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RESEARCH ARTICLE

Studying Inhibitory Effect of William Pear and Propolis against *Alernaria Alternate* with Diagnosis of Some Active Compounds

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Abstract

Alternaria alternate was isolated from orange juice, using potato dextrose agar (PDA) media, incubated for 5 days at 27 co, the evaluating anti fungal effects were seven different concentration (1.25, 2.5, 5, 10, 15, 20, 25) mg / ml of the extract alcohol: (William pear extract = WPE) and (Propolis extract = PE), the inhibitory efficacy of each extract against the fungus was increased by increasing its concentration. Also observed that the inhibitory activity of (PE) was greater than (WPE).The separation process of (PE) showed a presense: Flavone, 3-hydroxy flavone and Quercetin and absense these compounds in (WPE).

Keywords: *Propolis, Wiliam pear, A. alternate, TLC.*

Introduction

The most common method in recent years to prevent or inhibit the growth of microbes in foods is the use of natural products that work at the same time as flavors [1].William pear is rich in fruits, and contains many fiber and antioxidants, making it an important fruit for the body, it earns the strength and immunity to resist many diseases that may exposed to it. There are many benefits to the human body when eating pear fruit: Free Molecules Fight [2], preventing heart disease [3], Cancer protection, Control blood sugar [4], Strengthening the immune system, increase the power rate and Facilitate digestion [5].

William pear contents vitamin, vitamin K, potassium, calcium, iron, magnesium, riboflavin, vitamin B-6, and folate. Pears, especially those with red skin, also contain carotenoids, flavonols, and anthocyanins [2]. Propolis is a resinous substance collected by bees from trees and used a dielectric material in the cell to make the cell more insidious [6].The bees collect the resin particles from the stems, stalks, leaves, and various vegetable parts of Pine, Willow, Poplar and other trees, and with their cocoons, enter into the cell and mix with wax and salivary secretions, the propolis is produced annually by 10-300 g/h, Varies depending on the type of bees, the climate and the geographical area from which they were collected [7, 8] and [9].

We also find that the propolis varies in color from yellow to bleached to the brown and taste (bitter, bitterness, flavored) according to the geographical area and season in which it was collected [10].Propolis is collected by beekeepers through the process of traps. The traps are used when there are large amounts of propolis in order to reduce the degree of contamination or by certain tools used for scraping [7].

Propolis is a term of Greek origin consisting of two words (pro) meaning "before or before "Polis means the city [11]. Since ancient times, mankind has known the Propolis, and since the various miracles in Egypt, the propolis was well known by the priests of the pharaohs who were used to mummify the bodies of their dead. Prophets were also known by ancient Greeks [12].

In the Georgian folk medicine; it was also common to have a small piece of propolis on the navel of a newborn child. In the course of his study of wax, the Arab world Ibn Sina identified two kinds of wax:

- Wax clean: is the wax, which is the wall of the hive, where it is used by bees to incubate eggs and store honey.
- Black wax: It is considered the dirt of bees and so what was meant by Ben Sina black wax is the propolis [13].

In Italy, in the 17th century, propolis was used to paint stringed instruments and today it is still used in musical instruments (rosin resin) [14] and [15].

It was also used in the manufacture of arc wire and the dice industry [7]. In London, the Propolis was a private drug between the 17th and 20th century. Propolis became more common in Europe because of its anti-bacterial activity [16].

Not long ago the propolis was practically used by doctors to put it on the wounds during the Anglo-Boer War and the Second World War. In the former Soviet Union (USSR), it was also used for 30% [17].

In recent decades, propolis has been used in dentistry as a narcotic and as effective as cocaine and has been used in the manufacture of toothpaste and medical powders for dental and gingivitis and mouth infections [18], [19], [20], [21] and [22]. The chemical composition of the propolis is favoiods, phenols, carbohydrate, free amino acids, alkaloids, terpenoids, lipids and vitamin C. [23] and [24].

Material and Method

Strains

Alternaria alternate was isolated from orange juice, using potato dextrose agar (PDA) media, incubated for 5 days at 27 c^{o} [25].

