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Original Article

Preparation, Evaluation of Propolis Extract Gel and exploring its Antioxidant, Antimicrobial Activity

Shahad Myasar Alfaris*, Rasha Khalid Dhahir, Amina Mudhafar Al-Nima

Shahad Myasar Alfaris*, Rasha Khalid Dhahir, Amina Mudhafar Al-Nima

Department of Pharmaceutics, College of Pharmacy, University of Mosul, Mosul, IRAQ.

Correspondence

Shahad Myasar Alfaris

Department of Pharmaceutics, College of Pharmacy, University of Mosul, Mosul, IRAQ.

E-mail: shmn89@uomosul.edu.iq

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ABSTRACT

Background: Propolis is a wax-like resin which is produced by different types of bees by mixing resinous secretions of plants with beeswax and other products of bees' metabolism. However, this natural product contains a group of compounds that are responsible for various propolis activities including antimicrobial, anticancer, antioxidant, antiviral, and others. This study aims to prepare different gel formulae that contain various concentrations of propolis extract using two different polymers and to evaluate their physical properties, antioxidant, and antimicrobial activities. **Method:** Propolis was extracted depending on the hydroalcoholic method; six different gel formulae (P-1 to P-6) were prepared with different concentrations of propolis extract (0.5, 1, and 1.5%) using two different polymers; carbopol-934 and poloxamer-407, in concentrations of 0.5 and 25% respectively. The formulae were evaluated physically for their visual appearance, *p*H, viscosity, spreadability, and physiological compatibility with the skin. The anti-microbial activities were investigated by well diffusion method against various bacterial species including *Staphylococcus aureus (gram+), Pseudomonas aeruginosa (gram-), Enterococcus fecalis (gram+)* and against one fungal species which is *Candida albicans*. Also, the antioxidant activity was determined utilizing 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Finally, a stability test was carried out for the chosen formula. **Results:** The six formulae (P-1 to P-6) were successfully prepared and evaluated and they were acceptable in their physical appearance and consistency, *p*H reading was in the range of 5.33±0.08 to 5.72±0.02 (p>0.05), a study of the skin irritation revealed no undesirable side effect, while the viscosity was in the range of 128.99±0.07 to 248.54±1.04 Pa.s (p>0.05), also the carbopol-934 containing formulae revealed good spreadability (1.495±0.075 to 5.15±0.35 mg.cm/s, p>0.05), in comparison to poloxamer-407 containing ones. Both carbopol-934 and poloxamer-407 containing formulae exhibited antimicrobial activity but the P-3 formula had the largest inhibition zone against *C. Albicans* (16.1mm), and only P-2, P-5 and P-6 were active against *pseudomonas aeruginosa* (12mm in all), *while* P-2 had the largest inhibition zone against *enterococcus fecalis* (13.2 mm), P-2 and P-5 showed the largest activity against *staphylococcus aureus* (15.5 and 14.6 mm respectively). The carbopol-934-containing formulae exhibited higher antioxidant activity than those with poloxamer-407, and the best-calculated IC50 belonged to P-2 (1.19 mg/ml). **Conclusion:** All the prepared formulae showed antimicrobial and antioxidant activities with some controversial results. However, P-2 (1% propolis extract and 0.5% carbopol-934), was active against all types of the tested microorganism, and had the best-calculated IC50, in addition to that it was a stable formula.
Key words: Propolis, Carbopol-934, Poloxamer-407, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Propolis is a viscous, wax-like resin, called bee's glue due to its sticky nature, it can be produced by different types of bees including Stingless bees from the Meliponini tribe and Apis mellifera honeybee.[1,2] Propolis is collected by bees from different resinous secretions of plants such as gums, mucilage, lattices, buds and barks of trees.[3,4] After extraction, it is mixed with the secretion of the hypopharyngeal gland, pollen and bee's wax. Propolis word comes from Greek, in which "pro" means at the entrance to, and "polis" means city or community, so it refers to hives of defensive material.[3] Bees use propolis to smooth the inner surface of hives, prevent insects, fill gaps, and as an antiseptic against microbial infections as seen in figure 1.[3,4]

More than 300 various components were discovered in crude propolis, the latter consists of resins, waxes, essential oils, pollens, and other organic substances in different ratios.[3,5] Generally, propolis contains a mixture of components like flavonoids, terpenes, phenylpropanoids, lignans, stilbenes, coumarins, and their prenylated derivatives.[2] It is also full of vitamins such as A, B1, B2, B3, biotin, and bioflavonoids, its compositions and classifications depend on plant sources and geographical region of collection.[2,4,5]

Propolis was used for a long time ago for many purposes such as mouth disinfectant, wound treatment, anti-eczema, anti-myalgia, anti-rheumatism agent, and anti-myalgia.[2] However, over the last decades, research on propolis were intensified, and revealed multiple actions of propolis as antibacterial, antiviral, anti-inflammatory, anti-protozoal, anti-fungal, antioxidant, anticancer, anti-hepatotoxic, and anti-mutagenic.[6,7] It was documented that propolis is effective against microorganisms through both direct action for example on both Gram-positive and Gram-negative bacteria which was revealed during the *in-vitro* study, and by activation of the defensive mechanism of the organism.[7,8] Therefore, propolis has got a great interest in the treatment of different human infections and the development of drugs with biotechnological products.[8] It was reported by Conte *et al.* (2022) that poplar propolis is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), also Yildirim *et al.* (2004) demonstrated the effect

