GRADIVA

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

Exploring Sustainable Food Packaging: Development and Characterization of Biodegradable Composite Films by Incorporating Starch and Propolis Extracts for the Preservation of Strawberries

Fatiha Ferhoum [1]*, Khadidja Mahdi [2], Nassima Bourfis [3], Souhila Bensmail [4], Djamila Djouahra-Fahem [5] & Fatima Benaisa [6]

1, 2, 3. Department of Agricultural Sciences, Faculty of Nature and Life Sciences and Earth Sciences, University of Bouira, Algeria.
Research Laboratory: Biotechnologie and Protection of Agricultural and Natural Ecosystems, University of Bouira, Algeria.
* Corresponding Author Email: f.ferhoum@univ-bouira.dz
4, 5. Research Laboratory: Biotechnologie and Protection of Agricultural and Natural Ecosystems, University of Bouira, Department of Biology, Faculty of Nature and Life Sciences and Earth Sciences, Bouira, Algeria.
6. Institute Desbrest of Epidemiology and Public Health (IDESP), University of Montpellier and INSERM, Montpellier Department of Pneumology, Allergology and Thoracic Oncology, Monpelier University Hospital, Montpellier, France, Common Core Department of Natural and Life Sciences, Targa Ouzemour, University of Bejaia, Bejaia, Algeria.

Abstract

Modern scientific research has demonstrated that beehive products contain bioactive substances with medicinal applications. Among these products, propolis is a resinous substance harvested by bees on certain buds and barks of specific trees. The composition of propolis ensures various biological properties, including antibacterial, antifungal, antioxidant and anti-inflammatory effects. Propolis can find application in food packaging, utilizing its composition rich in bioactive compounds. A food film based on starch and propolis serves as an alternative to replace plastic materials, known to cause diseases such as cancer due to the harmful chemicals they contain and facing criticisms related to environmental impact. The primary focus of this study is on the physicochemical characteristics of propolis, especially polyphenols and flavonoids. The antioxidant activity of Algerian propolis ethanolic extract, obtained through different extraction methods, is evaluated. Antimicrobial activity was evaluated by measuring inhibition-zones diameters in millimeters for agar diffusion, along with determining the Minimum Inhibitory Concentration (MIC). Significant differences were observed between the three-propolis ethanolic extracts (PEEs) in the sensitivity to various tested strains. In terms of antioxidant activity, one of the three PEEs proves to be the most effective. Moreover, this study focuses on developing a food packaging film based on starch and PEF that has been most effective in preliminary analyses. The characteristics of the film, including thickness, moisture content, and biodegradability, were examined. The film's impact on the shelf life of strawberries has been explored, revealing an increased shelf life compared to market food film.

Keywords: *Propolis, Bioactive Compounds, Food Film, Biodegradability, Preservation, Starch.*

1. INTRODUCTION

Since antiquity, natural products, especially those of plant origin, have always been an important source of therapeutic agents. Currently, about 25-30% of all drugs available for the treatment of diseases are derived from natural products (from plants, animals, bacteria and fungi) [1]

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

Bee products have been exploited by humans for thousands of years. They come from natural substances, produced by bees. Their uses ensure a good market and represent an additional income for the beekeeper [2]. Among these products, the propolis, it was called the tears of trees at the time of Aristotle [3]. Indeed, it is a natural resinous substance collected by bees either on tree buds such as poplar, oak, alder, etc. [4]. It has a balsamic smell and a variable color depending on its plant origins; it varies from light yellow to very dark brown [5].

Throughout the world, several studies are devoted to propolis, an important source of phenolic compounds, in particular flavonoids; numerous propolis substances harvested from different regions of the world have been identified; whose phenolic acids, flavones, flavonols and flavanones mark their permanent presence (standard elements of propolis). Furthermore, the antimicrobial activities of propolis have generally been attributed to flavonoids, although other components found in propolis may also exhibit inhibitory actions against microorganisms [6]. For this purpose, propolis is used in medicine, cosmetology, veterinary medicine and food industry [5].

Food packaging can be an area of use for propolis, which consists of maintaining the hygienic, nutritional and organoleptic qualities of food for as long as possible, by acting on the various mechanisms of alteration to slow down or eliminate their effects. Many conservation technologies have been developed based on the development of packaging materials based on wood, paper, metals, glass and synthetic polymers from petrochemicals (plastic) [7].

Environmental, economic and food safety challenges have led packaging scientists and producers to partially replace petrochemical-based polymers with other biodegradable ones. In order to reduce the volume of waste from synthetic packaging based on polymers of petrochemical origin which pose an environmental problem [8].

Biodegradable packaging is made up of macromolecules of natural and renewable origin, called biopolymers, such as proteins, lipids and polysaccharides (celluloses and derivatives, starches and derivatives, etc.), which can also play an active role in improving the organoleptic, biochemical, hygienic, microbiological and/or physicochemical qualities of the foods thus packaged. This role consists of serving as a support for active substances (vitamins, antioxidants, antimicrobials, etc.) [9], which is the case in our study.

This study is carried out in order to investigate the antioxidant and antimicrobial properties of propolis and utilize its active compounds in creating an effective, easily digestible, and environmentally friendly food film. This initiative is driven by:

- The desire to valorize a by-product of the hive;
- Determine the influence of the extraction technique on the composition of propolis;
- Develop an active, biodegradable and digestible packaging film;
- Preserve the environment of packaging from petrochemicals.

2. MATERIAL AND METHODS

The sample of propolis used in this study was provided to us by beekeepers from Takerboust village, Aghbalou commune, Bouira wilaya, Algeria. The harvest was carried out in January 2022 by scraping the frames. After receiving the raw propolis is prepared (cleaning, grinding) according to the method [10].

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

Several extractions with ethanol were carried out on propolis in order to compare the yields and antioxidant activity. These preliminary tests aimed to determine the best extraction method to support this study.

2.1. Extraction Methods

Extraction by maceration is carried out according to the method of Boryana et al. [11] with some modifications. Put 1 g of propolis in 50 ml of 96% Ethanol. After magnetic stirring for 24 hours away from light, the mixture is then filtered by a number 1 Wattman filter, the filtrate is concentrated in a rotary steamer at 50 °C then stored at 4 °C until use.

To extract the active ingredients from propolis by ultrasound, we opted for the protocol described by Wafa et al. [12] by making some modifications: Sonication of 1(g) of propolis in 50(ml) of 96% ethanol for 3 hours at 50°C, Filtration with Whatman number 1 filter paper, Concentration with a rotary rotavapor at 50°C under vacuum.

Soxhlet extraction is based on a distillation process which is widely used in laboratories and food and non-food industries [13]. 1g of propolis is introduced into the Soxhlet cartridge. 150 ml of 96% Ethanol are poured into the flask and 25ml into the extractor, the extraction is carried out for 6 hours until the material is exhausted. Then Concentration in a rotary rotavapor at 50°C under vacuum.

The extraction yield is calculated by the formula given by [14]:

R (%) = 100 Mext/Mech.

Where:

- R is the yield in%;
- Mext is the mass of the extract after evaporation of the solvent in mg;
- Mech is the dry mass of the plant sample in mg.

