



Original article

Biological control of yeast contamination of industrial foods by propolis

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ABSTRACT

Bee glue (Propolis, PR), mixture of beeswax and resin is collected from honeybee (*Apis mellifera*) of different plant parts. The antimicrobial potential of PR against food borne yeast was reported. The experiment was designed to examine the way of antimicrobial impact of PR on food borne yeasts (*Cryptococcus laurentii* and *Candida famata*) and its usage use as biological strategy for the preservation of soft foods against microbial spoilage. The study also highlights, the ability of ethanol and water- PR extracts, discouraged growth of tested yeast. Antifungal properties were also determined using electron microscope while biochemical analysis was determined using free and proteinic amino acid technique and oxidative enzymes were determined using HPLC analysis. Antioxidant enzymes were determined using ELISA assay. The highest effect was recorded on *C. laurentii* however, the lowest effect shows on *C. famata*. The electron microscopic studies clearly disclosed the effect of water PR distillate on the external shape and internal organs of some tested yeast e.g. *C. laurentii* and *C. famata*. The result indicated some differences on concentrations of bio-chemical analyses for these tested yeasts treated with 70% water- PR extracts of different food materials. Moreover, biochemical analysis results also reported that the treated yeast indicated natural preservative to food products and considered as best alternative to the (chemical) preservatives currently employed.

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1. Introduction

Bee glue (Propolis), (PR) is a combination of a Greek term “Pro” meaning “opposite of entry”, and “polis” meaning “the city or community” represents as ingredient which acts in protection of the hoard (Salatino et al., 2005). PR is a combination of beeswax and resins collected by *Apis mellifera* (honeybee) from different plant parts e.g., flower, buds, nectar and other plant exudates. PR from different geographic locations has been found to possess various biological happenings such as uncontaminated, antiviral, and antifungal. PR (bee glue) is a resinous waxbee hive by part produced by bees (*Apis mellifera*). It possessed various pharmaceutical properties and till now has been used in folk medicines as bio-cosmetics and health foods (Bankova, 2005). PR is produced by honeybees from the collected plant parts mixed with bee-resin

and the metabolic compounds of honeybee and is used to stabilize the honeycombs cells; act as defense against invaders and cold weather (Golder, 2004). PR composed of enzymes and salivary secretions significant for fill cracks, cover hives or gaps to protect themselves from microorganism's entry, fungi and bacteria into the hive (Bankova, 2000). Moreover, the PR can be used to line the honeycomb, facilitate the smooth laying of eggs by the queen, and to preserve and petrify the decay organisms (beetles and insects) that commonly not part the hive and used by honeybees. (Castaldo and Capasso, 2002). Moreover, PR chemistry is viscous in nature includes essential oil (10%), beeswax (30%), resin (50%), pollen (5%) and organic and mineral compound (5%) (Fokt et al., 2010). Many scientists reported that (Bankova et al., 2000; Teixeira et al., 2010; Valencia et al., 2012), about 300 compounds are present include phenolic acids, cinnamic acid, caffeic acid, terpenes, flavonoids, esters, amino acids, sugar, sterols, steroid hydrocarbons, minerals, aliphatic hydrocarbons, sesquiterpene and triterpene hydrocarbons in PR. PR is basically a lipophilic substance i.e., in colder environment, it's hard, brittle while as in warm conditions, it's soft, flexible and very sticky, hence called “beeswax” (Marcucci, 1995). It has a distinct odor, its oil shows adhesive properties and it has a strong reaction with skin proteins (Sforcin, 2007). Its composition is very complex (Boyanova,

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2006). Moreover, vegetal origin of collected material affected by collection time as well as chemical composition of resinous material (Fernandes et al., 2007). The color of PR varies from yellowish green to dark brown, depending upon the location viz; savannah, tropical forests, desert, coastal and mountainous regions, where it is produced (Piccinelli et al., 2011). PR is attracting and more popular as a natural preserving material in food industry. However, it has been added to foods and drinks as bioactive compounds to increase life standard (Mishima et al., 2005; Moreira et al., 2008).

'Hurdle technology' planned by Leistner and Gorris (1995), advocates an intellectual use of mixtures of different preservations methods to realize multi-target, mild but preservation effects should be more safe, nutritious and economical foods. An extract of PR acts as successful antifungal agent in minute quantities against spoiled fruit juice yeasts. Focus in its antifungal properties was targeted on human health (Cafarchia et al., 1999). Na⁺ benzoate preservative system is less efficient than PR as it more active against yeast (Moreira et al., 2008). The antifungal mechanism of PR was examined for the growth, aflatoxins production, lipids and digestion (Hashem et al., 2012). It has also been reported by Takaisi-Kikuni and Schilcher (1994) cell division restriction was caused by PR and might have inhibited the DNA replication of cells. Gas chromatographic analysis results of cellular fatty acids revealed that PR increased the saturated fatty acids accumulation and suggested the defense mechanism of fungal membrane by reducing cell flexibility and resistance (Hashem et al., 2012). Han and Park (2002) have reported that PR and its various products have been mainly used for health benefits but not for fruit juice processing and preservation yet. Currently, the focus of the use of PR in developed countries has been on its utilization as a consumer suited health supplement. This is the reason it has nowadays been recognized as a natural, healthy and beneficial product for human use (Espina et al., 2012). Several studies have been conducted pertaining to the composition and antifungal and antioxidant activities of PR from many topographical regions, such as Brazil, Bulgaria, Greece, Cyprus, France, Italy, and Croatia. (Marcucci et al., 2001; Prytyk et al., 2003; Salomão et al., 2004; Bastos et al., 2008; Kalogeropoulos et al., 2009). However, little is known about Saudi PR, and its extracts. In current study, we explored the chemical composition, antioxidant activities, and the anti-yeast properties of Saudi PR extracts. So, the current study was designed to characterize the bioactive properties of Saudi PR (regarding phenolic content and antioxidant activity) and to evaluate the potential effectiveness of PR extract in different concentration against food-borne yeast, for biochemical analysis of treated yeast and indicated the application of natural preservative to food products as an alternative to the (chemical) preservatives currently employed. The PR antimicrobial potential against food borne yeast were reported. Moreover, it was also investigating the mechanism of antimicrobial impact of PR on food borne yeast and possibility to use PR as an alternative biological strategy to preserve soft foods against yeast.

2. Materials and methods

2.1. Collecting and extract of PR

Raw Saudi PR was purchased from local bee farm (Al Soudah, Abha, Kingdom of Saudi Arabia). PR was collected manually from the beehives during the period of the dry season (May–July 2017). After collection, PR was desiccated in dark and kept at a temperature of 4 °C until its processing.

2.2. Ethanolic extraction of PR (EEP)

PR material was crushed into very small pieces and 80% ethanol was added to it by 1:10 (weight/volume) ratio. Afterwards the

blend was put in a closed flask at 25 °C for 48 h. The materials were shake from time to time and distillate was then clean using Whatman No. 1 filter paper type 4, and the distillate was a very dense material like alcoholic extract (yaghobi et al., 2007).

2.3. Water extraction of PR (WEP)

PR samples were grinded using mortar and pestle and were then diverse with purified water (1 g of PR per 10 ml of purified water). PR water blend was then heated at 40 °C temperature on a hot plate till properly dissolved. The solution was then kept for cooling at room temperature and vortexed for 15 min. Afterwards, the solution was later filtered using Whatman filter paper no. 6. The filtrate was then evaporated at 40 °C using a hot oven and remaining filtrate solution was finally at 4 °C in the dark until tested for antifungal activity (Siqueira et al., 2009). Different concentrations (30, 50, 70, 100%) of water extract PR were prepared according to.

2.4. Chemical analysis of PR

2.4.1. Flavonoids assay

For the preparation of Solution, A dissolving 10 g of powder PR was liquified in 50 ml of 95% ETOH solution followed by its filtration. Solution B was prepared by adding 10 ml of ETOH (50%) to 10 ml of NaOH (50%) followed by mixing equal volumes of both solutions A & B, the appearance of yellow color is the evidence of flavonoids (Kosalic et al., 2005).

2.4.2. Phenolic compounds assay

Phenolic compound assay was prepared by adding 3 ml of PR extract to 2 ml of ferric chloride solution (1%). The presence of a bluish-green hue is evidence of the presence of phenols.

2.4.3. Resins assay

For the preparation of resin assay 10 g of powder PR was liquefied in 50 ml of 95% ETOH solution and left for two minutes in a water bath at 100 °C. The solution was then filtered, and 100 ml of water was added to solution followed by acidification with hydrochloric acid 4%. The appearance of turbidity in the solution was evidence of resins (Orsi et al., 2007).

2.4.4. Antifungal activity of PR extract

The agar well diffusion methods determined the antifungal activities of PR extract as described by (Cafarchia et al., 1999).

2.4.5. Agar well diffusion method

Yeast samples were isolated from food spoilage strains and maintained at 30 °C on Sabouraud dextrose agar (SDA). The three culture plates seeded with tested yeasts could solidify and were punched to make open wells with a sterile cork borer (7.0 mm diameter). 0.05 ml of PR extract were then filled with these open wells. The culture plates were then heated at 30 °C for 72 h and inhibition zones were observed. Evaluation of the Fungistatic and Fungicidal Aspect after Exposure to Ethanolic and Water extraction of PR on Sabouraud dextrose Agar. The clear zone of inhibition was swabbed using a sterile cotton swab. The same swab was used to spread over the new plates containing SDA medium in order to figure out fungistatic and fungicidal aspect of the exposed PR to ethanolic and water extraction.

