ANTIBACTERIAL, ACTIVITY OF PROPOLIS FROM DIFFERENT LOCALITY OF TURKEY

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Abstract - Propolis has traditionally been used in curing infections and healing wounds unburns.Current researches have shown that propolis has antibacterial, anti-fungal and antiviral actions however, This activity of propolis is highly variable depending on its geographic origin. In this in-vitro study, the antibacteriall activity of theme than olicextracts of the Turkish propolis from Sivas and Tokat provinces was evaluated against Xanthomonasaxonopodispv. phaseoli, Xanthomonas campestrispv. vesicatoria, Pseudomonassyringae pv. tomato, Pseudomonas savastanoipvphaseolicola, and Clavi bactermichiganensis sub. sp. michiganensis at different (5%, 3%, 1% and0.5% v/v) concentrations. Sivas and Tokat propolisextracts showed similar pattern of inhibitory effects on the bacterial species at each concentration. Both propolis extracts exhibited 100% growth inhibition on the bacterial species tested at 5% v/v concenratin and efficasy was decreased with decrease in concentrations. At the lowest dose (0.5%) inhibition rate of 55.43% was obtained on X. campestrispv. vesicatoria. It seems that propolis has exellent antibacterial activitiy.

Keywords - Propolis; antibacterialactivity,Xanthomonas, Pseudomonas,Clavibacter

I. INTRODUCTION

For centuries honey bees and their products attracted the attantions of mankind; A large number of different products were produced using honey bees and these products were utilized in different forms. Propolis, one of the honey bee products, is extremely important both for its antimicrobial properties as well as its health benifit because of its important vitamins, minerals and elements contents. To date, about 180 different compounds have been identified in propolis. The chemical content of propolis is highly variable depending on the plant species visited by the honey bees. In addition, the variability of bee wax content also affects the chemical content of crude propolis (Crane, 1990). Propolis contain B1, B2, C and vitamin E. Also in propolis, copper, calcium aluminum, strontium and vanadium elements and myristic acid, benzoic acid, benzyl alcohol, caffeic acid, vanillin, cinnamic acid, acacetin, camphoride and isovanilin have been determined. (Speciale, et al., 2006; Bankova, et al. 1982; Aksoy and Dığrak, 2006). Propolis is expressed as resinous substances secreted from the leaves, buds and stems of many different plants. These resinous materials collected by the worker bees are transported in pollen baskets of bees and stored in different places of the hive and used by bees at appropriate times and places (Ghisalberti, 1979; Krell, 1996). According to the season, honey bees are mainly used the propolis for disinfection of honeycomb and barrel parts in the hive as well as for covering the openings in the hood (Ali, et al., 1991). Because of their important contribution to the health of honey bees, propolis is a very important product for bees. Raw propolis contains approximately 40-60% resin and vegetal balsam (phenols, phenolic acids, esters flavanons, dihydroflavonones, flavones, flavonols, chalcones, phenolic glycerides, aliphatic and phenolic acids, alcohols, aldehydes and ketones), 20-35% wax, 5% essential oils (mono and sequiterpenes), 5% pollen and 5% minerals (Ca, Mg, K, Na, Fe, Cu, Zn, Mn) and other organic substances (Doğan and Hayoğlu, 2012). Propolis is solid and brittle in cold and soft and sticky in warm conditions. The propolis has a wide range of colors ranging from light yellow, green and dark brown depending on the plant source. Propolis can partially dissolve in ether, chloroform, acetone and other organic solvents and 95% alcohol (Bogdanov, 2012; Kumova et al., 2002; Tekeli, 2007; Kara et al., 2014)

Propolis is a bee product used in medicine, dentistry, and cosmetics industry because of it's so many different biological activities such as antibacterial, antifugal, antiviral, anti-inflammatory, anti tumor and local anesthetic effects (Bankova, et al., 2000).In the propolis extract, the antimicrobial effect of compounds such as Pinobanksin-3-acetate (flavonoid), Benzyl p-coumarate, Pinocemprin, Galangin and caffeic acid was determined.Significant antimicrobial effects of propolis against Bacillus subtilis, Staphylococcus aureus, Candida albicans and Trichophyton mentagrophytes were observed (Starzyk, et al., 1977)