2.2. Quantification of Some Bioactive Compounds of Propolis

2.2.1. Quantification of Total Polyphenols

The determination of total polyphenols is carried out by the method described by Basyirah et al [15] with some modifications. 5 ml of distilled water are added to 0.5 ml of ethanolic extract of propolis, mixed well; 0.5 ml of the folin-ciocalteus reagent is added and left to stand for 3 min. 0.5ml of sodium carbonate (10%) is added, mixed well, then incubated for one hour at room temperature and protected from light, then the absorbance is measured at 760 nm.

The calibration curve (Y=aX+ b) is obtained with gallic acid at different concentrations practiced under the same operating conditions as the samples.

2.2.2. Quantification of Flavonoids

The estimation of the content of total flavonoids contained in the Ethanolic extracts of propolis is carried out by the method described in the [14].

A volume of 1ml of extract of dried pumpkin waste or pulp is added with 1ml of aluminum chloride (AlCl3 at 2%), the absorbance is measured at 430nm, after 1 hour of incubation. The quantity of flavonoids is calculated in mg quercetin equivalent per 1 g of sample (mg EQ / g Ech), from the standard curve prepared with quercetin.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The calibration curve (Y=aX+ b) obtained with quercetin at different concentrations practiced under the same operating conditions as the samples

2.3. Evaluation of Antioxidant Activity: The antioxidant activity of ethanolic extracts of propolis is evaluated by two different methods: the anti-radical activity against the DPPH radical and the ferric reduction capacity of plasma (FRAP: Ferric Reducing Antioxidant Power).

2.3.1. Anti-Radical Activity

The demonstration of the antioxidant power of propolis extracts via the DPPH test is carried out by the method described by Shi et al [16], with a few modifications: 1ml of the ethanolic solution of DPPH (0.05 mM) is added to 2 ml of the solution of ethanolic propolis extracts (different concentrations), then the tubes are incubated at room temperature and in the dark for 30 minutes. Readings are taken at 517 nm.

The absorbance's were converted into radical-scanning rates of DPPH according to the equation

$$:\%Inhibition\ of\ DPPH = \frac{Ac-As}{Ac} \times 100$$

Ac: Absorption of control.

As: Absorbance of the sample.

At different concentrations, the calculation of IC50 (The median inhibitory concentration) is carried out, because this value allows us to interpret the results of this method.

2.3.2. Evaluation of Anti-Radical Activity using the FRAP (Ferric Reducing Antioxidant Power) Method

The demonstration of the antioxidant power of propolis extracts via the FRAP method is carried out by the method described by Benzie and Strain [17], with some modifications:2.5ml of the phosphate buffer solution (ph: 6.6) and 2.5ml of potassium ferricyanide (K3Fe (CN)6 (1%) are added to 2.5ml of the ethanolic extract of propolis. the mixture is incubated at 50°C for 20 min, then 2.5 ml of trichloroacetic acid (10%) is added and centrifuged at 5000 rpm. 2.5 ml of supernatant are added with 2.5 ml of distilled water and 0.5 ml of iron chloride FeCl3 (0.1%). Read the absorbance at λ=700 nm.

2.4. Antibacterial Activity of Ethanolic Extract of Propolis

2.4.1. Bacterial Strains

Antimicrobial activity was evaluated against the following microorganisms: Bacillus subtilis (ATCC 6633), Enterococcus Feacalis (ATCC 29219), MRSA (ATCC 43300), Pseudomonas aeroginosa (ATCC 6633), Candida albicans (ATCC 10231).

2.4.2. Inoculum Preparation

A 24-hour young culture was carried out on nutrient agar plates for each species. Then, the bacterial suspensions were prepared in physiological water and adjusted according to the 0.5 McFarland standard using a spectrophotometer in order to have a microbial load of 10 8 CFU/ml. The bacterial suspension thus prepared was used to test the sensitivity of the strains to the samples under study [18].

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

2.4.3. Agar Well Method

The agar well diffusion test was carried out according to the methods described by Valgas [19]. A standardized bacterial inoculum was distributed evenly on a Mueller-Hinton agar surface using sterile cotton swabs. Immediately, wells of approximately 6 mm in diameter were made on the agar surface using a sterile metal cylinder.

2.4.4. The Minimum Inhibitory Concentration

MIC determination was carried out by the broth micro-dilution method according to CLSI M07-A9 guidelines (CLSI, 2012) and Mbosso et al. (2010). Initially, the ethanolic extracts obtained from each extraction method were dried to obtain a dry extract, then 50 mg of each dry extract was dissolved in 1 ml of ethanol to produce stock solutions of 25 mg. /mL. A volume of 200 µL of extract mixed with M-H broth was added to the first wells of each row. Then, a two-fold serial dilution was performed up to the eighth well in each row. Finally, 50 µL of bacterial inoculum diluted in M-H broth was added to the wells. The plates were incubated aerobically at 37°C for 24 h. The 11th to 12th wells were reserved as controls for viability (with and without ethanol) and sterility (with and without extracts) [20].

The MIC was considered to be the lowest concentration of the extract that prevented visible growth due to the formation of acidic metabolites corresponding to microbial growth [20].

2.5. Elaboration of a Starch-Based Bioplastic Film :

Elaboration was carried out using the method described by Inyoung et al [21] with a few modifications:

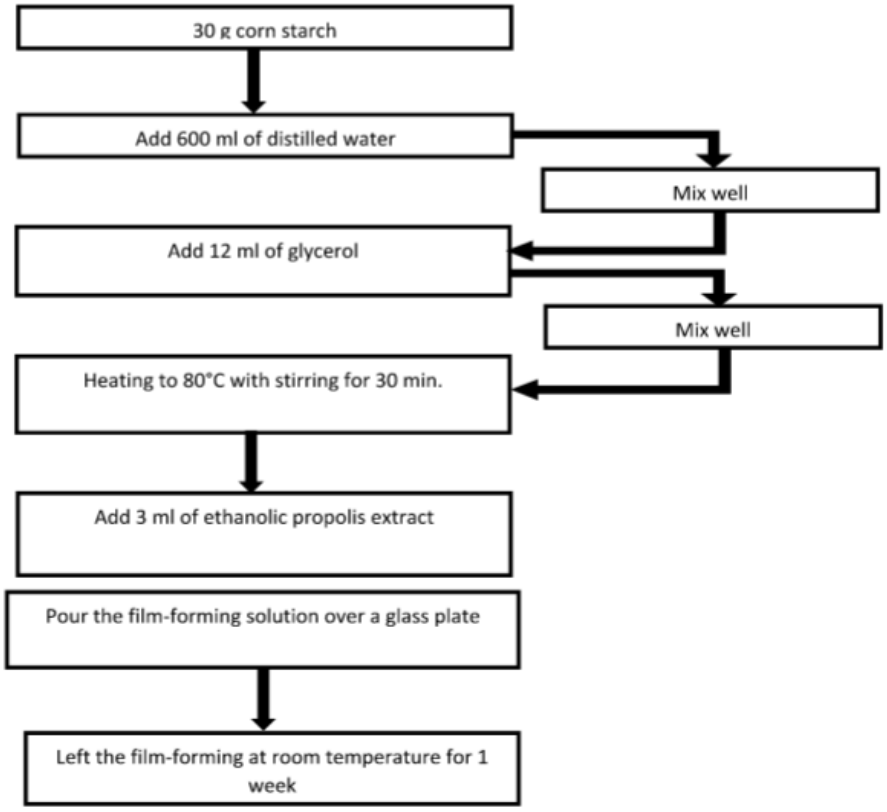

Figure 1: Manufacturing Diagram for a Starch-Based Bioplastic

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

2.5.1. Film Thickness

The thickness of the starch-based biodegradable film was determined using a digital micrometer from the average of at least three random measurements made on the film [22].

