# Solvent polarity fractionation for extracting mulberry leaf powder as silkworm attractant

Jia-Ying Liu[1], Jia-Le Tian[1], Zhan-Peng Zhang[1], Lu-Chan Gong[1, 2], Jun Wang[1, 2, *]

[1] Jiangsu Key Laboratory of Sericultural and animal biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, China.
[2] Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture and Rural Affairs, The Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212100, China.

Keywords: *Bombyx mori*, Extract, Attractant; Pheromone, Behavioral experiment

Presenting author email: wangjun@just.edu.cn

Among various environmental cues, chemical information is the most important factor for insect survival and reproduction. Insects usually rely on pheromone signals in homogenous communication, the main signals are sexual, gathering and feeding signals (Ono et al.,2021). Current research on insect attraction has focused on sex pheromones and aggregation pheromones: it has been shown that pregnant females of *H. armigera* (Hubner) have a mixture of pheromone mixtures (Z) -11-cetolein and (Z) -9-cetolein, eliciting a stronger response than unmated males (Bakthavatsalam et al., 2017). The clustering pheromones of *Locusta migratoria* and *Schistocerca gregaria* were also identified. Locustol (2-methoxy-5-ethylphenol) has been identified from the feces of migrating *L. migratoria* and is thought to play an active role in traits associated with the social period (Nolte et al.,2017). However, there are few studies on the application of plant-derived feeding pheromones in artificial feed promotion. The development of new insect feeding attractors can not only attract insects but also provide the effect of promoting feed intake, which is the need of green and sustainable development.

At present, the main methods for analyzing the quality of plant-derived attractants include volatile matter analysis, extraction component analysis, etc. (Cagliero et al.,2023). For example, there have been studies that analyzed the attractants of cotton bollworm by means of GC-MS isolation, transcriptomic sequencing, and taxis experiment. A four-component mixture with strong attractant effect was identified, which provided ideas for the development of a new poison bait for the olfactory pathway of cotton bollworm (Di et al.,2017). Another study used supercritical $CO_2$ to extract fat-soluble components from wood through GC-MS separation and behavioral analysis. Synthetic-mellein has been identified as an effective attractant of *Tribolium confusum* (Hori et al.,2017). Therefore, it can be seen that the separation of components and the analysis of components is a reliable means to dig plant source attractants, and the development of a new separation method also provides a theoretical basis for the separation of attractants.

In this study, organic reagents of different polarities were used to extract mulberry leaves in different stages, and behavioral experiment were carried out on silkworms to obtain graded components with different attractant effects on silkworms. A better attractant component was selected through comparative analysis between extracts of different polarities. Among them, the separation method of different polarity is according to the difference of polarity and solubility, petroleum ether, ethyl acetate, chloroform and methanol were used to extract the fat-soluble components from mulberry leaves.

Table 1. Extraction efficiency and post-extraction residue of mulberry leaf powder

| Extractive | Petroleum ether extraction | Ethyl acetate extraction | Acetone extraction | Methanol extraction |
|---|---|---|---|---|
| Extraction efficiency (%) | 7.55±0.2 | 3.82±0.35 | 2.53±0.5 | 14.84±1.2 |
| Post-extraction residue (g) | 92.54±0.2 | 89.7±0.32 | 86.75±0.45 | 73.87±1.04 |

As shown in Table 1, four kinds of extracts were obtained by fractional extraction: petroleum ether extract, ethyl acetate extract, acetone extract and methanol extract. The extraction rate of methanol extract was 14.84%, and the extraction rate of petroleum ether extract was 7.55%. Although the extraction rate of methanol is higher, the solid solution content of petroleum ether is higher.