2.5. Evaluation of the antifungal properties of PR

2.5.1. Electron microscope studies

Preparation of isolated yeast after growing in PR extract according to Afrikian et al., (1973). Scanning Electron Microscopes (SEM):

The effect of PR extract on structure of vegetation yeast cells after growing in PR extract according to Bozzola and Russell (1999). Examination of the yeast cells was carried out by using JEOL JSM-7610F Scanning Electron Microscope at magnification of 900 \times , 8000 \times , 10,000 \times , 16,000 \times and 20,000 \times .

Transmission Electron Microscopes (TEM): The Effect of PR extract on structure of vegetation yeast cells after growing in PR extract (Bozzola and Russell 1999). Examination of the yeast cells was carried out by using JEOL JEM-1400plus TEM at magnification of 20000 \times , 30000 \times , 40000 \times , 50000 \times and 60000 \times .

2.5.2. Extraction and analysis of amino acids

The cell free extract after thoroughly washed with 70% ethanol was use in small column (0.8 \times 12 cm) of dowex 50 (H form) to hold the free amino acids. The free amino acids were eluted with 25 ml ammonia in 75 ml of 75% ethanol. The amino acid was dissolved in 0.2 ml distilled water immediately after drying them in vacuum in a rotary evaporator. Proteinic Amino acid HPLC analysis was carried according to.

For proteinic amino acid analysis, 1 g yeast cells were digested with 6 N Hydrochloric acid, then dried and dissolved in methanol and filtered through 0.45 μ m membrane filter before HPLC injection. The samples were finally redissolved in borate (pH 8.8) buffer (for derivatization with AQC (6-aminoquinolyl-N-hydroxysuccinimide carbamate). GBC HPLC system was used for the quantification of hydrolyzed amino acids. This system consists of two GBC LC 1110 pumps, a helium solvent degassing system, a 150 \times 3.8 mm RP18 steel column, using fluorescence detector, a Win Chrom Chromatography Ver. 1.3 and having software for data procurement and a temperature control module. For the separation of AQC derivative amino acids, eluent mobile phase acetonitrile: water ratio (72:28; v/v) were used and 1.6 ml/min flow rate was applied at 37 °C temperature. Excitation and emanation wavelengths were set at 250 and 395 nm, respectively.

2.5.3. Determination of oxidative enzymes

The harvested yeast cells were washed double with sterile double-distilled water. For oxidative enzymes, 1 g from the yeast cells was suspended in 2 ml phosphate buffer (pH 7). The yeast cells suspension was moved into an extraction buffer, contained 20 mM of a potassium phosphate buffer (pH 7) and a protease inhibitor cocktail. The cells were sonicated in ice-cold normal saline (1/9, w/v) in Virsonic[®] ultrasonic cell disruptor for 10 min., then centrifuged at 5000 rpm for 5 min at 4C and the supernatant (cell lysate) was stored at –80C until the assays were performed (Timur et al., 2005). Superoxide dismutase (SOD) [using ELISA kit purchased from Cayman company USA cat No. 706002] determined at 450 nm. Catalase [using ELISA kit Cayman company USA cat No. 707002] determined at 540 nm. Glutathione Reductase [using OxiSelect[™] ELISA kit purchased from Cell Biolabs company San Diego, CA, USA cat No. STA-812] determined at 405 nm. Ascorbate Peroxidase activity were determined at 340 nm [using ELISA kit Cohesion Biosciences company USA cat No. CAK1052]. The said activity was according to instruction manuals and the absorbance were measured using 800 TS Microplate Reader Bio-Tek company, USA.

3. Results

3.1. Antifungal potential of ethanolic and water PR extract

For the determination of the antifungal effects of ethanolic and water extract PR on the tested yeasts, the zone of inhibition was divided according to (Saubolle and Hoeplich., 1978) and the scale of the results used in the current study was shown in Table 1. The weekly effect for ethanolic and water PR extracts for yeast were in range of (\leq 16 mm) in diameter, intermediate effect was

in range between (>17 to 23 mm) in diameter and highly effect for ethanol and water propels extract were in range of (>24 mm) in diameter. According to size of inhibition zone, the highest effect of PR extract was on *C. laurentii* while as the least impact of PR extract was on *C. famata*. The analysis of variance (ANOVA) table for zone size inhibition based on error mean square value was describe in the Table 2. All the zone size showed significant effect at 5% probability.

3.2. Scanning electron microscope

After examining different yeast using scanning electron microscope, the micrograph in Fig. 1 (A: B) showing effect of non-treated and treated water PR on *C. laurentii* which indicated decreasing number of yeast cells when treated with the said extract. The scanning electron micrograph of non-treated *C. laurentii* cells in (Fig. 2 A, B, C). showed regular oval shape yeast cells. On the other hand, crease on some cell walls with some deformities in the external shape of cells were observed after treatment of *C. laurentii* by water PR extract, shown in (Fig. 2 D, E, F). Again, after the treatment of *C. laurentii* by water PR extract, the micrograph was shown in (Fig. 3 A, B) indicated some appendages on yeast cell wall, some deformities in the external shape of cells and exit of the components of some cells. Also, the scanning electron micrograph of non-treated *C. famata* yeast cells was shown in (Fig. 4 A), while as the micrograph of *C. famata* after treated by water PR extract, showed some decreasing number of cells was shown in (Fig. 4 B). Finally, the scanning electron micrograph of non-treated *C. famata* cells represented by (Fig. 5 A) showed Oval to Globose shape of yeast cells. On the other hand, *C. famata* cells after treatment by water PR extract observed deformities in the external shape of cells and some appendages on yeast cell wall shown in (Fig. 5 B).

3.3. Fungistatic and fungicidal of PR (ethanolic and water extraction)

The data was recorded according to growth (fungistatic, FS) or non-growth (fungicidal, FC) on S.D.A and shown in (Table 3). Results discovered that of all the established isolates, PR water extract gave FS effect against the isolates of *Candida famata*, *C. parapsilosis* and *Cryptococcus terreus* and FC effect against the rest of the isolates. While as the PR ethanol extract gave FS effect against the isolates of *Rhodotorula mucilaginosa*, *C. famata*, *C. pelliculosa*, *C. parapsilosis* and *Cryptococcus terreus* and FC effect against the rest of isolates. The data regarding mean value of fungistatic and fungicidal effects of ethanolic and water extraction of PR against yeast cells isolated from industrial foods was presented in the Table 4. The maximum fungicidal effect was recorded in EPE 30 *Saccharomyces cerevisiae*, *Trichosporon mucoides*, *Rhodotorula mucilaginosa* recorded 16.0 which was followed by the yeast *Candida parapsilosis* recorded (15.50). The minimum fungicidal effect (12.0) was recorded for *Candida famata* for zone size 30. While for EPE 50 the maximum fungicidal effect was (25.0) recorded against yeast *Candida magnoliae* and *Cryptococcus laurentii* which was followed by the yeasts *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa*, *C. zeylanoides*, *C. pelliculosa*, *C. sphaerica* and *C. lusitaniae* recorded (24.0) using EPE50. The maximum fungicidal effect for EPE70 was recorded 28.0 under *Cryptococcus laurentii* which was followed by *Candida zeylanoides*, *Candida pelliculosa* and *Kloeckera* spp, recorded 27.0. The minimum was (18.0) recorded under *Candida famata* using EPE 70 zone size. The maximum *Cryptococcus laurentii* recorded (32.0) using zone size 100 which was followed by the 30.5 recorded *C. magnoliae* and *C. zeylanoides*. The minimum fungicidal effect was 24.5 recorded using *C. famata* yeast cells under EPE 100.

Table 1
Sensitivity pattern of yeasts to Ethanollic and water propolis extract.

Isolates	Inhibition zone (Diameter mm)								Isolates	Inhibition zone (Diameter mm)							
	EPE30	EPE50	EPE70	EPE100	EPE30	EPE50	EPE70	EPE100		EPE30	EPE50	EPE70	EPE100	EPE30	EPE50	EPE70	EPE100
<i>Saccharomyces cerevisiae</i>	16 R	24 S	25 S	26 S	15 R	23.5 S	24 S	25 S	<i>Candida sphaerica</i>	14 R	24 S	25 S	26 S	13.5 R	21 I	25 S	27.5 S
<i>Cryptococcus humicola</i>	14 R	16 R	20 I	28 S	15.5 R	16 R	22 I	30.5 S	<i>Candida guilliermondii</i>	14.5 R	23 I	24.5 S	25.5 S	14 R	20 I	24 S	25 S
<i>Trichosporon mucoides</i>	16 R	20 I	23 I	25 S	16 R	22 I	23 I	24.5 S	<i>Candida lusitanae</i>	15 R	24 S	25 S	26 S	14.5 R	24 S	26 S	27.5 S
<i>Rhodotorula mucilaginosa</i>	16 R	24 I	25 S	27 S	16 R	23 I	25 S	26 S	<i>Kloeckera spp</i>	15.5 R	23 I	27 S	30.5 S	15 R	26 S	27 S	29 S
<i>Candida krusei/inconspicua</i>	15 R	16 R	23 I	29.5 S	14 R	16 R	24 S	25 S	<i>Candida magnoliae</i>	16 R	25 S	26 S	27.5 S	15.5 R	23 I	24.5 S	25 S
<i>Candida zeylanoides</i>	16 R	24 S	27 S	30.5 S	15.5 R	24 S	28 S	25 S	<i>Cryptococcus terreus</i>	13 R	19 I	24 S	26 S	16 R	17 I	26 S	30 S
<i>Candida famata</i>	12 R	15 R	18 I	24.5 S	13 R	14.5 R	17 I	24 S	<i>Cryptococcus laurentii</i>	16 R	25 S	28 S	32 S	15 R	24 S	30 S	35 S
<i>Candida pelliculosa</i>	16 R	24 S	27 S	30 S	16 R	19 I	25 S	30 S	<i>Candida colliculosa</i>	16 R	24 S	26 S	30 S	16 R	26 S	29 S	32 S
<i>Candida parapsitosis</i>	15.5 R	18.5 I	23 I	25 S	14 R	16 R	22.5 I	25 S	<i>Geotrichum kelbahii</i>	14 R	16 R	23 I	26 S	13.5 R	18 I	24 I	26 S
<i>Candida rugosa</i>	16 R	23 I	24 S	25 S	16 R	22 I	24.5 S	25 S									

S= > 24 mm in diameter: I = 17 to 23 mm in diameter: R= ≤ 16 mm in diameter (Saubolle and Hoerprich., 1978).