2.5.2. Water Solubility

Film samples (30 mm × 10 mm) were dried at 100°C for 20 min. The initial dry matter of each specimen was determined by examining the sample weight and described as (Wi). This was confirmed by immersing each specimen in a beaker containing distilled water (100 ml). The beaker was then fixed and continuously stirred under constant agitation for a period of 1 h at room temperature. Finally, the sample fraction that did not dissolve was isolated and left to dry for 20 min in an oven at at 100°C, then weighed (Wo). Using the equation, the water solubility of each sample each sample was determined [23].

$$Solubility(\%) = \left(\frac{Wi - Wo}{Wi}\right) \times 100$$

2.5.3. Water Absorption

The study of water absorption was carried out using the ASTM D 570-98 (1998) approach. Film samples were oven-dried at 100°C for 20 min, then cooled in a desiccator to ensure constant weight. The samples were then weighed at room temperature, immersed in distilled water for 30 min. The soaked film samples were cleaned and reweighed with a clean piece of cloth. Using the initial and final masses and final masses recorded, the mass difference was calculated using equation [24]:

$$Waterabsorption(\%) = \frac{Mfinal - Minitial}{Minitial} \times 100$$

2.5.6. Film Degradation

Research into biodegradation properties is essential for the implementation of biodegradable films in the environment. In this study, soil burial tests were carried out for EEP-based starch film for 1 week.

Briefly, biodegradation is defined as the breakdown of material by fungi, bacteria or other biological decomposers [25]. Through an enzymatic or metabolic process, these microbial organisms break down the polymer into compounds with a lower average molecular weight.

2.6. Statistical Analysis

The results obtained in the present work are represented as means ± standard error

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

It emerges through the observation of the extraction yields, from Figure 2 that the extraction by Soxhlet technique revealed the highest extraction yield compared to the other techniques which is 73.67% on average. On the other hand, the maceration technique and ultrasound showed a low value of extraction yield which are 65% and 63.3% respectively. These results are comparable to those obtained by Nur Basyirah et al [26]. Longer extraction time resulted in higher extraction yield due to longer time of samples and solvent in contact with each other and greater mass transfer. After 4 hours of extraction, the extraction yield gradually decreases as they reach final equilibrium [27].

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

According to Kouamé et al (2021)[28], the method can influence the extraction yield, maceration is an extraction technique that is conducted at room temperature, therefore some metabolites cannot be extracted effectively when they are poorly soluble at ambient temperature.

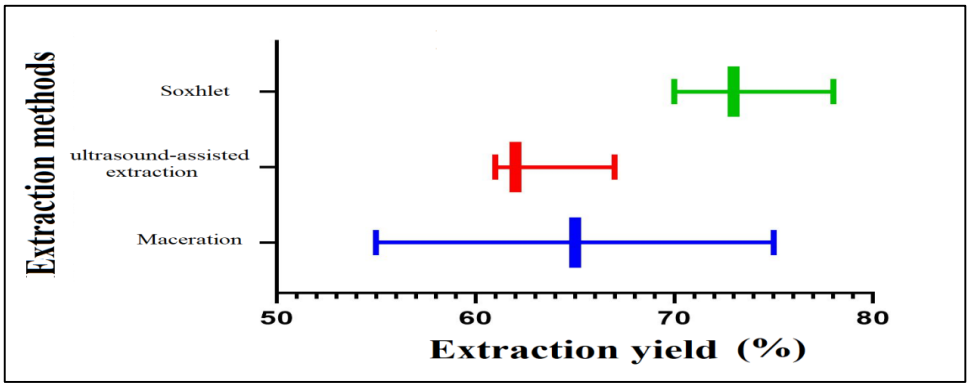

Figure 2: Extraction Yield of Different Methods

Maceration consisted only of soaking the samples without vibration, which reduced the contact between the sample and the solvent compared to soxhlet extraction. The ultrasound extraction technique usually does not present a high yield, which is the case in our study. Due to the high temperature exposure and continuous recycling of solvents during the extraction process, it helps to increase the solubilization of raw material components [29].

In the soxhlet extraction, the small amount of solvent, solvent recycle and reduced extraction time are the main advantages of the method compared to maceration. Several research groups have found that 4 6 h Soxhlet extraction with absolute ethanol at 60° C led to the highest yields and higher total phenolics and total flavonoids. [30]

3.2. Quantification of Some Main Compounds of Propolis

The results of the determination of total polyphenols and flavonoids in the propolis sample analyzed are presented in Table No. 1. The values of the concentration and the corresponding standard deviation for the polyphenols are expressed in mg of acid equivalent Gallic per gram of raw propolis (mg EAG / g M raw), for flavonoids are expressed in mg of quercetin per gram of raw propolis.

Table 1: Polyphenol and Flavonoid Content of Different Propolis Extraction Methods

Extraction méthodes	Quantity of polyphenols in mg/g of propolis	Quantity of flavonoïdes en mg/g of propolis
Macération	130.07 ± 2.24	11.39 ± 2.61
Ultrasons	100.99 ± 0.79	13.27 ± 1.26
Soxhlet	40.45 ± 1.14	31.98 ± 2.55

The total polyphenol contents of the different propolis extraction methods present significant differences, the variation goes from 40.45 (Standard deviation: 1.14) mg EAG /g of raw propolis to 130.07 (Standard deviation: 2.24) mg EAG /g of raw propolis.All three methods showed that maceration extraction produced a higher phenolic content than the other two extraction methods, and also this technique contributed to an increase in polyphenol content even though it produced a low extraction yield.However, the total content of phenolic compounds with the soxhlet technique showed the lowest value compared to other methods.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The extraction time extended by sonication up to 1.5 h leading to degradation of phenolic content in propolis and supported by Gullian & Terrats [125, 31] who stated that total phenolic in samples will generally be degraded due to a longer extraction and exposed to high temperature, which is the case in our experience, for the soxhlet extraction technique, it lasted 6 hours at a temperature of 70°C, for ultrasound it lasted 3 h at a temperature of 50°C. While maceration was carried out at room temperature for 24 hours. Which explains the reason for the high polyphenol content of the latter; it was not exposed to high temperature compared to the other two extraction methods.

The total polyphenol content of Brazilian propolis is of the order of 232 (Standard deviation: 22.3) mg EAG/g [32], this value is higher than that found on propolis extracted by maceration.

Work carried out on Iranian propolis [33] reported phenolic compound contents of around 8.46% (Standard deviation: 0.03); 7.11% (Standard deviation: 0.19) and 3.08% (Standard deviation: 0.02) of raw propolis respectively for Tehran, Isfahn and Khorasan. These values are lower than that found in the present study for extraction by maceration (10 to 16 g/100 g of propolis).