The Extraction

Extraction of William pear peel and propolis were performed according to the method described by (Junjian, etal.2013). William pear peel and propolis were extracted with 500 ml of alcohol extract, in an ultrasonic bath at 37 c^{o} for 40 min. Two produced extracts were dried under negative pressure in rotary evaporation at 40 c^{o} and then re-dissolved 4 g in 40 ml of edible alcohol. The two extracts of (William pear extract = WPE) and (Propolis extract = PE) were filtered through a 0.45-um membrane (Millipore) and stored in refrigerator at 4 c^{o} until analysis. The evaluating anti fungal effects were seven different concentration (1.25, 2.5, 5, 10, 15, 20, 25) mg / ml.

Separation Process

The analysis was performed on precoated 20x20 cm (0.25 mm thick) thin layer chromatography (TLC) plates. The plates were developed at room temperature in a vertical separating chamber to the height of approximately 18 cm from the start. The chamber was previously saturated with the appropriate mobile phase (saturation time was 1 hour). Mobile phase was: toluene: ethyl acetate: formic acid, 36:21:5 ml [26].

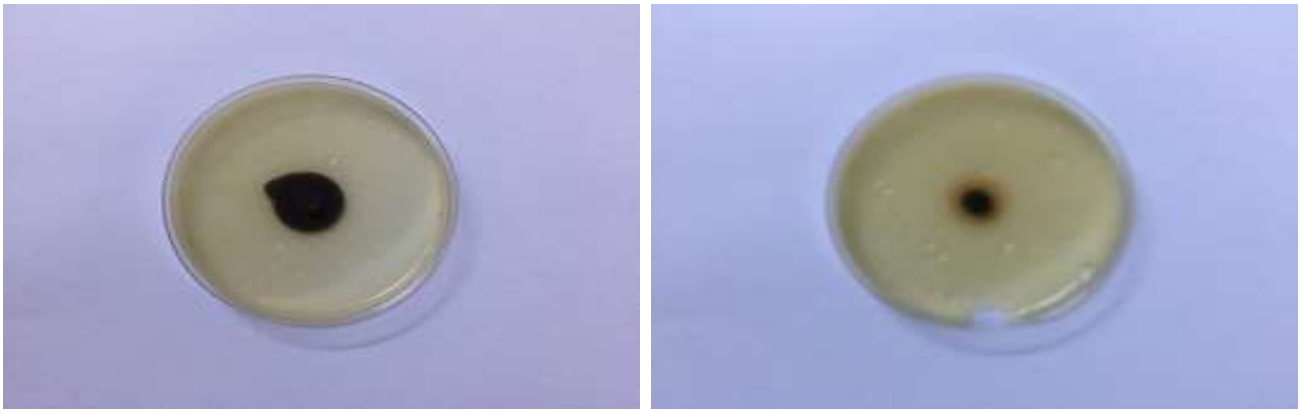
Results and Discussion

After evaluating the antifungal effects of seven different concentration of dilution. The positive control compared with two extracts of (PE) and (WPE) produced significantly inhibition against *Alternaria.alternate*, the inhibitory efficacy of each extract against the fungus was increased by increasing its concentration. Also observed that the inhibitory activity of (PE) was greater than (WPE) as shown (Table 1).

Table 1: Inhibitory effect of (PE) and (WPE) against *A. alernate.*

Concentration mg/ml (PE)	Average Diameter cm	Inhibitory Present %	Concentration mg/ml (WPE)	Average Diameter cm	Inhibitory Present %
1.25	1.8	61	1.25	4.6	0.0
2.5	1.0	79	2.5	4.6	0.0
5	0.0	100	5	4.5	3.0
10	0.0	100	10	3.5	24
15	0.0	100	15	1.9	59
20	0.0	100	20	1.5	68
25	0.0	100	25	0.0	100
CO.	4.6	0.0	CO.	4.6	0.0

Where the rate of inhibition of (PE) at the concentration (1.25, 2.5) mg/ml was (61, 79) % respectively and the highest rate of inhibition was 100% at concentration 5 mg/ml (Figure 1). While (WPE) did not show the effect at the concentration (1.25, 2.5) mg/ml and the highest rate of inhibition was 100% at concentration 25 mg/ml (Table 1).