Table 2
Analysis of variance (ANOVA) for zone size based on error mean square (EMS) value.

SOV	EPE30	EPE50	EPE70	EPE100	EPE30	EPE50	EPE70	EPE100
Treatment	4.27**	37.57**	18.33**	16.32**	3.07**	39.94**	23.78**	28.02**
Rep	2.37	1.46	1.35	0.17	0.11	0.17	1.27	0.75
Error	1.40	1.23	1.04	0.89	0.72	0.94	0.60	0.95

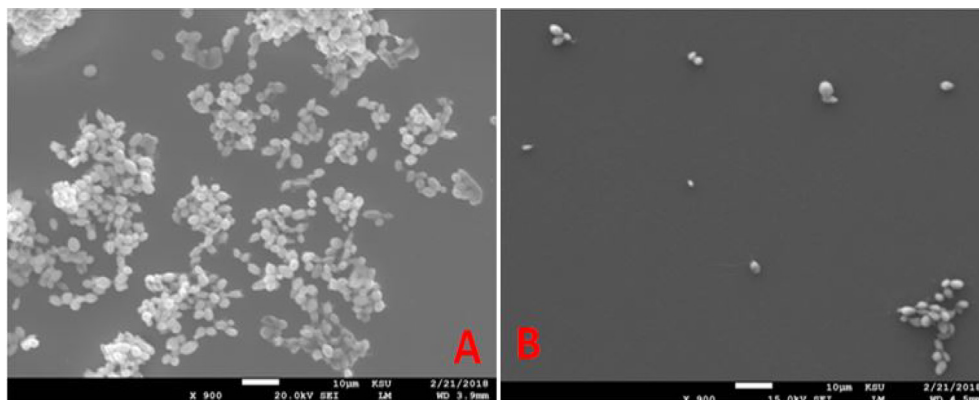


Fig. 1. (A-B). Scanning Electron Micrograph (SEM) of *Cryptococcus laurentii*, (A) Non treated yeast cells (NT) of *Cryptococcus laurentii* (B) Yeast cells of *Cryptococcus laurentii* after treated water propolis extract and reduced cell numbers.

3.4. Transmission Electron Microscope (TEM)

After examining different yeast using transmission electron microscope it can be concluded that the transmission electron micrograph of non-treated and treated *Cryptococcus laurentii*

shown in (Fig. 6A, B) and (Fig. 6C, D) respectively indicated full lyses of yeast cells when treated with water PR extract. Also, (Fig. 6A) represented non-treated *Cryptococcus laurentii* cells while as (Fig. 6B, C) showing decomposition of internal organs of cells with some deformities in the shape of cells when treated with

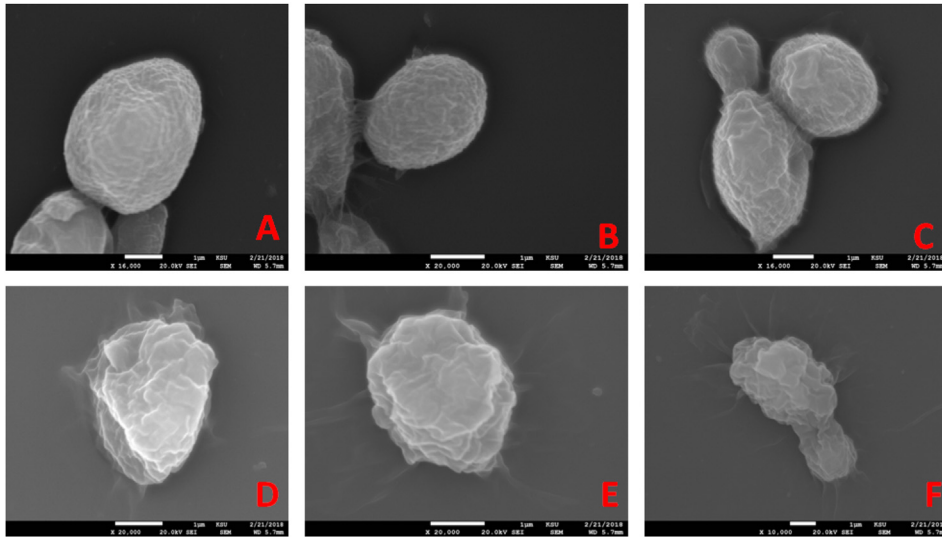


Fig. 2. (A-F). Scanning Electron Micrograph (SEM) of *Cryptococcus laurentii*, (A,B,C) Non treated yeast cell (NT) of *Cryptococcus laurentii* showed regular oval shape of yeast cells; (C,D,E) yeast cell of *Cryptococcus laurentii* after treated with water propolis extract.

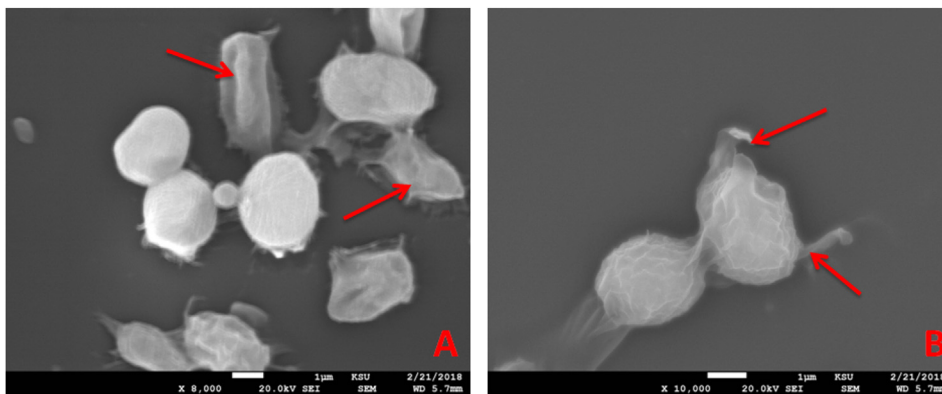


Fig. 3. (A-B). Scanning Electron Micrograph (SEM) of *Cryptococcus laurentii*, (A, B) yeast cell of *Cryptococcus laurentii* after treated with water propolis extract, which illustrated exit of the components of some cells, some appendages on yeast cell wall and some defor.

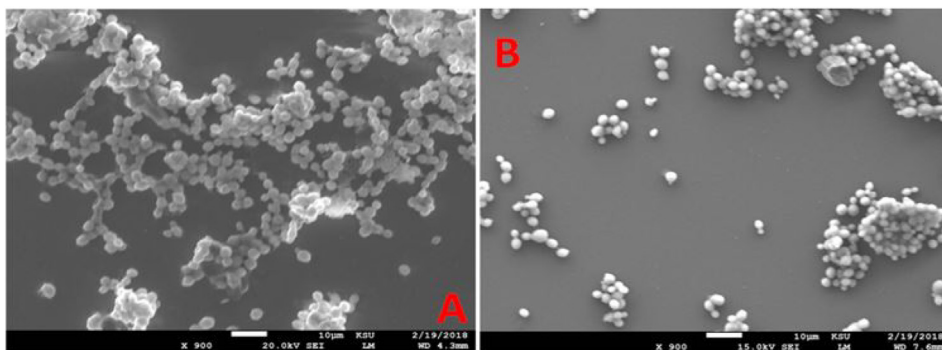


Fig. 4. (A-B). Scanning Electron Micrograph (SEM) of *Candida famata*, (A) Non treated yeast cells (NT) of *Candida famata*; (B) yeast cells of *Candida famata* after treated by water propolis extract and which illustrated some decreasing number of cells.

water PR extract. The transmission electron micrograph of non-treated *C. famata* yeast cells shown in (Fig. 7A, B) while as the other micrograph of *C. famata* shown in (Fig. 7C) when treated by water PR extract, indicated some lyses of cells and deformities in the

shape of cells. Transmission Electron Micrograph (TEM) of *Cryptococcus laurentii*, (A, B) Non treated yeast cell (NT) of *Cryptococcus laurentii*; (C) yeast cell of *Cryptococcus laurentii* after treated with water propolis extract was presented in the (Fig. 8A, B, C, D).

