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Antibacterial and antifungal effects of Korean propolis against ginseng disease

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Abstract

We investigated the anti-microbial activity of propolis against the pathogenic bacteria and fungi on ginseng. We selected six microbials that caused postharvest root rots in ginseng. Propolis extracts were prepared by using the ethanol extraction method. We seeded the bacteria and fungi related to ginseng disease on a specific culture medium, and treated it with propolis extracts by using the paper disc method. Propolis extracts indicate the anti-microbial activity against *Paenibacillus polymyxa*, *Fusarium solani*, *Rhizoctonia solani* AG-1 and *Pythium ultimum*. However, the anti-fungal activity of propolis is weak on *Pseudomonas fluorescens* subsp. *Cellulosa* and *Colletotrichum gloeosporioides*. As a result, the antimicrobial effects of propolis against microbial that prevent ginseng growth were confirmed. The antimicrobial effects are shown according to the concentration of propolis against root rot. The fungi also showed antibacterial effects in a dose-dependent manner.

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Introduction

Panax ginseng(ginseng) is a perennial herb of the family Araliaceae (Choi, 2008; Yun, 2001). The roots of ginseng have been used a health functional food. Various medicine and pharmacological effects of ginseng such as anti-cancer, anti-circulatory shock effects, promotion of hematopoiesis and regulation of immune has been studied recently (Shin *et al*., 2006; Sung *et al*., 2000; Tode *et al*., 1999; Yu *et al*., 2003). These functions are mostly derived from the saponin of a ginseng root (GuangLie *et al*., 2013; Wang *et al*., 2013). Due to these health functions of ginseng, the ginseng agriculture focused on increasing the saponin content and control of ginseng disease such as postharvest root rots. In particular, postharvest root rots has been a major concern for ginseng farmers.

Propolis is a resinous material produced by honeybee through mixing saliva and beeswax with a variety of trees and plants exudate. Honeybee uses the propolis to protect their hive (Burdock, 1998). Propolis contains many physiologically active substances such as flavonoids and phenolic compounds which is antioxidant, anti-inflammation, anti-tumor, and anti-microbial activities (Cavalaro *et al*., 2019; Duca *et al*., 2019; Jeong, 2004; Khayyal, 1993; Kim *et al*., 2018; Kunimasa *et al*., 2010; Sforcin, 2000). It has also been used by the early civilization to cure

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wounds due to its functional properties. Especially, anti-microbial activity of propolis is widely known and, it is commonly used in the industry and medicine (El-Tayeb *et al*., 2019; Nazeri *et al*., 2019; Przybylek and Karpinski, 2019). In agriculture and food industry, propolis also has been applied to plant growth and food preservation.

In this study, our data suggested that propolis, a honeybee product, could be possible to protect ginseng from the ginseng disease bacteria and fungi. Six species of bacteria and fungi were tested for anti-microbial activity of propolis. Propolis has anti-microbial activity for *Paenibacillus polymyxa*, *Rhizoctonia solani* AG-1, *Fusarium solani* and *Pythium ultimum* on dose-dependent. However, *Pseudomonas fluorescens* subsp. *Cellulosa* and *Colletotrichum gloeosporioides* had not anti-microbial activity. Our results suggest that propolis can be used as a protection agent against ginseng disease bacteria during growth stage of ginseng.

Materials and Methods

Propolis extracts

To extract 1 kg of raw material propolis, 3.5 L of 80% ethanol was used. The extracted solution was filtered by using Whatman No. 2 Filter paper. After removing the impurities, extracted propolis was concentrated to 18%, and its total flavonoid content has 1%.

Bacteria and fungi selection

The microorganisms that were distributed at the Korean Agricultural Culture Collection (KACC) were *Paenibacillus polymyxa* (KACC 10098), *Pseudomonas fluorescens* subsp. cellulosa (KACC 10195), *Fusarium solani* (KACC 43387) and *Pythium ultimum* (KACC 40705) caused postharvest root rots. *Rhizoctonia solani* AG-1 (KACC 40101), *Pythium ultimum* (KACC 40705) and *Colletotrichum gloeosporioides* (KACC 40003) caused damping-off and bitter rots respectively. Among the microbial, *Paenibacillus polymyxa* and *Pseudomonas fluorescens* subsp. *Cellulosa* were classified as bacteria. While *Fusarium solani, Pythium ultimum*, *Rhizoctonia solani* AG-1 and *Colletotrichum gloeosporioides* were classified as fungi. The six species of bacteria that were selected and tested in this study caused serious damage to ginseng farms.

Inoculation of the bacteria and fungi

To inoculate the microbial, we prepared a bacteria culture medium by adding 20 g potato dextrose agar medium (PDA, BD biosciences) to 1 L of distilled water. This culture medium was autoclaved for 15 min at 121℃ on 2 psi. After autoclave, the culture medium solidified on a 10x10 mm petri dish.

The bacteria and fungi inoculation method was different. Bacteria species were spread on to culture medium with spreader. Fungi species were inoculated onto center of culture medium with platinum loop. The period for bacteria culture was two days and while it took seven days for fungi.