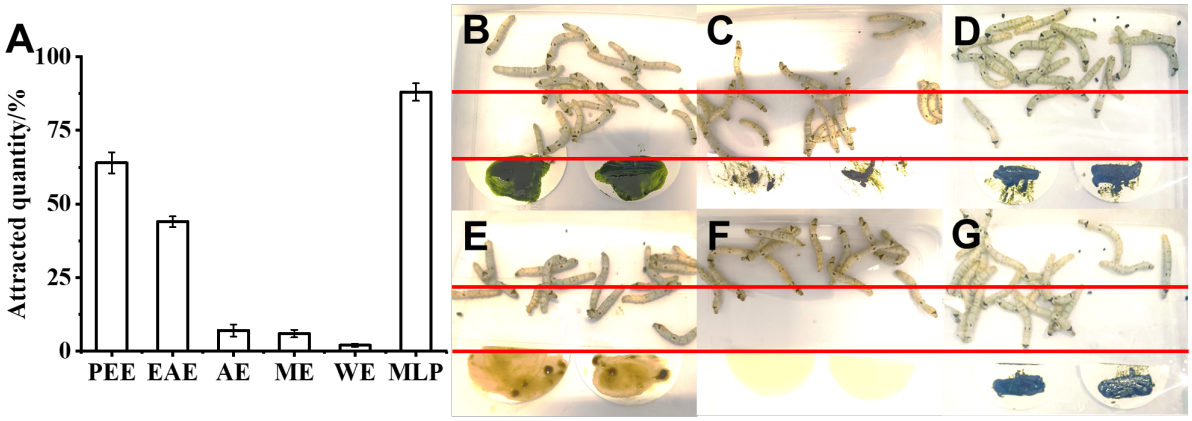


Figure 1. Attractant effect of different extract components on *Bombyx mori* (A)
PEE: petroleum ether extract (B), EAE: ethyl acetate extract (C), AE: acetone extract (D), ME: methanol extract (E), WE: water extract (F), MLP: mulberry leaf powder (G)

Figure 1 shows the attractive effects of different extracts extracted by different polarities on silkworms, among which petroleum ether extract has the strongest attraction and attracts the largest number of silkworms, followed by ethyl acetate extract, while acetone and methanol extract have only weak attraction. The water extract was not attractive at all, while the mulberry leaf group as a control was the most attractive. Petroleum ether can usually extract oil, chlorophyll, volatile oil and other substances in plants (Tian et al.,2018). In this experiment, the low-polarity fat-soluble substance extracted from petroleum ether has the best attraction effect on silkworm, while the water-soluble extract has basically no attraction effect, which is consistent with the reported that the substance attracting insects is often fat-soluble volatile matter. Therefore, in this experiment, the fat-soluble substance extracted from petroleum ether has the best attraction effect on silkworm. However, the analysis of the compounds in the components still needs further research.

This study established a new classification method for silkworm feeding attractants, and obtained the components with good feeding effect. The components were subsequently detected by GC-MS to obtain specific compounds, and the verification of the compounds needed to be further studied, providing a new classification method for component extraction and a new idea for the development of novel silkworm feeding attractants.

**Funding:** This work was supported by the Jiangsu Agricultural Science and Technology Innovation Fund (CX(20)2029).

**References:**

[1] Alcantara, A R, de Maria P D, Littlechild J A, Schurmann M, Sheldon R A, Wohlgemuth R. Biocatalysis as key to sustainable industrial chemistry [J]. *Chemsuschem*, 2022, 15(9): e202200709.
[2] Domach M M. Perspectives and prospects for whole cell catalysis[J]. *Catalysis Letters*, 2015, 145:346-359.
[3] Gallus S, Peschke T, Paulsen M, Burgahn T, Niemeyer C M, Rabe K S. Surface display of complex enzymes by in situ SpyCatcher-SpyTag interaction[J]. *Chembiochem*. 2020, 21:2126-2131.
[4] Hatlem D, Trunk T, Linke D, Leo J C. Catching a SPY: Using the SpyCatcher-SpyTag and related systems for labeling and localizing bacterial proteins[J]. International Journal of Molecular Sciences, 2019, 20(9):2129.
[5] Pinto A, Contente ML, Tamborini L. Advances on whole-cell biocatalysis in flow[J]. *Current Opinion in Green and Sustainable Chemistry*. 2020, 25:100343.
[6] Plavec TV, Berlec A. Surface anchoring on lactococcus lactis by covalent isopeptide bond[J]. *Acta Chimica Slovenica*. 2019, 66:18-27.
[7] Ye M, Ye Y, Du Z, Chen G. Cell-surface engineering of yeasts for whole-cell biocatalysts[J]. *Bioprocess and Biosystems Engineering*. 2021, 44:1003-1019.
[8] Tian C, Gao X, Yang J. Chemical compositions, extraction technology, and antioxidant activity of petroleum ether extract from *Abutilon theophrasti Medic.* leaves[J]. *International Journal of Food Properties*, 2018, 21(1): 1789-1799.