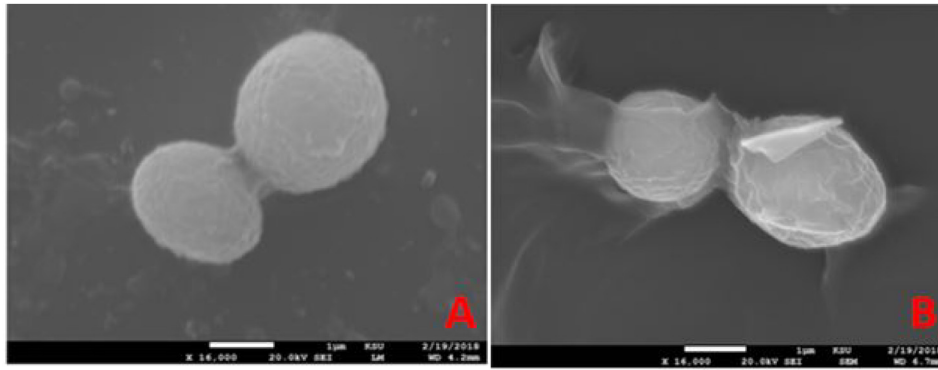


Fig. 5. (A-B). Scanning Electron Micrograph (SEM) of *Candida famata*, (A) Non treated yeast cell (NT) of *Candida famata* showed Oval to Globose shape of yeast cells; (B) yeast cell of *Candida famata* after treated with water propolis extract, which illustrated deformities.

Table 3

Determination of fungistatic and fungicidal effects of ethanolic and water extraction of propolis against yeast cells isolated from industrial foods.

Yeast	Yeast Extract		Yeast	Yeast Extract	
	EPE	WPE		EPE	WPE
<i>Saccharomyces cerevisiae</i>	FC	FC	<i>Candida sphaerica</i>	FC	FC
<i>Cryptococcus humicola</i>	FC	FC	<i>Candida guilliermondii</i>	FC	FC
<i>Trichosporon mucoides</i>	FC	FC	<i>Candida lusitaniae</i>	FC	FC
<i>Rhodotorula mucilaginosa</i>	FC	FC	<i>Kloeckera spp</i>	FC	FC
<i>Candida krusei/inconspicua</i>	FS	FC	<i>Candida magnoliae</i>	FC	FC
<i>Candida zeylanoides</i>	FC	FC	<i>Cryptococcus terreus</i>	FS	FS
<i>Candida famata</i>	FC	FC	<i>Cryptococcus laurentii</i>	FC	FC
<i>Candida pelliculosa</i>	FS	FS	<i>Candida colliculosa</i>	FC	FC
<i>Candida parapsilosis</i>	FS	FS	<i>Geotrichum kelbahnii</i>	FC	FC
<i>Candida rugosa</i>	FC	FC			

*FC = Fungicidal effect, FS = Fungistatic effect

Table 4

Mean value of Fungistatic and fungicidal effects of ethanolic and water extraction of propolis against yeast cells isolated from industrial foods.

Sr. No	Treatments	EPE30	EPE50	EPE70	EPE100
1	<i>Saccharomyces cerevisiae</i>	16.0a	24.0ab	25.0cd	26.0efg
2	<i>Cryptococcus humicola</i>	14.0bc	16.0d	20.0f	28.0cd
3	<i>Trichosporon mucoides</i>	16.0a	20.0c	23.0e	25.0g
4	<i>Rhodotorula mucilaginosa</i>	16.0a	24.0ab	25.0cd	27.0def
5	<i>Candida krusei/inconspicua</i>	15.0ab	16.0d	23.0cd	29.5bc
6	<i>Candida zeylanoides</i>	16.0a	24.0ab	27ab	30.5ab
7	<i>Candida famata</i>	12.0d	15.0d	18.0g	24.5g
8	<i>Candida pelliculosa</i>	16.0a	24.0ab	27.0ab	30.0b
9	<i>Candida parapsilosis</i>	15.50ab	18.50c	23.0e	25.0g
10	<i>Candida rugosa</i>	16.0a	23.0b	24.0de	25.0g
11	<i>Candida sphaerica</i>	14.0bc	24.0ab	25.0cd	25.0g
12	<i>Candida guilliermondii</i>	14.50bc	23.0b	27.0ab	26.0efg
13	<i>Candida lusitaniae</i>	15.0ab	24.0ab	25.0cd	25.50fg
14	<i>Kloeckera spp</i>	15.50ab	23.0ab	27.0ab	26.0efg
15	<i>Candida magnoliae</i>	16.0a	25.0a	26.0bc	30.5ab
16	<i>Cryptococcus terreus</i>	13.0cd	19.0c	23.0e	26.0efg
17	<i>Cryptococcus laurentii</i>	16.0a	25.0a	28.0a	32.0a
18	<i>Candida colliculosa</i>	16.0a	24.0ab	26.0bc	30.0b
19	<i>Geotrichum kelbahnii</i>	14.0bc	16.0d	23.0e	26.0efg
LSD		1.96	1.84	1.69	1.56

3.5. Biochemical analysis of treated Yeast

Free amino acids and their derivatives

Sample 1. Non-treated *Cryptococcus laurentii*: The data regarding chromatogram trace of free amino acids of (non-treated *Cryptococcus laurentii*) produced by the automated amino acid analyzer was presented in the Fig. 9 (A, B, C, D). The detailed analysis results of free amino acid of (non-treated *Cryptococcus*

laurentii) was presented in the supplementary Tables 1–4. The retention time, concentration ug/ml and concentration (%) was presented in the supplementary Tables 1–4.

Cryptococcus laurentii after treated by (WEP)

The data regarding Chromatogram trace of free amino acids of (*Cryptococcus laurentii* after treated by WEP 70%) produced by the automated amino acid analyzer was presented in the Fig. 11 (A, B, C, D). The details analysis results of total free amino acid treated

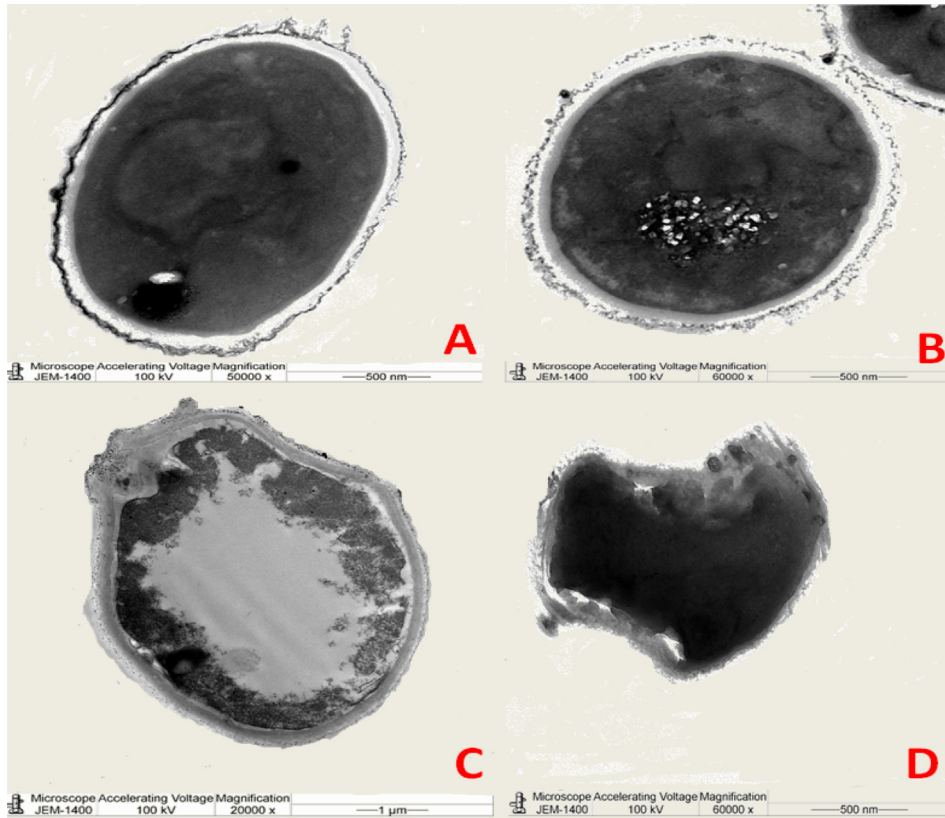


Fig. 6. (A-D). Transmission Electron Micrograph (TEM) of *Cryptococcus laurentii*, (A, B) Non treated yeast cell (NT) of *Cryptococcus laurentii*; (C, D) yeast cell of *Cryptococcus laurentii* after treated with water propolis extract, which illustrated full lyses of yeast c.

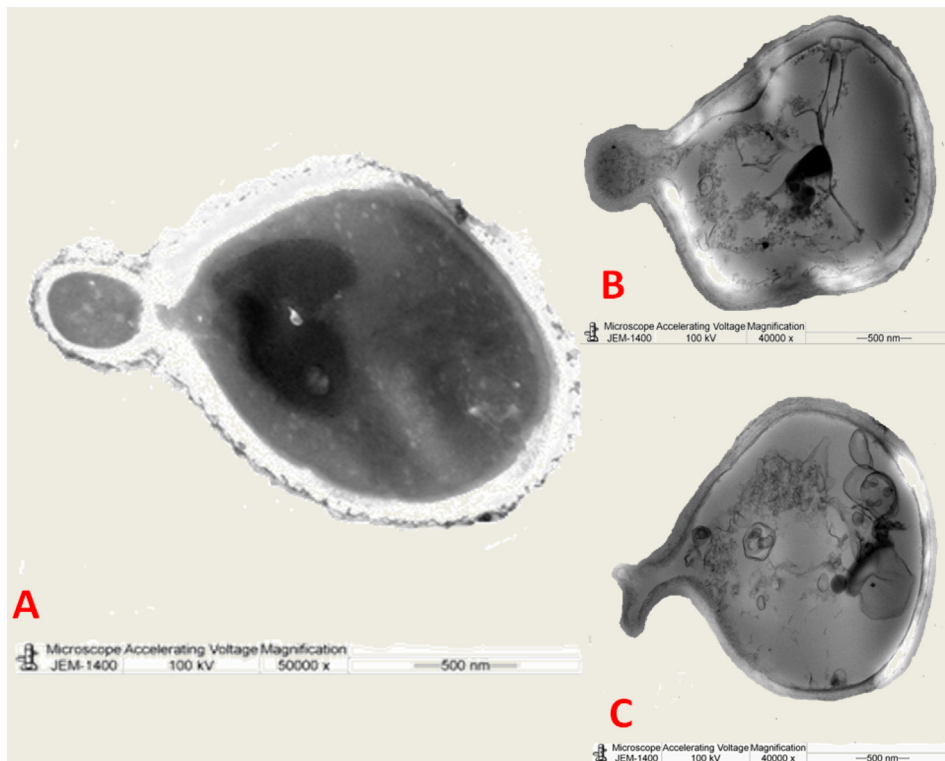


Fig. 7. (A-C). Transmission Electron Micrograph (TEM) of *Cryptococcus laurentii*, (A) Non treated yeast cell (NT) of *Cryptococcus laurentii*; (B, C) yeast cell of *Cryptococcus laurentii* after treated with water propolis extract, observed decomposition of internal organs.