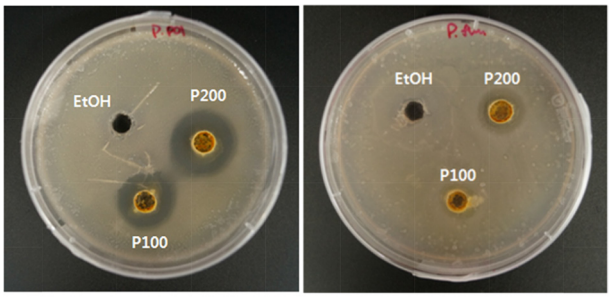
Verification of the anti-microbial activity

To investigate the anti-microbial effects of propolis, we used the filter paper disc method. Paper disc was purchased from ADVANTEC (8 mm, JAPAN). The filter paper disc was placed on top of a bacteria-spread medium, and the propolis was dropped on the paper at 100 and 200 μg. In the case of the fungi, the filter paper disc was placed on the center where the fungi are inoculated, and the propolis was dropped on the paper at 50 and 100 μg. An absolute EtOH was used as a solvent control for propolis extracts. We then confirmed the hollow for bacteria and the growth suppression for fungi culture.

Results and Discussion

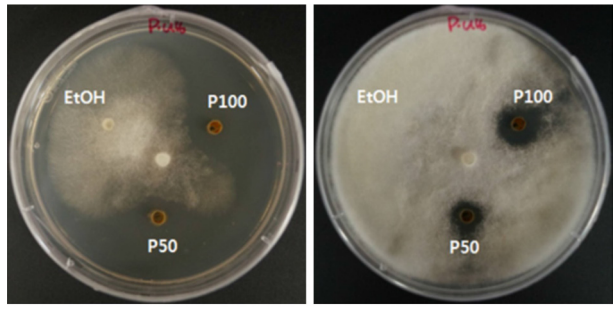
Anti-bacterial effects of propolis

The anti-bacterial activity of propolis was observed in *Paenibacillus polymyxa*. The hollow appeared in *Paenibacillus polymyxa* on dose-dependent. This means 100 μg of propolis is sufficient for anti-bacterial activity. However, propolis does not have anti-bacterial activity for *Pseudomonas fluorescens* subsp. *Cellulosa*. Even though 200 μg of propolis will be used, it still has a very weak hollow, which means that propolis does not indicate an anti-microbial activity for for *Pseudomonas fluorescens* subsp. *Cellulosa* (Fig. 1).



Paenibacillus polymyxa *Pseudomonas fluorescens* subsp. *cellulosa*

Fig. 1. The anti-bacterial effects of propolis for *Paenibacillus polymyxa* and *Pseudomonas fluorescens* subsp. c*ellulosa* after the spread of bacteria and treated with propolis on top of filter paper disc on dose-dependent.



2 days 7 days

Pythium ultimum

Fig 3. Maintenance of propolis activity wherein a *Pythium ultimum* was seeded on center of culture medium and propolis disc was set around the fungi. The duration of propolis activity was observed for seven days.

Anti-fungal effects of propolis

In the case of fungi, propolis prevents its growth on dose-dependent. As shown in Fig. 2, all fungi grow up in the ethanol treatment disc as a control. Especially, *Rhizoctonia solani* AG-1 and *Fusarium solani* did not grow in the disc containing propolis. These results showed that propolis has anti-microbial activity both in bacteria and in fungi. The anti-fungi activity of propolis is 100 μg. Despite that, propolis has a weak anti-microbial activity for *Colletotriclum gloeosporioides*.

We confirmed the anti-fungal activity of propolis on dose-dependent manner. Further, we confirmed how long the anti-fungal activity of propolis is. We seeded *Pythium ultimum* on the center of the medium and paper disc containing propolis set up around fungi. Then, we observed the growth of *Pythium ultimum* in seven days. As shown in Fig. 3, propolis inhibits growth of *Pythium ultimum* until seven days. This means that anti-fungal activity of propolis can be maintained for a longer period of time.

In this study, we confirmed that anti-microbial activity causes ginseng disease bacteria and fungi. The growth of bacteria and fungi were inhibited by propolis on dose-dependent conditions. In addition, propolis activity can be kept over time, which means propolis is an excellent anti-microbial and fungal agent for ginseng cultivation. This will help the stable cultivation of ginseng, and eventually increase the benefit of ginseng farmers.

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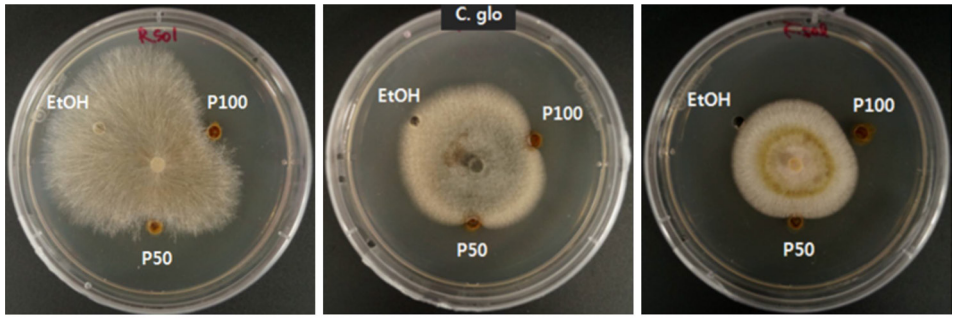

Rhizoctonia solani* AG-1** ***Colletotrichum gloeosporioides ***Fusarium solani***

Fig. 2. Three samples of propolis that inhibit fungi growth using a propolis-treated paper disc on dose-dependent (50 μg and 100 μg). Each fungus was seeded on center of culture medium, than propolis extract was placed around the fungi seeding.

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