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Figure 1: Bees making a proposed hole.[4]

of Turkish propolis against various types of mycobacteria.[6,9] The antimicrobial activity of propolis is related to its content of phenolic group especially flavonoid content, the antioxidant characteristics of propolis were also found to be associated with its content of phenolic compounds, by trapping free radical and chelating metal ions.[9]

Propolis had been utilized for a variety of formulations like creams, ointments, and gels, in the treatment of burns and wound healing. Carbopol or carbomer polymers are widely used as suspending, thickening, and emulsifying agents to change rheological properties and gel formulations. Their stability, transparency, and nontoxicity make them widely used as additives for pharmaceutical and cosmetics preparations.[10] Carbopol-934 has been used for the topical gel as synthetic hydrophilic polymers in a concentration of 1-5% (m/v).[11] Berretta *et al.* (2012) developed a gel for wound healing containing 3.6% propolis, based on poloxamer-407 polymer.[12] Poloxamer-407 which can be used for the development of *in situ* gelling systems, is a triblock copolymer with a central hydrophobic block of polypropylene glycol flanked by two hydrophilic blocks of polyethene glycol, it acts as a gelling polymer in concentrations of 15-50% (m/v). it is widely used with poorly water-soluble compounds because it is effective as a surfactant and it is non-toxic.[13] However, the final consistency of poloxamer-407-containing gel could be affected by different factors including its concentration, constituent types, salts, and the presence of a water-miscible organic solvent.[14,15]

This study aims to develop propolis gel formulae using two types of polymers, Carbopol-934 and poloxamer-407 in various concentrations of propolis and to evaluate their physical properties and their antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Propolis samples were collected from hives of honeybees of Babylon city in Iraq during the spring season of 2017 and 2018. Carbopol-934 (CARB-934) was purchased from HIMEDIA, India, and poloxamer-407 (POLO-407) was gifted from BASF SE, Ludwigshafen Germany. Propylene glycol was bought from THOMAS BAKER Co., India. Ethanol was imported from Tedia Company, USA, Muller-Hinton agar (Lab M, UK), 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) was purchased from industrial biotechnology, Germany, L (+)-ascorbic acid was purchased from VWR international BVBA, Leuven Belgium, and methanol was imported from scharlab S.L., Spain.

Extraction of propolis

The extraction process was done in order to discard unwanted wax materials like beeswax and get the required propolis constituents. The raw material was stored in a freezer cabinet to be ground later using a traditional knife into a few millilitre parts which were frozen again to be finally milled to a fine powder using an electric grinder (Royal-Japan). Then 45 g of prepared powder was put in manual shaking with 300 ml ethanol 70% (v/v) for 10 minutes and kept the mixture for three days for completing the extraction process. After that, the filtration process of the resulting mixture was done with Whatman filter papers (CITOTEST 150mm) to get pure extract free from waxy impurities. Finally, in order to get a dry extract an evaporation process was done by pouring the filtration product into glass Petri dishes at room temperature in which it was scraped and finally stored in a refrigerator.[16]

Selection and optimization of gelling agent

One of the main ingredients of the formula is the gelling agent (polymer). In order to optimize the type and concentration of a polymer, gel formulae were prepared with two different polymers: CARB-934 and POLO-407. However, different concentrations of these polymers were tested and the concentration that showed good appearance and consistency was selected. First, the CARB-934 in different concentrations was used as a gelling polymer, it was dispersed in a sufficient amount of distilled water with gentle and mechanical mixing. Later on, it was put for 3-4 hours on a magnetic stirrer (Fisher Scientific, Korea) at 350 rpm with a temperature of 55˚C and left for cooling and kept in a refrigerator overnight in order to drive out the resulting air bubbles. Finally, the *p*H was changed to 5–5.5 by adding a few drops of triethanolamine to form the gel formula.[17] On the other hand, different concentrations of POLO-407 were prepared. The preparation was done by dispersing the polymer in a sufficient amount of distilled water and vigorously hand mixing for 5 minutes, later it was put for 2 hours on a magnetic stirrer (Fisher Scientific, Korea) at 350 rpm with a temperature of 60˚C, then cooled at room temperature and kept overnight to remove air bubbles. Finally, all the prepared polymer dispersions were stored in a refrigerator.[18]

Propolis gel preparation

Propolis extract was dissolved in propylene glycol to prepare different concentrations (0.5, 1, and 1.5% (m/v)) by mixing by hand and with a magnetic stirrer for 2 hours. Then after, it was put in a sonicator (Lab Tech, DAIHAN LABTECH CO. LTD, Korea) at 55ºC until the complete dissolution of the propolis extract. After cooling, it was filtrated using gauze to remove some un-dissolved waxy impurities. The resulting solution was added to the above-prepared polymer dispersion and stirred for 2 hours with magnetic stirring to prepare different propolis gel formulae named (P-1 to P-6) that include two types of gelling agents (CARB-439 and POLO-407) and different propolis concentrations as shown in table 1.