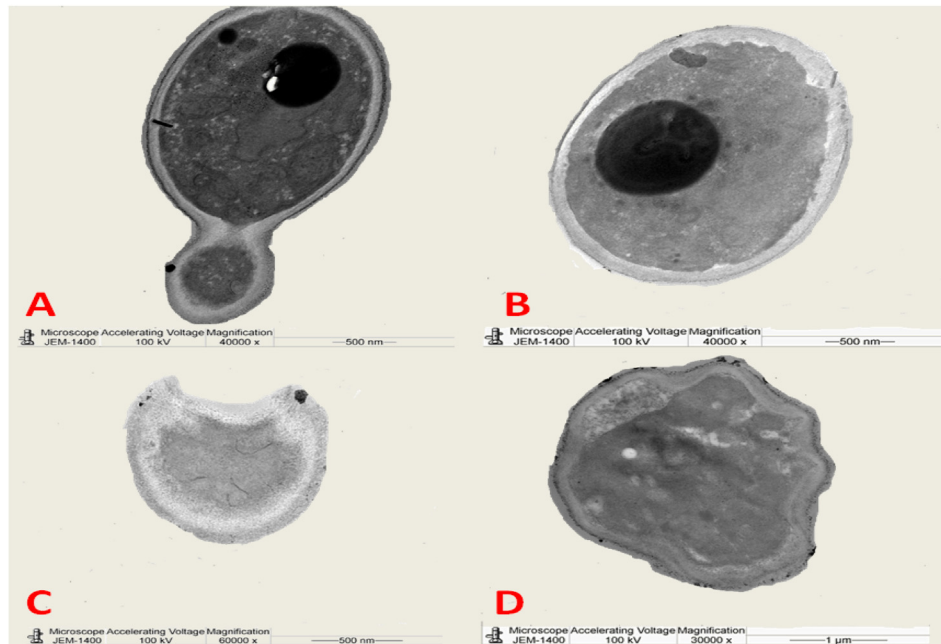


Fig. 8. (A-D). Transmission Electron Micrograph (TEM) of *Candida famata*, (A, B) Non treated yeast cell (NT) of *Candida famata*; (C, D) yeast cell of *Candida famata* after treated with water propolis extract, which illustrated some lyses of cells and deformities in the sh.

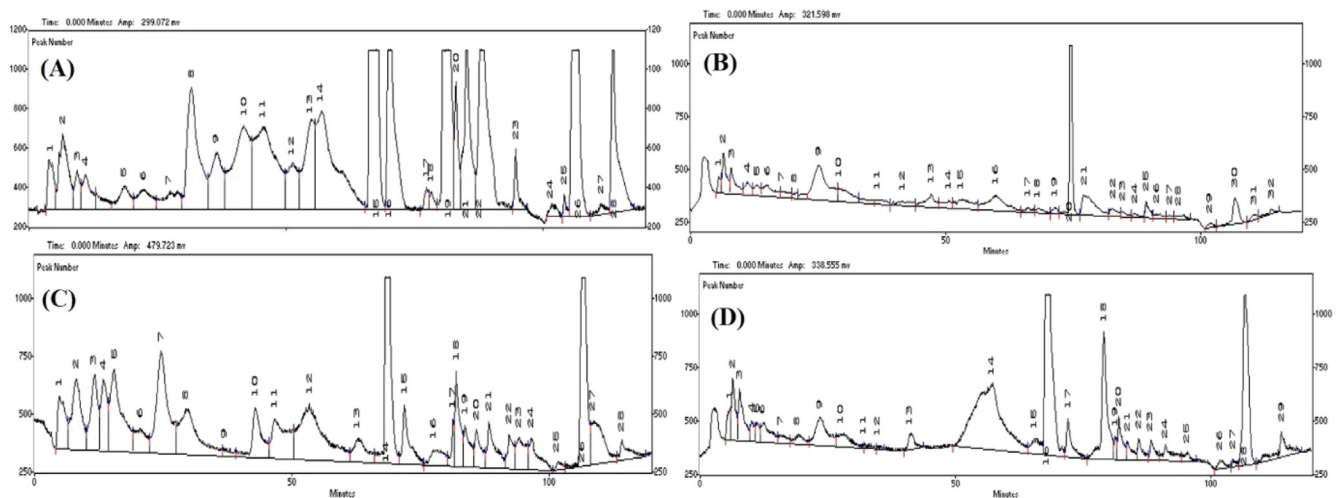


Fig. 9. (A-D). Chromatogram trace of free amino acids of (non-treated *Cryptococcus laurentii*) (A); (*Cryptococcus laurentii* after treated by WEP 70%) (B); (non-treated *Candida famata*) (C) (*Candida famata* after treated by WEP 70%) (D).

with (*Cryptococcus laurentii* after treated by WEP 70%) was consist of peak number, name of the amino acid, retention time, concentration and concentration (%) was presented in Fig. 11 (B).

Sample 3 non-treated *Candida famata*

Data regarding chromatogram trace of free amino acids of (non-treated *Candida famata*) produced by the automated amino acid analyzer was presented in the Fig. 11 (C). The detailed information of free amino acids of non-treated *Candida famata* was presented in Table 5.

Candida famata after treated by (WEP)

Data regarding chromatogram trace of free amino acids of (*Candida famata* after treated by WEP 70%) produced by the automated amino acid analyzer was presented in Fig. 11 (D). The detailed information of chromatogram trace of free amino acid, peak number, amino acid name, concentration and concentration (%) was presented in the Table 5.

The retention time, concentration and concentration (%) of four samples were presented in the S1, S2, S3, S4). The maximum amino acid content gamma-amino-n-butyric recorded retention time (87.70), concentration (1412.92 ug/ml) and concentration (18.99%) which was followed by the threonine (45.53), 1073.82 ug/ml and 14.43% RT, concentration and concentration (%) respectively. The followed by amino acid Glutamic acid and glycine recorded time (41.95; 56.92), concentration (858.77; 374.52) and (11.22; 11.54%) respectively. The arginine retention time (113.93) was recorded, concentration (867.47ug/ml) and concentration (11.66%) as shown in S1 Table. The phosphoserine, taurine and histidine were recorded same range of concentration (1.26; 1.62 and 1.92%). The retention time and concentration for phosphoserine, taurine and histidine are (4.29; 93.44), (6.70; 120.48) and (94.78; 142.67) respectively. The minimum retention time, concentration (ug/ml) and concentration (%) was recorded for phospho-

Table 5

Mean fungistatic and fungicidal effects of ethanolic and water extraction of propolis against yeast cells isolated from industrial foods.

Sr. No	Treatments	EPE30	EPE50	EPE70	EPE100
1	<i>Saccharomyces cerevisiae</i>	15.0abc	23.5bc	24.00fg	25.0fg
2	<i>Cryptococcus humicola</i>	15.50ab	16.0ij	22.00 h	30.50bc
3	<i>Trichosporon mucoides</i>	16.0a	22.0cd	23.0gh	24.50fg
4	<i>Rhodotorula mucilaginosa</i>	16.0a	23.0bc	25.0ef	26.0ef
5	<i>Candida krusei/inconspicua</i>	14.0a	16.00ij	24.0fg	25.00fg
6	<i>Candida zeylanoides</i>	14.0cde	24.0b	28.00bc	25.0fg
7	<i>Candida famata</i>	13.0e	14.50j	17.0i	24.00g
8	<i>Candida pelliculosa</i>	16.0a	19.0fg	25.0ef	30.0c
9	<i>Candida parapsilosis</i>	14.0cde	16.0ij	22.50h	25.00fg
10	<i>Candida rugosa</i>	16.0a	22.0cd	24.50f	25.00fg
11	<i>Candida sphaerica</i>	13.50de	21.00de	25.0ef	27.50de
12	<i>Candida guilliermondii</i>	14.0cde	20.00ef	24.00fg	25.00fg
13	<i>Candida lusitanae</i>	14.50bcd	24.00b	26.0df	27.50de
14	<i>Kloeckera spp</i>	15.0ab	26.00a	27.0cd	29.0cd
15	<i>Candida magnoliae</i>	15.50ab	23.0bc	24.50f	25.0fg
16	<i>Cryptococcus terreus</i>	16.0a	17.0hi	26.0de	30.0c
17	<i>Cryptococcus laurentii</i>	15.0abc	24.00b	30.0a	35.00a
18	<i>Candida colliculosa</i>	16.0a	26.00a	29.0ab	32.00b
19		13.50de	18.0gh	24.0fg	26.0ef
LSD		1.41	1.41	1.29	2.02

ethanolamine, urea, methionine, leucine, carnosine and lysine (9.63; 38.88; 0.52%), (11.24; 60.83 ug/ml; 0.82%), (77.51; 12.62; 0.17%), (78.41; 14.26; 0.19%), (101.56; 61.28; 0.82) and (104.18; 32.37; 0.44) was recorded respectively. The total amino acid concentration 7440.80 ug/ml free amino acids of (non-treated *Cryptococcus laurentii*).

