanoidin).

What's the active component(s) in honey?

Honey contains about 80% sugar and about 20% water with minor and trace elements. Those composition depends on source plant, harvest season, and so on.

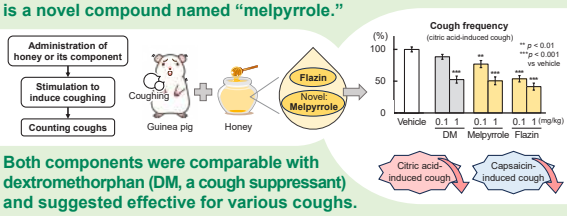
Polysaccharides, flavonoids and/or phenolic compounds have been reported to suppress inflammation in upper respiratory tract infection [3,4]. However, the active components and mechanisms of the cough-suppressing effect of honey have not yet been clarified.

[1] Paul IM, *et al. Arch Pediatr Adolesc Med.* 2007;161:1140-6.
[2] Raeessi MA, *et al. Iran J Otorhinolaryngol.* 2011;23:1–8.
[3] Šutovská M, *et al. Int J Biol Macromol.* 2009;44:236–9.
[4] Somerville VS, *et al. Adv Nutr.* 2016;7:488–97.

Key Findings

1 **In this study, we developed a system to evaluate the cough suppressant effects of honey and its components in guinea pigs.**

2 **Two active components were discovered for the first time, one of which is a novel compound named "melpyrrole."**



3 **Both components were comparable with dextromethorphan (DM, a cough suppressant) and suggested effective for various coughs.**

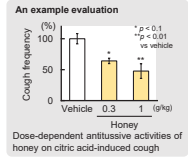
These findings provide aspects for evidence-based standardization of honey products and future development of a new type of cough medicine.

Tani H, *et al. J Agric Food Chem.* 2023;71:13805-13.

System Setup for Cough-Suppressant Evaluation

Method: Thirty minutes after intragastric administration of test samples, guinea pigs inhaled 100 mM citric acid or 10 μM capsaicin aerosol (5 min, ultrasonic nebulizer). Coughs were counted for 30 min from inhalation onset.

Figure 1. Citric acid- or capsaicin-induced guinea pig cough model



Dose-dependent antitussive activities of honey on citric acid-induced cough

Evaluation — Cough Suppressant Activity of Honey

Method: DM (positive control) and honey were tested using the evaluation system.

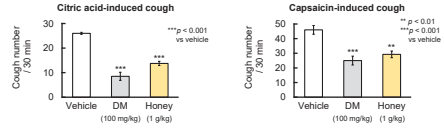


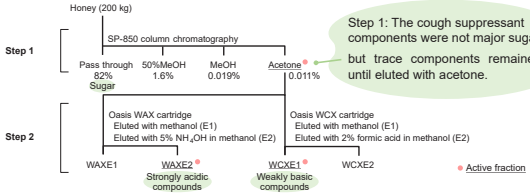
Figure 2. Antitussive activity of DM and honey in citric acid- and capsaicin-induced guinea pig cough models

DM and honey significantly reduced the cough number induced by citric acid (stimulates Aδ-fibers) and capsaicin (stimulates C-fibers).

Screening, Identification and Evaluation of Active Components

Screening 1 — Search for Active Fractions of Honey

Method: Honey was separated on Sepabeads SP-850 column chromatography and two types of ion-exchange cartridges, WAX and WCX. Cough suppressant activity of each fraction was evaluated.



Step 1: The cough suppressant components were not major sugars but trace components remained until eluted with acetone.

Figure 3. Activity-guided fractionation schemes of honey

Step 2: Amphoteric compounds (possessing both acidic and basic functional groups) have been suggested as active components.

Screening 2 — Search for Active Components

Method: Candidate active components were identified by LC/MS/MS-based multivariate analysis. Peaks common to active fractions with ≥2-fold change ($\log_2$ FC > 1) and $p < 0.01$ ($-\log_{10} p > 2$) were selected and shown in orange (○).