The study carried out on propolis from Portugal shows total polyphenol contents which oscillate between 151.00 (Standard deviation: 0.01) and 329.00 (Standard deviation: 0.01) mg EAG/g of propolis respectively for the region of Fundao and Borne [34].

The study carried out on propolis from Colombia, shows total polyphenol contents of the order of 80.19 (Standard deviation: 0.11) mg EAG/g of propolis, for the ethanolic extract obtained by soxhlet, which is superior to the result obtained in our experiment.[35]

The work carried out on propolis collected from the hive farm, Sultan Zainal Abidin University (Besut Campus) [26], shows total polyphenol contents similar to our study concerning the extract obtained by soxhlet.

The total flavonoid contents of the different propolis extraction methods present significant differences, the variation goes from 11.39 (Standard deviation: 2.61) to 31.98 (Standard deviation: 2.55).

The three methods showed that soxhlet extraction produced a higher flavonoid content than the other two extraction methods which is 31.98 ± 2.55 mg of quercetin/g of propolis. The ethanolic extract of propolis obtained by maceration showed a total flavonoid content of 11.39 ± 2.61 mg quercetin/g of the propolis, while the flavonoid compound extracted from the sonication technique was 13.27 ± 1.26 mg quercetin/g propolis (table 6). According to its two extraction methods, the propolis extract from sonication showed a higher flavonoid content than the maceration technique.

The work carried out on the sample from Brazil indicates a flavonoid content of around 43 (Standard deviation: 0.1) mg EQ / g of raw propolis [126?36], for the extract obtained by maceration.

The total flavonoid content of Chinese propolis obtained by maceration varies from 8.3 (Standard deviation: 3.7) to 188 (Standard deviation: 6.6) mg EQ / g of propolis [37].

The study carried out on samples from the beekeeping farm, Sultan Zainal Abidin University (Besut Campus) [26], presents flavonoid contents extracted by soxhlet similar to our experience.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The results obtained in the experiment carried out by Gloria et al [38], show a flavonoid content of the extract obtained by Soxhlet of 2.65 (Standard deviation: 0.04) mg EQ / g of propolis, which is lower to the results obtained in our study.

The study carried out by [39], explains that the content of total flavonoids and polyphenols depends on the botanical region and the breed of bee.

3.3. Evaluation of Antioxidant Activity

Antioxidants can react at different stages of the oxidation process and they can have more than one mechanism of action; there is no in vitro reference test to evaluate the antioxidant activity of a sample. For this, the combination of different tests is an indicator of the antioxidant capacity of the sample to be tested [40].

In the present study, the antioxidant activity of the ethanolic propolis extracts studied was determined using two different methods:

3.3.1. Determination of Reducing Power

In this study, the DDPH assay was used to analyze the antioxidant activity of propolis. The DPPH test is a method that accepted the electron or hydrogen to become a stable free radical thatnoticed by the purple to yellow color changes [41]. The results are expressed in IC50 (figure). The lower value of IC50 indicates the stronger antioxidant activity in propolis.

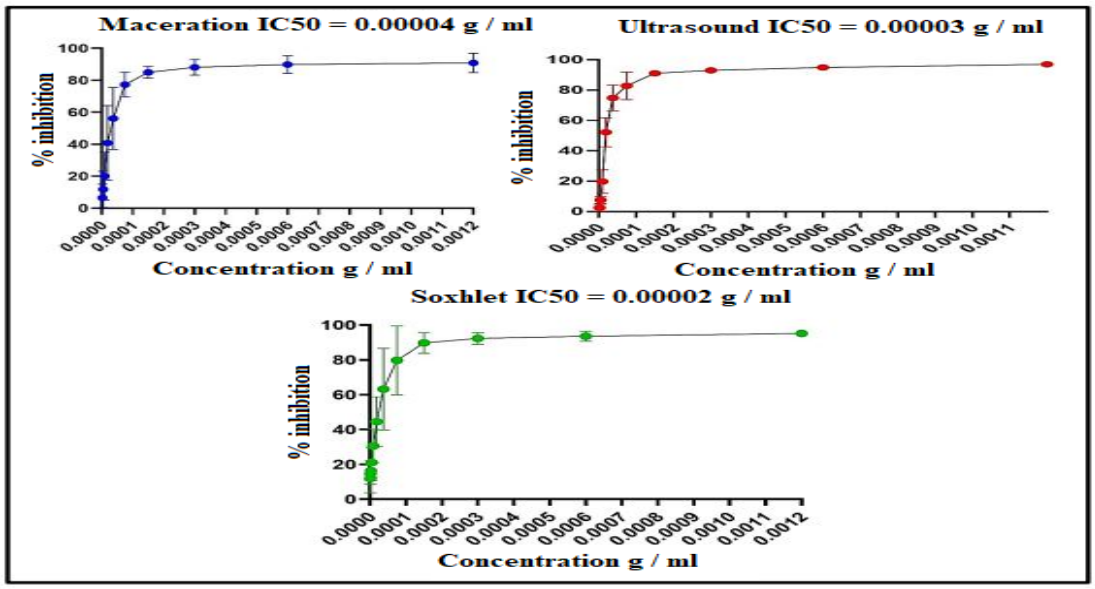

Figure 3: Percentage of Inhibition of Different Extraction Methods

From the curves illustrated previously, we note that the percentage of antioxidant activity increases with increasing concentration for all propolis extracts. It seems that the anti-radical activity is strongly dependent on the concentrations of propolis extracts. The more concentrated the extract, the higher the percentage of activity.

By comparing the average of the IC50 of the three extraction methods (Figure 3), we can deduce that the soxhlet extraction method presents the best anti-radical power estimated at (IC50 = 0.00002 g / ml) followed by the ultrasound extraction (IC50 = 0.00003 g/ml). Extraction by maceration has a value of IC50 = 0.00004 g/ml.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The study carried out on ethanolic extracts of propolis from Portugal obtained by maceration showed that the IC50 values are of the order of 0.00006 g/ml and 0.00025 g/ml respectively for Bornes and Funddao propolis [42]. Results superior to those found in our study, therefore our ethanolic extract of propolis obtained by maceration has a greater anti-radical power than propolis from Portugal.

The work carried out on the ethanolic extracts of propolis from the beekeeping farm, Sultan Zainal Abidin University (Besut Campus) [26], showed that the IC50 value is 0.000031 g/ml, which is lower to the result found in our study, therefore the propolis used by Nur, Basyirah, extracted by maceration has a greater anti-radical power than our propolis. While the result obtained on the ethanolic extracts obtained by soxhlet which is 0.000023 g / ml is similar to our result which is 0.00002 g / ml. While the results found for the ultrasound technique are lower than the results obtained in our study, which means that our ultrasound extract has low anti-radical power.