This study was aimed at evaluation of anti bacterial activities of Sivas and Tokat propolis methanolic extracts against plant pathogenic bacteria, Clavibacter michiganensis sub.sp. michiganensis (Cmm), Pseudomonas syringae pv. tomato (Pst), Xanthomonas axonopodis pv. vesicatoria (Xav), Xanthomonas axonopodis pv. phaseoli (Xap) and Pseudomonas savastanoi pv. phaseolicola (Psp)

II. MATERIAL AND METOD

One isolate from each of Clavibacter michiganensis sub.sp.michiganensis (Cmm), Pseudomonas syringaepv. tomato (Pst), Xanthomonas axonopodispv. vesicatoria (Xav), Xanthomonas axonopodispv. phaseoli (Xap) and Pseudomonas savastanoipv. phaseolicola (Psp) was used in this study. These bacteria were obtained from Çukurova University, Department of Plant Protection and Black Sea Agricultural Research Institute.

Approximately 100 g of propolis collected by Apismellifera, were obtained directly from the beehives from Tokat and Sivas province of Turkey. The samples of propolis were dried and ground to a fine powder. Methanolic extracts of propolis (PME) were prepared by adding 100 g of the speciments of propolisto 200 ml obsulate methanol in 500 ml flask and shaking on an orbital shaker (at 120 rpm) for one day. After extraction, the mixture was passed through four- layer of cheese clothes to seperate solid materials. The methanol was evaporated at 32 ^{0}C using rotary evaporator. The solid phase was disolved in 10% acetone to obtain 20% concentration of propolis extract as an stocksolution (Takaisi-Kikuni, et al. 1994).

All bacterial strains were cultivated for 2-3 days at 28°C on King B plates2-3 days and the bacterial cultures were diluted in sterile saline and the optical density was adjusted to the inoculum dencity of 1x10^{6}cfu/ml. Each bacterial suspension was streak on surface of plates containing King B, amended with 5%, 3%, 1%, and 0.5% propolis extracts. All tests were performed in dublicate, using a 10% acetone solution without propolis as a control to test the inhibitory effect of the solvent. Rifampicin (33.3 µg/ml) was also used to compare the efficiency of propolis with antibiotic. The propolis and antibiotic were added to King B medium after auto claving when the agar had cooled to approximately 45 ^{0}C. There were three replicates per species at each propolis concentration. After 2-3 days of incubation period at 28 ^{0}C in darkness, The results were read spectrophotometrically. The percentage growth inhibition with respect to the control was calculated as,

P = (C-T/C) x100,

where, P is Inhibition (%), C is Average growth in the control (mm) and T is average growth in the treated ones (Yanar et al., 2005).

Statistical analysis: The data significance test was performed at 5% level (Tukey's multiple range test) using SPSS.

III. RESULTS and DISCUSSION

In the present study, the antibacterial activity of methanol extracts of propolis collected at Sivas and Tokat provinces of Turkey is reported and the impact of collection site on the biological activities was evaluated. The bacterial susceptibility to propolis varied and considering the highest growth inhibition rate measured for each one when 5% of extract was applied. The Xanthomonas species tested were the least susceptible when the Sivas methanolic extracts of propolis were used at 0,5% and 1% concentrations (Table 1), However, the only Xav was the least susceptible bacterial species to Tokat propolis extracts at 0,5% and 1% extract concentrations (Table 2). The controls (10% acetone) did not show an inhibitory effect on any of the test bacteria. Good dose responses were observed for the inhibitory effects of the propolis extracts. Both Sivas and Tokat propolis extracts gave similar results that of the rifampicin (33.3 µg/ml) at 3% and 5% concetrations (Table 1 and 2). In general, Clavibacter michiganensis sub. Sp .michiganensis, Pseudomonas syringae pv. tomato and Pseudomonas savastanoi pv. phaseolicola were more susceptible to both propolis extracts than the Xanthomonas species at 0,5% and 1% concentration but at concetrations of 3% and 5% there were no significant differences among bacterial species tested. All the bacterial species tested exhibited over 90% growth inhibition at 3% and 5% propolis extracts concentrations (Table 1 and 2). Propolis contain some phenolic substances which include cinnamic acid derivatives and some flavonoids (Marcucci et al., 2001). The main compounds available in propolis are acids such as caffeic, p-Coumaric acid, 3-prenyl-4-hydroxycinnamic, 3,5-diprenyl-4-hydroxycinnamic, 2,2- dimethyl-8-prenyl-2H-1-benzopyran-6-propenoic, a kaempferol derivative (flavonoid) and 2,2-dimethyl-6- carboxyethenyl-2H-1-benzopyran (Bankova et al., 2000; Marcucci et al., 2001(Burdock, G.A., 1998; Takaisi-Kikuni, et al. 1994; Albayrak S., and Albayrak S., 2008).