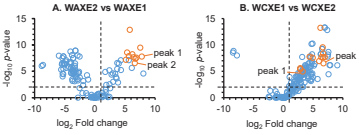


Figure 4. Volcano plot of the comparison for active fractions vs inactive fractions

Table 1. Candidate active components

	m/z	Estimated formula
peak 1	308.10	$C_{17}H_{13}O_3N_3$
peak 2	309.09	$C_{17}H_{12}O_4N_2$

Peak 2 (*m/z* 309.09), the most intense peak in the active fractions, was selected. Peak 1 (*m/z* 308.10), with a lower MS signal, differed from peak 2 by approximately one mass unit and had a formula in which one O atom replaced by an N atom.

Identification — Structural Determination of Active Components

Method: Structures were predicted based on the fragmentation patterns observed in LC-MS/MS and confirmed either by direct comparison with an authentic standard or by chemical synthesis.

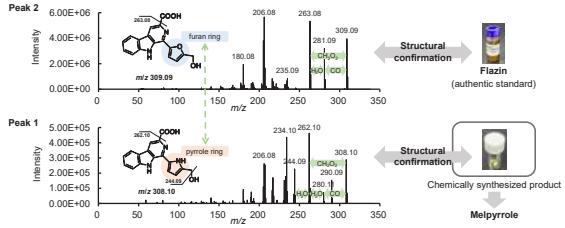


Figure 5. Positive ESI MS/MS spectra of candidate antitussive components in honey

Peak 2 was identified as flazin. Peak 1 was a novel compound in which the furan ring of flazin was replaced with a pyrrole ring, and named "melpyrrole."

Evaluation — Cough Suppressant Activity of Melpyrrole and Flazin

Method: DM, melpyrrole and flazin were tested using the evaluation system.

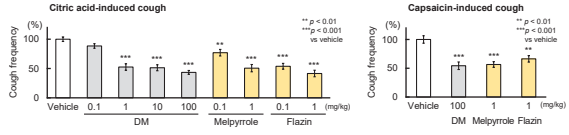


Figure 6. Antitussive activity of melpyrrole and flazin in citric acid- and capsaicin-induced guinea pig cough models

Melpyrrole and flazin suppressed cough in the citric acid-induced cough model, with results comparable to or even better than DM. They also significantly reduced cough in the capsaicin-induced cough model, suggesting broad-spectrum cough suppressant effects.

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BACKGROUND: Honey has long been used as a folk remedy for cough, and numerous studies have demonstrated its ability to improve both the frequency and severity of cough compared to usual care. However, the active components responsible for honey's cough-suppressing effect have not been elucidated.

OBJECTIVES: The objectives of this study were to evaluate the antitussive effect of honey in a controlled experimental setting and identify its active components.

METHODS: Antitussive effects of honey and its components were evaluated by in vivo experiments with guinea pigs, in which cough responses were induced by inhalation of citric acid (which stimulates Aδ-fibers) or capsaicin (which stimulates C-fibers). Fractionation of honey was conducted using Sepabeads SP-850 and anion-exchange column chromatographies. Screen for candidate active components and prediction of their structures were performed based on the fragment patterns observed in LC MS/MS and subsequently confirmed either by direct comparison with authentic standards or by chemical synthesis.

RESULTS AND DISCUSSION: Acacia honey, administered at 1 g/kg, significantly reduced the frequency of cough induced by both citric acid and capsaicin compared with vehicle-treated controls. These findings suggest that intragastrically administered honey suppresses both types of cough evoked by the stimulation of Aδ- and C-fibers, as well as dextromethorphan (DM). LC-MS/MS-based multivariate analysis of the honey fractions yielded two active candidate components. One component was identified as flazin by direct comparison with an authentic standard. The other was estimated to be a novel compound in which the furan ring present in flazin is replaced by a pyrrole ring, as deduced from its LC-MS/MS fragment patterns. The structure of this novel compound was subsequently confirmed through chemical synthesis, and it has been named “melpyrrole." When administered individually at doses of 0.1 and 1 mg/kg, both flazin and melpyrrole exhibited significant antitussive activity in guinea pigs, with effects equal to or greater than those observed with DM. These findings provide a basis for the evidence-based standardization of honey products and would lead to the future development of a new type of cough medicine.