3.3.2. Reducing Power Test on Potassium Ferrocyanide (FRAP)

The reducing power results are illustrated in Figure N 4

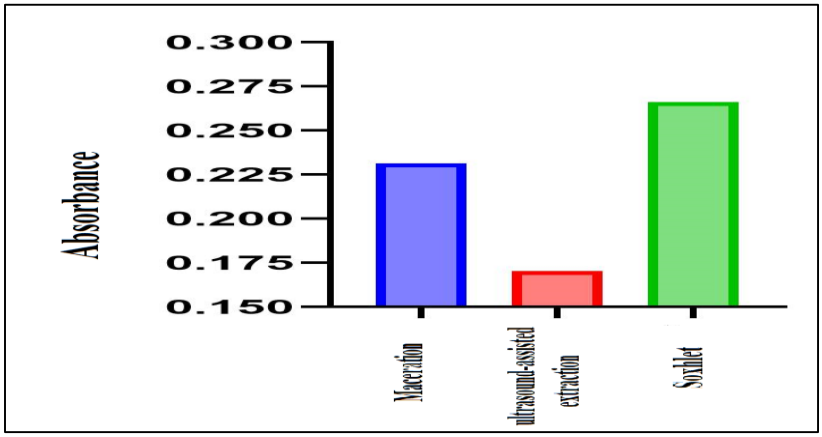

Figure 4: Absorbance of Different Propolis Samples

From the results obtained (Figure 4), we note that the extract obtained by the soxhlet technique has the highest absorbance compared to the other extracts, which indicates the presence of hydroxyl group in high proportion in the extract obtained by the soxhlet method.According to what we found in the present study, the amount of flavonoids in propolis reflects the antioxidant capacity.

The results obtained in the study carried out by Sulaiman et al [43], showed that the quantity of phenols and flavonoids in propolis does not testify to the antioxidant capacity.

According to [44], the biological activity of propolis is linked to the quantity of flavonoids and phenols, which are responsible for its antioxidant capacity.

3.4. Evaluation of Anti-Microbial Activity

3.4.1. The Sink Diffusion Method

The results obtained following diffusion tests on agar extracts from the strains are represented in the table below:

An antibiotic was used as a positive control (Ciprofloxacin) which presents the sensitivity of all strains.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

Table 2: Diameters of the EEP inhibition zones and the control

The EEP The strains	Macération	Ultrason	Soxhlet	Ciprofloxacine
Bacillus subtilis (ATCC 6633)	-	12 mm	9,66 mm	38 mm
Enterococcus Feacalis (ATCC 29219)	-	9,66 mm	12,33 mm	37 mm
SARM (ATCC 43300)	15 mm	12,66 mm	10 mm	34 mm
Pseudomonas aeroginosa (ATCC 6633)	9,5 mm	13 mm	-	28 mm
Candida albicans	+	+	+	+

According to the results obtained, the majority of strains show sensitivity to extracts from different extraction methods.

Our results show that:The ethanolic extract of propolis obtained by ultrasound gave antibacterial activities for all strains, with diameters greater than 12 mm on MRSA (ATCC 43300) and *Pseudomonas aeroginosa* (ATCC 6633).The EEP obtained by soxhlet recorded the greatest antibacterial activity on *Enterococcus Feacalis* (ATCC 29219).

Bacillus subtilis (ATCC 6633) is sensitive to extracts obtained by ultrasound and soxhlet but resistant to EEP obtained by maceration.

The EEP obtained by ultrasound showed that it has antibacterial activity for GRAM + bacteria (Bacillus subtilis (ATCC 6633), MRSA (ATCC 43300) and Enterococcus Feacalis (ATCC 29219)) and GRAM – bacteria (Pseudomonas aeroginosa (ATCC 6633)).

The positive control used is ciprofloxacin, showed strong antibacterial activity compared to our EEP obtained by ultrasound, which is considered the best extract with antibacterial activity compared to other extracts. While ethanol, which is used as extraction solvent, is considered negative controls because it did not give any antibacterial activity on all strains.

All EEP extracts showed antifungal activity against Candida albicans (ATCC 10231)

3.4.2. Minimum Inhibitory Concentration

The results obtained after testing the MIC of the extracts on the strains are represented in the table below.

MIC values ranged between 0.2 and 1.56 mg/ml for the tested microorganisms. The minimum inhibitory concentration of all EEPs which inhibits the growth of MRSA (ATCC 43300) and Candida albicans, is 0.78 mg/ml and 0.20 mg/ml respectively.

Table 3: The Results of the MICs of the EEP Extracts

EEP / The strains	Macération	Ultrason	Soxhlet
Bacillus subtilis (ATCC 6633)	-	0.78 mg/ml	0.78 mg/ml
Enterococcus Feacalis (ATCC 29219)	-	0.20 mg/ml	0.39 mg/ml
SARM (ATCC 43300)	0.78 mg/ml	0.78 mg/ml	0.78 mg/ml
Pseudomonas aeroginosa (ATCC 6633)	1.56 mg/ml	1.56 mg/ml	-
Candida albicans	0.20 mg/ml	0.20 mg/ml	0.20 mg/ml

For the Pseudomonas aeroginosa strain (ATCC 6633), the MIC to inhibit this strain is 1.56 mg/ml for the EEP obtained by maceration and Ultrasound, while the EEP obtained by soxhlet does not present any antibacterial activity.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The MICs of Bacillus subtilis strains (ATCC 6633 is of the order of 0.78 mg/ml for the two EEP extracts obtained by ultrasound and soxhlet, also the latter presents a MIC of the order of 0.20 mg/ml and 0.39 mg/ml respectively on the strain Enterococcus Feacalis (ATCC 29219). Whereas the EEP obtained by Maceration does not show any activity towards these two strains.

According to these results, EEP obtained by ultrasound presents the best antibacterial activity, compared to other extracts.

According to the work carried out by Mihaela et al [45], showed that all aqueous extracts of propolis showed antimicrobial activity against the strains used, which are E. coli, S. aureus, B. cereus, P. aeruginosa, C. albicans.

The study carried out by Freires et al [46], showed that ethanolic extracts of Brazilian propolis present strong antifungal activity against Candida spp.

According to the work carried out by Syed Ahmad et al [47], showed that T. itama propolis from Malaysia have antibacterial activity against the strains: E. coli ATCC 25922 and S. aureus ATCC 25923.

3.5. Study of Film Characteristics

Glycerol-containing starch (potato) films were developed and characterized in terms of characterized in terms of thickness, moisture content, density, solubility and water water absorption. These characterizations are presented below.

Table 4: Characteristics of Processed Film

Characteristics / Elabored film	Thickness	Water solubility	Absorption	Degradation
Film based on starch and EEP	0.19 mm	25.70 %	75.9 %	58.4 %

The film produced with the lower concentrations of glycerol tends to be brittle and difficult to handle. Brittle and difficult to handle, whereas the film with 12 ml glycerol is flexible and easy to demold. The reason why this concentration is maintained.

3.6. Film Thickness

The thickness of starch-based biodegradable films developed was determined using a micrometer. Films developed with twice the concentration of potato starch with glycerol had the greatest thickness. The thickness of our developed film is 0.19 mm.

Film thickness generally depends on the size, shape and amylose content of the starch granules. Starch granules [48]. Under the same solid concentrations, higher amylose contents can enhance molecular interactions between polymers, increasing crystallinity of formation and heterogeneity of the film matrix and making the film thicker than the low-amylose film [49].