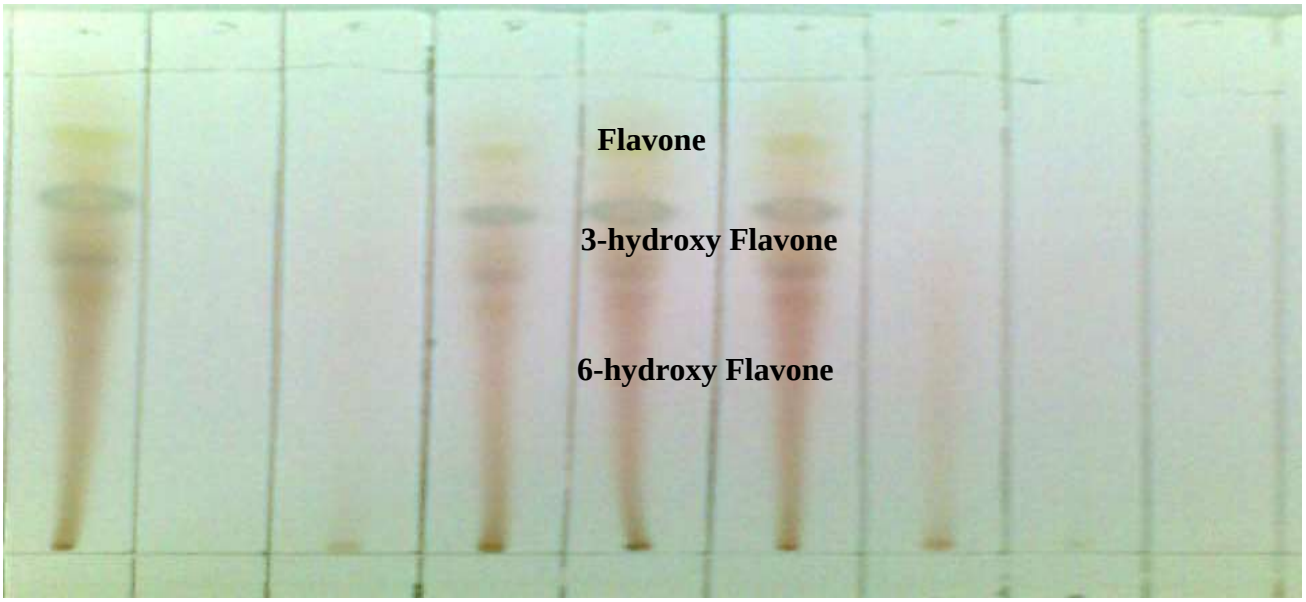


Control **(PE): at concentration 5 mg**

Figure 1: *A. alterate*

This study agrees somewhat with Oczan *et al* 2004 when they observed the antifungal properties of pollen and propolis extracts were determined on *Alternaria alternata* and *Fusarium oxysporium*, the inhibitory effect of propolis extracts on growth of F. oxysporium and A. alternata were generally higher than pollen extract. Kurt and Sahinler showed that (PE) were inhibitory against *Verticillium dahliae, Fulvia fulva* and *Penicillium digitatum* [27]. Propolis extract was also shown inhibitory against mycelial growth of *Aspergillus parasiticus* [28].

As for william pear: A study showed that the pear fruit has inhibitory capacity against (*Penicillium expansum*, *Botrytis cinerea* and *Dothiorella gregaria*) by An inhibitor of fungal polygalacturonase [29].The separation process of (PE) showed a presense: Flavone, 3-hydroxy flavone and Quercetin and absense these compounds in (WPE) (Figure 2), With this result I agree with Maric et al, 2004 when they prove that Propolis contains phenolic compounds such as (Flavanone, Naringenin, Flavone, 3-Hydroxyflavone, 6-Hydroxyflavone, Morin, Chrysin, Quercetin, Galangin, Apigenin. ect).



The separation process of (PE)

Figure 2: The separation process of (PE)

These compounds have antibacterial and antifungal activities and classified as flavonoids, in study: A number of antifungal compounds isolated from plants. These compounds belong to six chemical groups, such as phenolics and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids and steroidal

alkaloids, one of those plants (*Parthenium*) had an antifungal effect against *Aternaria Solani* and *Alternaria zinnia*. [30, 31].The inhibitory superiority of propolis extract against *Altrnaria alternate* is due to its high concentration of flavoniod compounds, Where the proportion of those compounds in the propolis up to 60 % [32].

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