Proteinic Amino acid HPLC analysis

Proteinic amino acid chromatogram of mixture proteinic amino acids in (non-treated *Cryptococcus laurentii*), are described in the Fig. 11 (A). Proteinic amino acid typical chromatogram of mixture proteinic amino acids in (*Cryptococcus laurentii* after treated by WEP 70%) Chromatographic conditions are described in Fig. 11 (B). proteinic amino acid Chromatogram of mixture proteinic amino acids in (non-treated *Candida famata*) were presented in the Fig. 11 (C). proteinic amino acid for typical chromatogram of mixture proteinic amino acids in (*Candida famata* after treated by WEP 70%) was presented in the Fig. 11 (D). The proteinic Amino acid ornithine (0.03;0.02; 0.03 and 0.05), Threonine (2.16; 1.72; 2.31; 2.64), Serine (4.10; 3.91; 3.17; 3.72), Isoleucine (1.67; 2.67; 2.49; 2.46), aspartate (2.58; 2.79; 3.35; 3.77), glutamic acid (3.26; 3.42; 3.57; 3.87), arginine (4.98; 4.87; 5.03; 5.54), Valine (2.37, 2.81, 3.65, 4.36), Glycine (0.02, 0.06, 1.57, 2.20), proline (1.25, 1.03, 4.19, 4.95), Alanine (1.02, 0.09, 1.48, 1.27), methionine (0.3, 0.12, 0.04, 0.03), Leucine (1.94, 1.15, 2.53, 2.24), Tyrosine (1.39, 1.43, 0.02, 0.06), Histidine (1.42, 1.57, 2.67, 2.45), asparagine (3.74, 3.58, 4.97, 5.37), phenyl alanine (3.51, 3.76, 3.75, 4.88), Glutamine (3.36, 2.63, 2.94, 4.52), Lysine (1.05, 1.97, 2.28, 2.91) and Citrulline (1.21, 1.42, 3.13, 4.73) as shown in Table 6.

3.6. Oxidative Enzymes

Four samples had been submitted for determination of Ascorbate Peroxidase using ELISA assay and data regarding for ascorbate peroxidase was presented in the Table 7. The treatment non treated *Cryptococcus laurentii* recorded 254 U/g, *Cryptococcus laurentii* after treated by (WEP) recorded 231.33 U/g, which was followed by the treatment (Non-treated *Candida Famata*) recorded ascorbate peroxidase 226.33 U/g. The minimum ascorbate peroxidase 201.66 U/g was recorded where *Candida famata* after treated by (WEP) (Table 7). The means values for oxidative enzymes using ELISA assay was presented in the Fig. 10 (A, B, C, D).

The non-treated *Cryptococcus laurentii* recorded 503.66 U/g, which was followed by the treatment non-treated *C. famata* recorded 351.66 U/g and least behind *C. famata* after treated by

(WEP) recorded 291.33 U/g catalase. The minimum catalase was recorded 184.66 under *Cryptococcus laurentii* after treated by (WEP). The maximum glutathione reductase 664 U/g was recorded where non-treated *Cryptococcus laurentii* which as followed by the treatment non-treated *C. famata* recorded 391.66 U/g and least behind *C. famata* after treated by (WEP) recorded 320.33 U/g. The minimum glutathione reductase 309.66 U/g was recorded *Cryptococcus laurentii* after treated by (WEP). The maximum superoxide dismutase 771 U/g was recorded where non-treated *Cryptococcus laurentii* which as followed by the treatment non-treated *C. famata* recorded 664 U/g and least behind *C. famata* after treated by (WEP) recorded 485.66 U/g. The minimum glutathione reductase 313.66 U/g was recorded *Cryptococcus laurentii* after treated by (WEP). The oxidative enzymes activity was decrease by using PR treated i.e. *C. famata* and reduced the ascorbate peroxidase, catalase activity, superoxide activity and glutathione reductase activity in yeast.

The data regarding proteinic amino acid using HPLC analysis for four samples (1. Non-treated *Cryptococcus laurentii* 2. *Cryptococcus laurentii* after treated by (WEP); 3 non-treated *Candida famata*; 4 *Candida famata* after treated by (WEP) results were presented in the Fig. 11 (A, B, C, D). The proteinic Amino acid ornithine (0.03;0.02; 0.03 and 0.05), Threonine (2.16; 1.72; 2.31; 2.64), Serine (4.10; 3.91; 3.17; 3.72), Isoleucine (1.67; 2.67; 2.49; 2.46), aspartate (2.58; 2.79; 3.35; 3.77), glutamic acid (3.26; 3.42; 3.57; 3.87), arginine (4.98; 4.87; 5.03; 5.54), Valine (2.37, 2.81, 3.65, 4.36), Glycine (0.02, 0.06, 1.57, 2.20), proline (1.25, 1.03, 4.19, 4.95), Alanine (1.02, 0.09, 1.48, 1.27), methionine (0.3, 0.12, 0.04, 0.03), Leucine (1.94, 1.15, 2.53, 2.24), Tyrosine (1.39, 1.43, 0.02, 0.06), Histidine (1.42, 1.57, 2.67, 2.45), asparagine (3.74, 3.58, 4.97, 5.37), phenyl alanine (3.51, 3.76, 3.75, 4.88), Glutamine (3.36, 2.63, 2.94, 4.52), Lysine (1.05, 1.97, 2.28, 2.91) and Citrulline (1.21, 1.42, 3.13, 4.73) (Tables S1–S4).

4. Discussion

PR a bee product also called city's guardian. In some citation also called Russian Penicillin. It is a natural sticky material, composed of honey-bees, flower resin, tree leaves and plants (Gupta et al., 2007). Different types of compounds were found in the PR and located in different geographic regions. The most important is the flavonoids and significant agents have the properties anti-inflammatory, anti-viral, anti-allergic, anti-cancer, anti-bacterial and anti-oxidant properties. Due to its wide

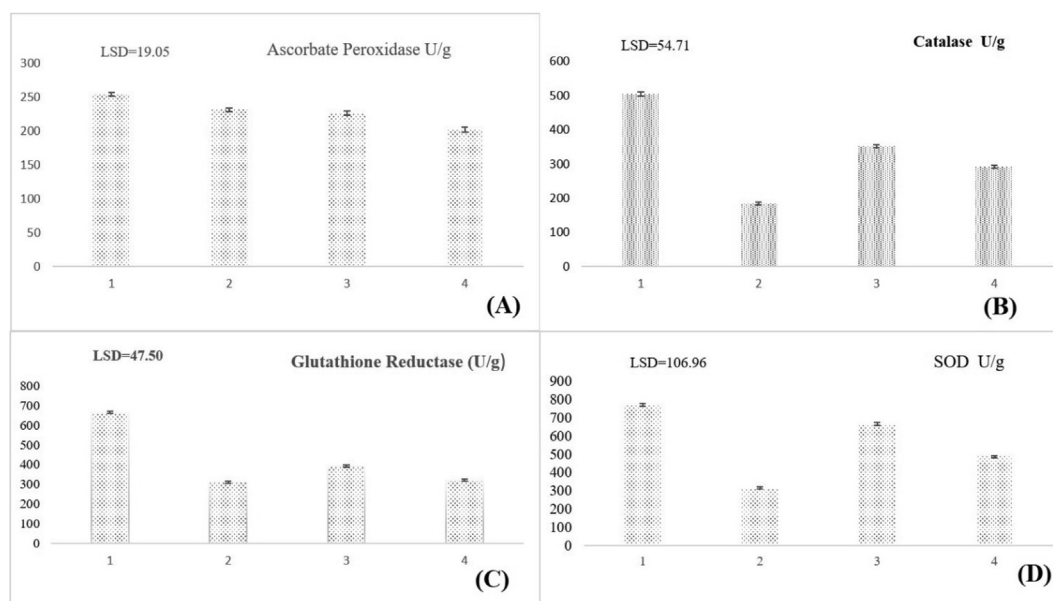
Table 6
Proteinic Amino acid HPLC analysis.