Many authors have observed a higher apparent permeability the lower the thickness. This phenomenon is much more pronounced for hydrophilic coatings. There however, there is a critical thickness value beyond which water vapour flux decreases linearly as thickness increases

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

3.7. Water Solubility

Water solubility is an important property of edible films because some food applications food applications may require good water insolubility in order to improve product integrity and water resistance [50].

The water solubility of films influences their possible applications. In some cases, pre-food water-soluble films are useful in a variety of applications, such as encapsulation such as encapsulation or food coatings. However, in other cases, insoluble films are preferred to improve the water resistance, integrity and shelf life of food products [51].

Table 9 shows the water solubility of low EEP edible film developed immersed in distilled water at room temperature for 1 h, is 25.70%.

According to the results obtained in the study carried out by Inyoung et al [52], on food films based on sweet potato starch, showed a solubility ranging from 20.60% to 99.16 %, which is comparable to the result obtained in our work.

The study carried out by Hazrati et al [53], showed water absorption results which ranging from 33.23% to 47% for D. hispida fiber-reinforced bio-composite films. higher than the results obtained in our study.

3.8. Water Absorption

Table 9 shows the results of the water absorption test for starch-based edible film and EEP. Based on a Fickian diffusion process, the weight gain due to water absorption water absorption undergone by the film.

Vilay et al [54] revealed that temperature, size of incorporated compound, permeability, surface area affected water absorption analysis. The temperature and size of were controlled in this study.

According to Table 9, the EEP-based edible film absorbed a large amount of water after 30 min immersion in water at room temperature, due to the hydrophilicity of starch.

According to our results, the processed film has an absorption value of 75.9%, which is lower than the results obtained in the study by Hazrati et al [53], which ranged from 81.23% for the control film, to 96.8% for the film containing D. hispida fibers.

A study by Salaberria et al [55], which used chitin as a filler in thermoplastic starch-based thermoplastic composites and found that the water absorption rate of the bio composites was proportional to the percentage of filler in the starch matrix thermoplastic matrix. Bio-composites with low and high filler contents were more and less resistant to water absorption. Less resistant to water absorption than the thermoplastic starch matrix, respectively.

3.9. EEP-based Biodegradation of Starch Film

Research into biodegradation properties is essential for the introduction of biodegradable films in the environment. In this study, soil burial tests were carried out were carried out for EEP-based starch film. In brief, biodegradation is defined as the decomposition of the material by fungi, bacteria or other biological decomposers [48]. Through an enzymatic or metabolic process, these microbial organisms break down the polymer into smaller compounds with a lower average molecular weight.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

The results revealed that the film produced would have no effect on the environment, implying that the film was degraded. The film was degraded. The initial film weight was 208.4 mg and after one week the weight weight became 121.7 mg.

Comparing our result with the work carried out by Hazrati et al [53], the film produced in our study was degraded by 58.4% after one week, whereas the films produced by Hazrati et al were fully degraded after 10 to 12 days.

The absorption of water and the degree of crystallinity of the starch in the processed film are two factors that may have contributed to this situation. That may have contributed to this situation. Because of the film's physical properties, it has absorbed the water, resulting in their susceptibility to microbial attack. When in an aqueous environment, these microbial organisms infected the film, which can be described by the film's water absorption properties, which were 75.9%.

3.10. Packaging Application for Strawberry Preservation

The strawberries were packaged using the new film developed from propolis and edible paper available on the market. After 2 days' storage in the open air at an ambient ambient temperature of 39°C (in summer), we noticed that the strawberries wrapped in our filmkept a good structural aspect and no visual deterioration was noticed. While strawberries strawberries packaged with market film and those left out in the open became unsuitable for consumption, and mould appeared on the surface.

Figure 5: Coating the Strawberries with the Various Films

Figure 6: Strawberries after 2 days of storage (a- Elaborated Film; b-Market Food film; c- Open Air)

A big visual difference was noticed between the strawberries (a, b, c). Strawberries left in the open and those wrapped in the cling film present in the market were deteriorated and moldy.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

Another experiment was carried out to determine the shelf life of strawberries coated with starch-based biofilm and EEP. The results showed that the developed film preserved the strawberries strawberries at room temperature for 3 days (Figures 7 and 8).

Figure 7: Strawberry Packed With Developed Film

Figure 8: Strawberries Wrapped in Developed film after 3 days at Room Temperature

Figure 9: Strawberries Wrapped in Developed Film after 4 days at Room Temperature

After 4 days of storing strawberries at room temperature, mold has developed on their surface, causing them to spoil and become unfit for consumption. Surface, causing them to spoil and become unsuitable for consumption. Based on the results obtained, we can conclude that the active, edible, biodegradable food biofilm we've developed extends the shelf life of strawberries, demonstrating its antioxidant and antimicrobial effect, due to the incorporated EEP.

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

4. CONCLUSION

Based on the experimental findings, it can be deducted that the ethanolic extract of propolis (EEP) obtained by soxhlet exhibits superior antioxidant activity in comparison to alternative extraction methods. Conversely, the EEP obtained by ultrasound demonstrates the most effective antimicrobial activity among the various EEPs, this is attributed to the ultrasound extraction technique, which prioritizes the extraction of high-quality bioactive compounds responsible for the observed antimicrobial activity. In contrast, the compounds extracted using high temperatures (Soxhlet at 70°C) and prolonged extraction times (maceration for 24 hours) were degraded. The evaluation of the film production using the EEP obtained through ultrasound yielded promising results. The film produced has a slightly yellow color appearance which is due to the propolis incorporated; it gave appreciable results with the conservation of the strawberries.

References

1) Sforcin, J. M., & Bankova, V. (2011). Propolis: is there a potential for the development of new drugs? *Journal of ethnopharmacology*, *133*(2), 253-260. https://doi.org/10.1016/j.jep.2010.10.032

2) Moraru, D., Alexa, E., Cocan, I., Obiștioiu, D., Radulov, I., Hulea, A. ... & Pătruică, S. (2023). Chemical Composition, Antioxidant, Antimicrobial and Antiinflamatory Activity of Some Bee Products. https://doi:10.20944/preprints202307.0874.v1

3) Kuropatnicki, A. K., Szliszka, E., & Krol, W. (2013). Historical aspects of propolis research in modern times. *Evidence-based complementary and alternative medicine*, *2013*. https://doi.org/10.1155/2013/964149.

4) Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, *26*(2), 83-99. https://doi.org/10.1051/apido:19950202

5) Dias, L. G., Pereira, A. P., & Estevinho, L. M. (2012). Comparative study of different Portuguese samples of propolis: Pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. *Food and Chemical Toxicology*, *50*(12), 4246-4253. https://doi.org/10.1016/j.fct.2012.08.056

6) Duarte, S., Rosalen, P. L., Hayacibara, M. F., Cury, J. A., Bowen, W. H., Marquis, R. E., & Koo, H. (2006). The influence of a novel propolis on mutans streptococci biofilms and caries development in rats. *Archives of Oral Biology*, *51*(1), 15-22. https://doi.org/10.1016/j.archoralbio.2005.06.002

7) Gontard, N., Guillard, V., Gaucel, S., & Guillaume, C. (2017). L'emballage alimentaire ET l'innovation écologique dans toutes leurs dimensions. *Innovations agronomiques*, *58*, 1-9.