Dose (%)	**GrowthInhibitionRates (%)**				
	Cmm[1]	**Pst**	**Xav**	**Xap**	**Psp**
Con(10% ace)	0 a[2]	0 a	0 a	0 a	0 a
0,5	99,71 b	98,45 b	55,43 b	55,52 b	98,88 b
1	99,75 b	98,99 b	68,73 c	78,73 c	99,42 c
3	99,78 b	99,64 b	98,40 d	99,57 d	99,63 cd
5	100 c	100 b	100 d	100 d	100 d
Anti. (rifa.)	100 c	100 b	100 d	100 d	100 d

Table 1. Antibacterial activity activities of different concentrations of Sivas propolis methanol extract on the plant pathogenic bacteria.

[1]Clavibacter michiganensissub.sp.michiganensis(Cmm),

Pseudomonas syringaepv. tomato (Pst), Xanthomonas axonopodispv. vesicatoria (Xav), Xanthomonas axonopodispv. phaseoli (Xap) and Pseudomonas savastanoipv. phaseolicola (Psp)
[2]Means followed by same letter in each columnare not significantly different at p = 0.05 by Tukey multible comperison test. Percentage (%) growth inhibition was calculated with comparison to thegrowth of the control.

The antibacterial activity of propolis may be related to the presence of flavonoids (Bosio et al., 2000). Several researchers have reported that some foodborne bacteria and fungi may be inhibited by propolis extracts (Grange & Darvey, 1990; Marcucci et al 2001; Özcan, 1999, 2000). Takaisi-Kikuni and Schilcher (1994) and Nieva Moreno et al. (1999) have shown that propolis extracts have antibacterial properties against some micro-organisms. Basım et al. (2006) reported that all the propolis samples and polen used in their experiments were active against the plant pathogenic bacteria test strains.

The antibacterial properties would warrant further studies on the in vivo applications of propolis against plant apthogenic bacteria.

Dose (%)	**GrowthInhibitionRates (%)**				
	Cmm[1]	**Pst**	**Xav**	**Xap**	**Psp**
Control(10 % ace)	0 a[2]	0 a	0 a	0 a	0 a
0,5	94,17 b	98,82 b	34,63 b	99,26 b	98,43 b
1	95,46 b	99,46 bc	73,63 c	99,36 b	99,43 c
3	97,66 c	99,56 bc	98,06 d	99,60 b	99,46 c
5	100 c	100 d	100 d	100 c	100 c
Anti. (rifam.)	100 c	100 d	100 d	100 c	100 c

Table 2. Antibacterial activity of different concentrations of Tokat propolis methanol extract on the plant pathogenic bacteria.

[1]Clavibacter michiganensissub. Sp.michiganensis (Cmm), Pseudomonassyringaepv. tomato (Pst), Xanthomonas axonopodispv. vesicatoria (Xav), Xanthomonas axonopodispv. phaseoli (Xap) and Pseudomonas savastanoipv. phaseolicola (Psp)
[2]Means followed by same letter in each column are not significantly different at p = 0.05 by Tukey multible comparison test. Percentage (%) growth inhibition was calculated with comparison to the growth of the control.

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