Amino acids	Sample 1* ($\mu\text{g/gm}$)	Sample 2* ($\mu\text{g/gm}$)	Sample 3* ($\mu\text{g/gm}$)	Sample 4* ($\mu\text{g/gm}$)	RT
Ornithine	0.03	0.02	0.03	0.05	8.8 \pm 0.1
Threonine	2.16	1.72	2.31	2.64	9.35 \pm 0.1
Serine	4.10	3.91	3.17	3.72	10.1 \pm 0.1
Isoleucine	1.67	2.67	2.49	2.46	13.52 \pm 0.2
Aspartate	2.58	2.79	3.35	3.77	14.9 \pm 0.3
Glutamic acid	3.26	3.42	3.57	3.87	16.8 \pm 0.2
Arginine	4.98	4.87	5.03	5.54	18.8 \pm 0.1
Valine	2.37	2.81	3.65	4.36	19.01 \pm 0.1
Glycine	0.02	0.06	1.57	2.20	23.3 \pm 0.8
Proline	1.25	1.03	4.19	4.95	25.6 \pm 0.4
Alanine	1.02	0.09	1.48	1.27	29.4 \pm 0.3
Methionine	0.3	0.12	0.04	0.03	32.75 \pm 0.1
Leucine	1.94	1.15	2.53	2.24	35.9 \pm 0.1
Tyrosine	1.39	1.43	0.02	0.06	38.2 \pm 0.1
Histidine	1.42	1.57	2.67	2.45	40.5 \pm 0.1
Asparagine	3.74	3.58	4.97	5.37	42.83 \pm 0.1
Phenyl alanine	3.51	3.76	3.75	4.88	44.7 \pm 0.1
Glutamine	3.36	2.63	2.94	4.52	48.1 \pm 0.5
Lysine	1.05	1.97	2.28	2.91	51.3 \pm 0.3
Citrulline	1.21	1.42	3.13	4.73	56.2 \pm 0.7

*Sample 1 non treated *Cryptococcus laurentii* *Sample 2 *Cryptococcus laurentii* after treated by (WEP) *Sample 3 non treated *Candida famata* *Sample 4 *Candida famata* after treated by (WEP).

Table 7
Analysis of Variance (ANOVA) of oxidative enzymes based on error mean square (EMS) value using ELISA assay.

SOV	Df	Ascorbate Peroxidase	Catalase	Glutathione Reductase	Superoxide dismutase
Treatments	3	1382.89**	53214.3**	82437.6**	121535**
Replication	2	25.08	266.6	1573.1	1287
Error	6	90.97	749.9	565.3	2866

**Fig. 10.** (A–D). Ascorbate Peroxidase U/g; (B) Catalase U/g; (C) Glutathione Reductase U/g; (D) SOD U/g 1. (Non-treated *Cryptococcus laurentii*; 2. *Cryptococcus laurentii* after treated by (WEP) 3. Non-treated *Candida famata* 4. *Candida famata* after treated by (WEP).

characteristics, PR can also use in canal irrigation as endodontic treatments (Ahangari et al., 2018). Of the newly found medications, PR has attracted attention as a natural antimicrobial agent. To detect antibacterial activity of some different concentrations of ethanol and water PR extracts against isolated yeast from indus-

trial food, 30%, 50%, 70% and 100% concentration were used. All tested PR extracts were effective against all tested yeasts. This study showed the ability of ethanol and water PR extracts, to discourage the growth of tested yeast. The highest effect was on *Cryptococcus laurentii* and the lowest effect shows on *C. famata*. To

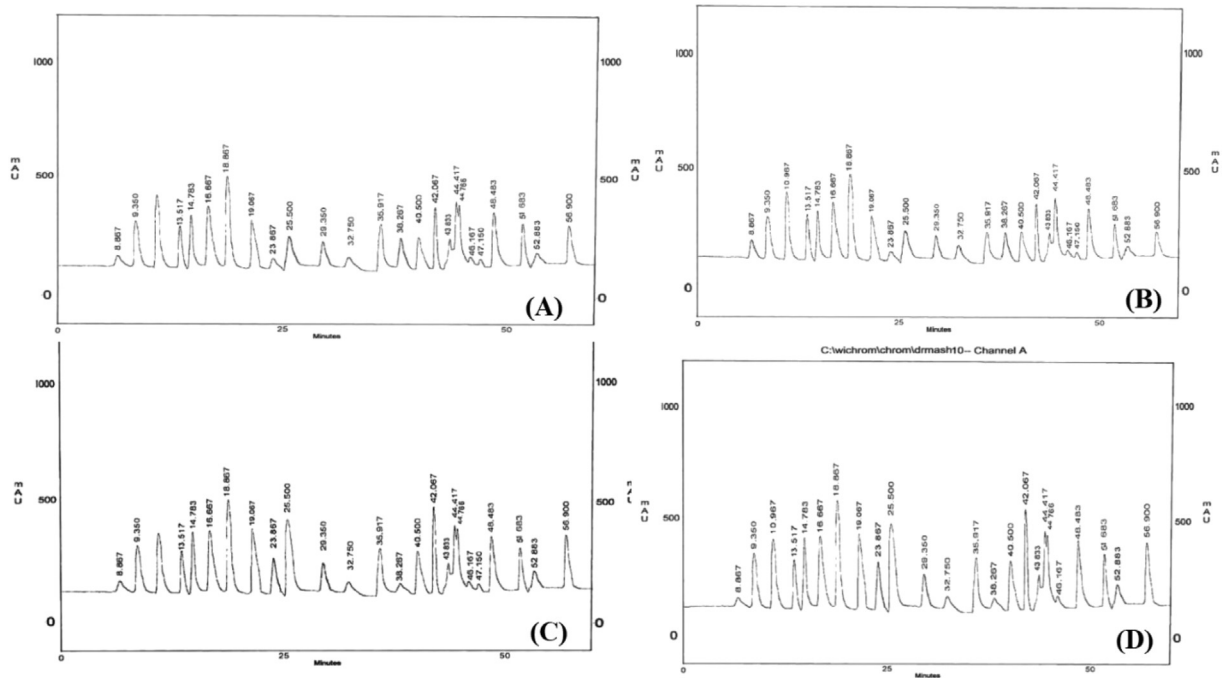


Fig. 11. (A–D). Chromatogram of mixture proteinic amino acids in (non-treated *Cryptococcus laurentii*) (A), (*Cryptococcus laurentii* after treated by WEP 70%) (B); (non-treated *Candida famata*) (C); *Candida famata* after treated by WEP 70%) (D).

evaluate the antifungal properties of PR, electron microscopic studies and some other biochemical analysis (i.e. determination of free and proteinic amino acids and determination of some oxidative enzymes) were used. Scanned electron microscope observations in current experiment visibly confirmed the effective fungicidal action used by PR. The cell rupture was recorded in our study was in agreement with Takaisi & Schilcher (1994) and who suggested that the possible mechanism of inhibition of cell division was due to antimicrobial action of PR and surface alteration of some cells are owing to a modification in cell permeability, which agrees with earlier ultrastructural recording. This shows that the first changes are limited at the plasmalemma and cell wall earlier the detection of any change in the cell interior. Transmission electron microscope studies with PR revealed that the plasmalemma permeability had been altered thus provoking osmotic imbalance, as was evident by showing the oozing of cations, amino acids, and proteins. That had explained the incision of the walls in collapsed cells corresponding to the pivotal areas where membranous material is deposited i.e., between the plasmalemma and the cell wall or in the cell wall itself resulted in causing an expanded presence of the cellular contour. The results are thoroughly corroborating to our preceding TEM findings of recurrent focal thickenings of the cell wall, deposition of irregular and dense presences in these walls. PR chemical composition depends upon, plant sources, seasons and geographical regions. About 300 different compounds were identified in PR i.e. aromatic acids, essential oils, waxes and amino acids (Anjum et al., 2018). Among other biochemical ingredients, different types of free amino acids, acid extraction and quantification of these amino acid are confirmed using gas–liquid chromatography. It was also reported that total concentration is about 40% w/w. moreover, it was also reported that arginine and proline composed of 50% crude acid extract. However, physiological consequence of arginine in PR can trigger mitosis and increase the protein biosynthesis and biochemical properties of proline. These free amino acids also help the plant to build collagen and elastin consider as an important component in the matrix of

connective tissues (Gabrys et al., 1986). A similar study was designed to determine the effect of antimicrobial result of PR and chemical content was done using GC–MS and inhibitory effect of PR was determine using disc diffusion method against 6 g positive, gram negative bacteria and yeast (fungi). The results reported that total flavonoid contents of PR were elevated than another compound. The oxidative enzymes activity was decrease by using PR treated i.e. *C. famata* and reduced the ascorbate peroxidase, catalase activity, glutathione reductase activity and Superoxide Dismutase activity in yeast. PR application reduced the ROS generation and lipid peroxidation by enhancing Cu/Zn-sod activity after oxidative stress. Moreover, it also protects the plants cells using synergistically with Cu/Zn-sod (Sa et al., 2013). Yonar et al (2014) reported that cells tolerance was not fully restored after treating with PR. It was also reported that simultaneous administration of PR and chromium, lipid peroxidation was decreased together while increasing antioxidant enzyme activity i.e. catalase, glutathione peroxidase, super oxide dismutase. The influence of ethanolic extract of PR was examined at proteomic level in mitochondria and results were analyzed after 1 h acquaintance of yeast cells to EEP and moderately polar fraction of EEP (E2). The results reported that antioxidative proteins were changed as well as proteins that were linked with ATP synthesis also found (Cigt et al., 2011). The inhibitory effects of PR extract are not fully known. A possible mechanism of PR activity would be through the inhibition of the synthesis of proteins and function of the membrane. Flavonoid quercetin is responsible for such kind of activity (Mirzoeva et al., 1997; Takaisi-Kikuni et al., 1994). Yeasts are less sensitive to PR than bacteria, and more sensitive than mold (Anjum, 2018). PR biological action were appearing to be related with and plasma membrane disruption and cell wall (Mello et al., 2006). Also, PR-mediated cell death has occurred in *S. cerevisiae* (De Castro et al., 2011). The antimicrobial properties of PR have been confirmed and reported by many scientists, but it may vary from lab to lab and geographic origin and is difficult to compare the PR composition and effects of antimicrobial study.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2020.01.023>.