8) Sid, S., Mor, R. S., Kishore, A., & Sharanagat, V. S. (2021). Bio-sourced polymers as alternatives to conventional food packaging materials: A review. *Trends in Food Science & Technology*, *115*, 87-104. https://doi.org/10.1016/j.tifs.2021.06.026

9) Mohamed, S. A., El-Sakhawy, M., & El-Sakhawy, M. A. M. (2020). Polysaccharides, protein and lipid-based natural edible films in food packaging: A review. *Carbohydrate polymers*, *238*, 116178. https://doi.org/10.1016/j.carbpol.2020.116178

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

10) Bankova, V., Bertelli, D., Borba, R., Conti, B. J., da Silva Cunha, I. B., Danert, C., ... & Zampini, C. (2019). Standard methods for Apis mellifera propolis research. *Journal of Apicultural Research*, *58*(2), 1-49. http://dx.doi.org/10.1080/00218839.2016.1222661

11) Trusheva, B., Popova, M., Bankova, V., Simova, S., Marcucci, M. C., Miorin, P. L. ... & Tsvetkova, I. (2006). Bioactive constituents of Brazilian red propolis. *Evidence-based complementary and alternative medicine*, *3*, 249-254.https://doi.org/10.1093/ecam/nel006.

12) Gargouri, W., Osés, S. M., Fernández-Muiño, M. A., Sancho, M. T., & Kechaou, N. (2019). Evaluation of bioactive compounds and biological activities of Tunisian propolis. *Lwt*, *111*, 328-336. https://doi.org/10.1016/j.lwt.2019.05.044

13) Cunha, I., Sawaya, A. C., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., & Carvalho, P. D. O. (2004). Factors that influence the yield and composition of Brazilian propolis extracts. *Journal of the Brazilian Chemical Society*, *15*, 964-970. https://doi.org/10.1590/S0103-50532004000600026.

14) Mokhtar, S. U. (2019). Comparison of total phenolic and flavonoids contents in Malaysian propolis extract with two different extraction solvents. *International Journal of Engineering Technology and Sciences*, *6*(2), 1-11. https://doi.org/10.15282/ijets.v6i2.2577

15) Mohammadzadeh, S., Sharriatpanahi, M., Hamedi, M., Amanzadeh, Y., Ebrahimi, S. E. S., & Ostad, S. N. (2007). Antioxidant power of Iranian propolis extract. *Food chemistry*, *103*(3), 729-733. https://doi.org/10.1016/j.foodchem.2006.09.014

16) Kumazawa, S., Hamasaka, T., & Nakayama, T. (2004). Antioxidant activity of propolis of various geographic origins. *Food chemistry*, *84*(3), 329-339. https://doi.org/10.1016/S0308-8146(03)00216-4.

17) Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

18) Arora, D. S., & Kaur, G. J. (2007). Antibacterial activity of some Indian medicinal plants. *Journal of natural medicines*, *61*, 313-317. DOI 10.1007/s11418-007-0137-8

19) Valgas, C., Souza, S. M. D., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, *38*, 369-380. https://doi.org/10.1590/S1517-83822007000200034

20) de Araújo, A. A., Soares, L. A. L., Ferreira, M. R. A., de Souza Neto, M. A., da Silva, G. R., de Araújo Jr, R. F., ... & de Melo, M. C. N. (2014). Quantification of polyphenols and evaluation of antimicrobial, analgesic and anti-inflammatory activities of aqueous and acetone–water extracts of Libidibia ferrea, Parapiptadenia rigida and Psidium guajava. *Journal of ethnopharmacology*, *156*, 88-96. https://doi.org/10.1016/j.jep.2014.07.031

21) Choi, I., Shin, D., Lyu, J. S., Lee, J. S., Song, H. G., Chung, M. N., & Han, J. (2022). Physicochemical properties and solubility of sweet potato starch-based edible films. *Food Packaging and Shelf Life*, *33*, 100867. https://doi.org/10.1016/j.fpsl.2022.100867

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

22) Nur Hanani, Z. A., Beatty, E., Roos, Y. H., Morris, M. A., & Kerry, J. P. (2012). Development and characterization of biodegradable composite films based on gelatin derived from beef, pork and fish sources. *Foods*, *2*(1), 1-17. https://doi.org/10.3390/foods2010001

23) Azlin, M. N. M., Sapuan, S. M., Zainudin, E. S., & Zuhri, M. Y. M. (2020). Natural fibre reinforced polyester composites: a review. In *7th Postgraduate Seminar on Natural Fibre Reinforced Polymer Composites*.

24) Tarique, J., Sapuan, S. M., Khalina, A., Sherwani, S. F. K., Yusuf, J., & Ilyas, R. A. (2021). Recent developments in sustainable arrowroot (Maranta arundinacea Linn) starch biopolymers, fibres, biopolymer composites and their potential industrial applications: A review. *Journal of Materials Research and Technology*, *13*, 1191-1219. https://doi.org/10.1016/j.jmrt.2021.05.047

25) Kliem, S., Kreutzbruck, M., & Bonten, C. (2020). Review on the biological degradation of polymers in various environments. *Materials*, *13*(20), 4586. https://doi.org/10.3390/ma13204586.

26) Zin, N. B. M., Azemin, A., Rodi, M. M. M., & Mohd, K. S. (2018). Chemical composition and antioxidant activity of stingless bee propolis from different extraction methods. *Int. J. Eng. Technol*, *7*, 90-95.

27) Roginsky, V., & Lissi, E. A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food chemistry*, *92*(2), 235-254. https://doi.org/10.1016/j.foodchem.2004.08.004

28) Kouamé, T. K., Siaka, S., Kassi, A. B. B., & Soro, Y. (2021). Détermination des teneurs en polyphénols totaux, flavonoïdes totaux et tanins de jeunes feuilles non encore ouvertes de Piliostigma thonningii (Caesalpiniaceae). *International Journal of Biological and Chemical Sciences*, *15*(1), 97-105.DOI:10.4314/ijbcs.v15i1.9

29) Yang, H., Dong, Y., Du, H., Shi, H., Peng, Y., & Li, X. (2011). Antioxidant compounds from propolis collected in Anhui, China. *Molecules*, *16*(4), 3444-3455. https://doi.org/10.3390/molecules16043444

30) Bankova, V., Trusheva, B., & Popova, M. (2021). Propolis extraction methods: A review. *Journal of apicultural research*, *60*(5), 734-743. https://doi.org/10.1080/00218839.2021.1901426.