References

- Ahangari, Z., Naseri, M., Vatandoost, F., 2018. Propolis: chemical composition and its applications in endodontics. *Iranian Endod. J.* 13 (3), 285–292.
- Anjum, S.I., Ullah, A., Khan, K.A., Attaullah, M., Khan, H., Ali, H., Bashir, M.A., Tahir, M., Ansari, M.J., Ghramh, H.A., Adgaba, N., 2018. Composition and functional properties of propolis (bee glue): a review. *Saudi J. Biol. Sci.* 26 (7), 1695–1703.
- Bankova, V., 2005. Recent trends and important developments in propolis research. *Evidence-Based Complement. Alternat. Med.* 2 (1), 29–32.
- Bankova, V.S., de Castro, S.L., Marcucci, M.C., 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie* 31 (1), 3–15.
- Bastos, E.M., Simone, M., Jorge, D.M., Soares, A.E., Spivak, M., 2008. *In vitro* study of the antimicrobial activity of Brazilian propolis against *Paenibacillus* larvae. *J. Invertebr. Pathol.* 97 (3), 273–281.
- Boyanova, L., Kolarov, R., Gergova, G., Mitov, I., 2006. *In vitro* activity of Bulgarian propolis against 94 clinical isolates of anaerobic bacteria. *Anaerobe* 12 (4), 173–177.
- Bozzola, J.J., Russell, L.D., 1999. *Electron Microscopy: Principles and Techniques for Biologists*. Jones and Bartlett, Boston, p. 670.
- Cafarchia, C., De Laurentis, N., Milillo, M.A., Losacco, V., Puccini, V., 1999. Antifungal activity of Apulia region propolis. *Parassitologia* 41 (4), 587–590.
- Castaldo, S., Capasso, F., 2002. Propolis, an old remedy used in modern medicine. *Fitoterapia* 73, S1–S6.
- Cigut, T., Polak, T., Gašperlin, L., Raspor, P., Jamnik, P., 2011. Antioxidative activity of propolis extract in yeast cells. *J. Agric. Food. Chem.* 59 (21), 11449–11455.
- De Castro, P.A., Savoldi, M., Bonatto, D., Barros, M.H., Goldman, M.H., Berretta, A.A., Goldman, G.H., 2011. Molecular characterization of propolis-induced cell death in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 10 (3), 398–411.
- Espina, L., Somolinos, M., Ouazzou, A.A., Condón, S., García-Gonzalo, D., Pagán, R., 2012. Inactivation of *Escherichia coli* O157: H7 in fruit juices by combined treatments of citrus fruit essential oils and heat. *Int. J. Food Microbiol.* 159 (1), 9–16.
- Fernandes, F.F., Dias, A.L., Ramos, C.L., Ikegaki, M., Siqueira, A.M., Franco, M.C., 2007. The “in vitro” antifungal activity evaluation of propolis G12 ethanol extract on *Cryptococcus neoformans*. *Revista do Instituto de Medicina Tropical de São Paulo* 49 (2), 93–95.
- Fokt, H., Pereira, A., Ferreira, A.M., Cunha, A., Aguiar, C., 2010. How do bees prevent hive infections? The antimicrobial properties of propolis. *Curr. Res. Technol. Educat. Top. Appl. Microbiol. Microbiol. Biotechnol.* 1, 481–493.
- Gabrys, J., Konecki, J., Krol, W., Scheller, S., Shani, J., 1986. Free amino acids in bee hive product (propolis) as identified and quantified by gas-liquid chromatography. *Pharmacol. Res. Commun.* 18 (6), 513–518.
- Golder, W., 2004. Propolis. The bee glue as presented by the Graeco-Roman literature. *Wurzburger Medizinhist. Mitteilung.* 23, 133–145.
- Gupta, S., Kundabala, M., Acharya, S.R., Ballal, V., 2007. A comparative evaluation of the antibacterial efficacy of propolis 30% sodium hypochlorite and 02% chlorhexidine gluconate against *Enterococcus faecalis*-An *in vitro* study. *Endodontology* 19 (2), 31–38.
- Han, S.K., Park, H.K., 2002. Accumulation of thiobarbituric acid – reactive substances in cured pork sausages treated with propolis extracts. *Journal of the Science of Food and Agriculture* 82 (13), 1487–1489. <https://doi.org/10.1002/jsfa.1216>.
- Hashem A., Abd Allah E.F., Alwathnani H.A. (2012). Effect of propolis on growth, aflatoxins production and lipid metabolism in *Aspergillus parasiticus* sp. *Pakistan J. Botany*, 44(3):1153–1158.
- Kalogeropoulos, N., Konteles, S.J., Troullidou, E., Mourtzinos, I., Karathanos, V.T., 2009. Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. *Food Chem.* 116 (2), 452–461.
- Leistner, L., Gorris, L.G., 1995. Food preservation by hurdle technology. *Trends Food Sci. Technol.* 6 (2), 41–46.
- Marcucci, M.C., Ferreres, F., Garcia-Viguera, C., Bankova, V.S., De Castro, S.L., Dantas, A.P., Valente, P.H., Paulino, N., 2001. Phenolic compounds from Brazilian propolis with pharmacological activities. *J. Ethnopharmacol.* 74 (2), 105–112.
- Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie* 26 (2), 83–99.
- Mello, A., Gomes, R.T., Lara, S., Silva, G., Alves, B., Cortés, M.E., Abreu, S.L., Santos, V. R., 2006. The effect of Brazilian propolis on the germ tube formation and cell wall of *Candida albicans*. *Pharmacologyonline* 3, 352–358.
- Mirzoeva, O.K., Grishanin, R.N., Calder, P.C., 1997. Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. *Microbiol. Res.* 152 (3), 239–246.
- Mishima, S., Inoh, Y., Narita, Y., Ohta, S., Sakamoto, T., Araki, Y., Suzuki, K.M., Akao, Y., Nozawa, Y., 2005. Identification of caffeoylquinic acid derivatives from Brazilian propolis as constituents involved in induction of granulocytic differentiation of HL-60 cells. *Bioorg. Med. Chem.* 13 (20), 5814–5818.
- Moreira, L., Dias, L.G., Pereira, J.A., Estevinho, L., 2008. Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food Chem. Toxicol.* 46 (11), 3482–3485.
- Orsi, R.O.I., Sforzin, J.M., Funari, S.R.C., Fernandes, J.R.A., Rodrigues, P., Bankova, V., 2007. Effects of propolis from Brazil and Bulgaria on *Salmonella* serovars. *Journal of Venomous Animals and Toxins including Tropical Diseases* 13 (4), 748–757. <https://doi.org/10.1590/S1678-91992007000400006>.
- Piccinelli, A.L., Lotti, C., Campone, L., Cuesta-Rubio, O., Campo, Fernandez M., Rastrelli, L., 2011. Cuban and Brazilian red propolis: botanical origin and comparative analysis by high-performance liquid chromatography–photodiode array detection/electrospray ionization tandem mass spectrometry. *J. Agric. Food. Chem.* 59 (12), 6484–6491.
- Prytyk, E., Dantas, A.P., Salomão, K., Pereira, A.S., Bankova, V.S., De Castro, S.L., Neto, F.R., 2003. Flavonoids and trypanocidal activity of Bulgarian propolis. *J. Ethnopharmacol.* 88 (2–3), 189–193.
- Sá, R.A., de Castro, F.A., Eleutherio, E.C., Souza, R.M., da Silva, J.F., Pereira, M.D., 2013. Brazilian propolis protects *Saccharomyces cerevisiae* cells against oxidative stress. *Brazil. J. Microbiol.* 44 (3), 993–1000.
- Salatino, A., Teixeira, É.W., Negri, G., 2005. Origin and chemical variation of Brazilian propolis. *Evidence-Based Complement. Alternat. Med.* 2 (1), 33–38.
- Salomão, K., Dantas, A.P., Borba, C.M., Campos, L.C., Machado, D.G., Aquino Neto, F.R., De Castro, S.L., 2004. Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. *Lett. Appl. Microbiol.* 38 (2), 87–92.
- Sforzin, J.M., 2007. Propolis and the immune system: a review. *J. Ethnopharmacol.* 113 (1), 1–4.
- Siqueira, A.B.S., Gomes, B.S., Cambuim, I., Maia, R., Abreu, S., Souza-Motta, C.M., De Queiroz, L.A., Porto, A.L.F., 2009. Trichophyton species susceptibility to green and red propolis from Brazil. *Letters in Applied Microbiology* 48, 90–96.
- Takaisi-Kikuni, N.B., Schilcher, H., 1994. Electron microscopic and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Med.* 60 (03), 222–227.
- Teixeira, E.W., Negri, G., Salatino, A., Stringheta, P.C., 2010. Seasonal variation, chemical composition and antioxidant activity of Brazilian propolis samples. *Evidence-Based Complement. Alternat. Med.* 7 (3), 307–315.
- Valencia, D., Alday, E., Robles-Zepeda, R., Garibay-Escobar, A., Galvez-Ruiz, J.C., Salas-Reyes, M., Jiménez-Estrada, M., Velazquez-Contreras, E., Hernandez, J., Velazquez, C., 2012. Seasonal effect on chemical composition and biological activities of Sonoran propolis. *Food Chem.* 131 (2), 645–651.
- Yaghoubi, S.M.J., Ghorbani, G.R., Soleimani, Z.S., Satari, R., 2007. Antimicrobial activity of Iranian propolis and its chemical composition. *DARU.* 15 (1), 45–48.
- Yonar, M.E., Yonar, S.M., Çoban, M.Z., Eroğlu, M., 2014. Antioxidant effect of propolis against exposure to chromium in *Cyprinus carpio*. *Environ. Toxicol.* 2, 155–164.