31) Gullian Klanian, M., & Terrats Preciat, M. (2017). Optimization of the ultrasound-assisted extraction of phenolic compounds from Brosimum alicastrum leaves and the evaluation of their radical-scavenging activity. *Molecules*, *22*(8), 1286. https://doi.org/10.3390/molecules22081286

32) Alencar, S. M. D., Oldoni, T. L. C., Castro, M. L., Cabral, I. S. R., Costa-Neto, C. M., Cury, J. A., & Ikegaki, M. (2007). Chemical composition and biological activity of a new type of Brazilian propolis: red propolis. *Journal of ethnopharmacology*, *113*(2), 278-283. https://doi.org/10.1016/j.jep.2007.06.005

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

33) Mohammadzadeh, S., Sharriatpanahi, M., Hamedi, M., Amanzadeh, Y., Ebrahimi, S. E. S., & Ostad, S. N. (2007). Antioxidant power of Iranian propolis extract. *Food chemistry*, *103*(3), 729-733. https://doi.org/10.1016/j.foodchem.2006.09.014

34) Moreira, L., Dias, L. G., Pereira, J. A., & Estevinho, L. (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food and Chemical toxicology*, *46*(11), 3482-3485. https://doi.org/10.1016/j.fct.2008.08.025

35) PALOMINO G, L. R., GARCÍA P, C. M., GIL G, J. H., ROJANO, B. A., & DURANGO R, D. L. (2009). Determination of phenolic content and evaluation of antioxidant activity of propolis from Antioquia (Colombia). *Vitae*, *16*(3), 388-395. DOI: 10.17533/udea.vitae.3020

36) Alencar, S. M. D., Oldoni, T. L. C., Castro, M. L., Cabral, I. S. R., Costa-Neto, C. M., Cury, J. A., & Ikegaki, M. (2007). Chemical composition and biological activity of a new type of Brazilian propolis: red propolis. *Journal of ethnopharmacology*, *113*(2), 278-283. https://doi.org/10.1016/j.jep.2007.06.005

37) Mok-Ryeon A., Shigenori K., Yumiko U., Jui N., Mitsuo M., Fang Z. and Tsutomu N. (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food chemistry*, vol. 101, pp. 1383-1392, 2007. https://doi.org/10.1016/j.foodchem.2006.03.045

38) (Suárez, G. A. P., Galindo, N. J. P., & Cuervo, O. H. P. (2022). Obtaining Colombian propolis extracts using modern methods: A determination of its antioxidant capacity and the identification of its bioactive compounds. *The Journal of Supercritical Fluids*, *182*, 105538). https://doi.org/10.1016/j.supflu.2022.105538

39) Kumazawa, S., Nakamura, J., Murase, M., Miyagawa, M., Ahn, M. R., & Fukumoto, S. (2008). Plant origin of Okinawan propolis: honeybee behavior observation and phytochemical analysis. *Naturwissenschaften*, *95*, 781-786. DOI: 10.1007/s00114-008-0383-y

40) Amić, D., Davidović-Amić, D., Bešlo, D., & Trinajstić, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica chemica acta*, *76*(1), 55-61.

41) Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules*, *27*(4), 1326. https://doi.org/10.3390/molecules27041326

42) (Moreira, L., Dias, L. G., Pereira, J. A., & Estevinho, L. (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food and Chemical toxicology*, *46*(11), 3482-3485. https://doi.org/10.1016/j.fct.2008.08.025

43) Sulaiman, G. M., Al Sammarrae, K. W., Ad'hiah, A. H., Zucchetti, M., Frapolli, R., Bello, E., & Bagnati, R. (2011). Chemical characterization of Iraqi propolis samples and assessing their antioxidant potentials. *Food and Chemical Toxicology*, *49*(9), 2415-2421. https://doi.org/10.1016/j.fct.2011.06.060

44) Aliyazıcıoglu, R., Sahin, H., Erturk, O., Ulusoy, E., & Kolayli, S. (2013). Properties of phenolic composition and biological activity of propolis from Turkey. *International Journal of Food Properties*, *16*(2), 277-287. https://doi.org/10.1080/10942912.2010.551312

Volume 63 | Issue 01 | January 2024
DOI: 10.5281/zenodo.10554698

45) Vică, M. L., Glevitzky, M., Tit, D. M., Behl, T., Heghedűş-Mîndru, R. C., Zaha, D. C., & Bungău, S. (2021). The antimicrobial activity of honey and propolis extracts from the central region of Romania. *Food Bioscience*, *41*, 101014. https://doi.org/10.1016/j.fbio.2021.101014

46) Freires, I. A., Queiroz, V. C. P. P., Furletti, V. F., Ikegaki, M., de Alencar, S. M., Duarte, M. C. T., & Rosalen, P. L. (2016). Chemical composition and antifungal potential of Brazilian propolis against Candida spp. *Journal de mycologie medicale*, *26*(2), 122-132. https://doi.org/10.1016/j.mycmed.2016.01.003

47) Yusop, S. A. T. W., Sukairi, A. H., Sabri, W. M. A. W., & Asaruddin, M. R. (2019). Antioxidant, antimicrobial and cytotoxicity activities of propolis from Beladin, Sarawak stingless bees Trigona itama extract. *Materials Today: Proceedings*, *19*, 1752-1760. https://doi.org/10.1016/j.matpr.2019.11.213

48) Basiak, E., Lenart, A., & Debeaufort, F. (2017). Effect of starch type on the physico-chemical properties of edible films. *International journal of biological macromolecules*, *98*, 348-356. https://doi.org/10.1016/j.ijbiomac.2017.01.122

49) Wang, K., Wang, W., Ye, R., Xiao, J., Liu, Y., Ding, J., & Liu, A. (2017). Mechanical and barrier properties of maize starch–gelatin composite films: Effects of amylose content. *Journal of the Science of Food and Agriculture*, *97*(11), 3613-3622. https://doi.org/10.1002/jsfa.8220.

50) Pérez-Gago, M. B., Nadaud, P., & Krochta, J. M. (1999). Water vapor permeability, solubility, and tensile properties of heat-denatured versus native whey protein films. *Journal of food science*, *64*(6), 1034-1037. https://doi.org/10.1111/j.1365-2621.1999.tb12276.

51) Lee, J. S., Choi, I., & Han, J. (2021). Mathematical modeling of cinnamon (Cinnamomum verum) bark oil release from agar/PVA biocomposite film for antimicrobial food packaging: The effects of temperature and relative humidity. *Food Chemistry*, *363*, 130306. https://doi.org/10.1016/j.foodchem.2021.130306

52) Choi, I., Shin, D., Lyu, J. S., Lee, J. S., Song, H. G., Chung, M. N., & Han, J. (2022). Physicochemical properties and solubility of sweet potato starch-based edible films. *Food Packaging and Shelf Life*, *33*, 100867. https://doi.org/10.1016/j.fpsl.2022.100867

53) Hazrati, K. Z., Sapuan, S. M., Zuhri, M. Y. M., & Jumaidin, R. (2021). Preparation and characterization of starch-based biocomposite films reinforced by Dioscorea hispida fibers. *Journal of Materials Research and Technology*, *15*, 1342-1355). https://doi.org/10.1016/j.jmrt.2021.09.003

54) Vilay, V., Mariatti, M., Taib, R. M., & Todo, M. (2008). Effect of fiber surface treatment and fiber loading on the properties of bagasse fiber–reinforced unsaturated polyester composites. *Composites Science and Technology*, *68*(3-4), 631-638. https://doi.org/10.1016/j.compscitech.2007.10.005

55) Salaberria, A. M., Labidi, J., & Fernandes, S. C. (2014). Chitin nanocrystals and nanofibers as nano-sized fillers into thermoplastic starch-based biocomposites processed by melt mixing. *Chemical Engineering Journal*, *256*, 356-364. https://doi.org/10.1016/j.cej.2014.07.009