Evaluation of propolis gel

Physical characterization

Visual evaluation: The resulting gel formulae (P-1 to P-6) were visually evaluated for their appearance, consistency, presence of bubbles, and colour.[19]

Determination of *p*H: A 1% solution of all the prepared gel formulae (P-1 to P-6) in distilled water was made and put on the magnetic stirrer for 1 hour. After that, *p*H measuring of the previous solutions was done using a digital *p*H meter (Eco Testr, Oakton Instruments, Singapore) which was adjusted by standard buffer solution *p*H 4, 7, and 10. However, three *p*H readings were done from each sample and the mean was registered.[20,21]

Skin irritation test: For probable future use, all prepared gel formulae (P-1 to P-6) were tested to ensure their skin compatibility. However, six volunteers were involved in this experiment that was proceeded by

Table 1: Composition of the prepared propolis gel formulae with their assigned codes.

Formula name	P-1	P-2	P-3	P-4	P-5	P-6
Propolis extract(%m/v)	0.5	1	1.5	0.5	1	1.5
CARB-934(%m/v)	0.5	0.5	0.5	-------	-------	-------
POLO-407(%m/v)	-------	-------	-------	25	25	25
Propylene glycol(ml)	50	50	50	50	50	50
Triethanolamine	q.s.	q.s.	q.s.	-------	-------	-------
DW (up to 100ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

P_{1-6}- Propolis gel formulae prepared using different gelling agents, DW=distilled water, q.s.=sufficient quantity

applying 1g of each formula on 2 square inches of the volunteer's skin area. Thereafter, looking for any warning points for irritation such as redness, swelling, scratching, and itching on the tested skin area for the next 24 hours, and the results were written.[18]

Viscosity determination: The gel viscosity is one of the measurements that should be done as it can give a clue about how much the gel can resist the flow after skin application. However, the prepared formulae (P-1 to P-6) were kept at room temperature for 1hr before starting the measurement. The evaluation was performed using a viscometer apparatus (Drawell scientific instrument Co., Ltd, Shanghai), that was supplied with spindle 4 and at 3 rpm. When the device started to work, the spindle rotated inside the different samples within clean beakers. Three measurements were taken for each formula and the mean was recorded.[22]

Spreadability test: Adequate spreadability is recommended for any topical gel formula. The test was done for all the prepared gels (P-1 to P-6) by using a handmade apparatus involving a block of wood with 2 symmetrical glasses in which the gel was put and its spreadability was measured depending on its "slip" and "drag "manner. On the surface of the glass, an extra weight of gel was put (about 2 g) and covered with an upper slide that was supplied with a hook. However, to perform a uniform film without excess gel and air, a 100g weight was put on the surface of the slides for 5 minutes. The upper slide was pulled by 20g weight with a sting on its hook to allow it to pass a 7.5 cm with the registration of the required time to pass this distance, the faster pass means superior spreadability. The next equation was used to calculate the spreadability of the prepared gel formula:

$$S = M.L/T$$

Where S = spreadability, M = weight that pull upper slide (g), L = glass slide length (cm) and T = time (sec) required for slide to pass.[17,23,24]

In-vitro antimicrobial activity

Antimicrobial susceptibility activity of all prepared formulae (P-1 to P-6) was studied by well diffusion method against bacterial species including *staphylococcus aureus (gram+), pseudomonas aeruginosa (gram-)*, *enterococcus fecalis(gram+)* and against one fungal type which is *candida Albicans*. A 39 g of Muller-Hinton agar was diluted with 1 Liter of distilled water in order to prepare Mueller-Hinton agar (Lab M, UK). Then after, the mixture was stirred and heated until boiling utilizing a hot plate stirrer (Stuart, UK) and left for 15min autoclaving at 115˚C with a portable autoclave (Guangzhou, China). The prepared agar was transferred into a petri dish for cooling and solidifying and then a 6 mm diameter well within the prepared agar was performed. Fresh cultures of *staphylococcus aureus, pseudomonas aeruginosa*, *enterococcus fecalis* and *candida Albicans* had been made ready with concentration 0.5 McFarland standard ($1.5x10^8$ CFU/ml) by introducing and spreading the required bacterial suspension on the top of agar plates using a sterile cotton swab. After 15 minutes, 100µl of different prepared gel formula was put in the resulting wells using standard antibiotic discs as a positive control and pure polymer gel as a negative control. Finally, the prepared plates were left for incubation at 37˚C for 18 hours in order to measure the inhibition zone with a specific digital calliper.[14,25]

In-vitro antioxidant activity using a (DPPH) assay

The antioxidant activities of the prepared gel (P-1 to P-6) were studied using the DPPH technique, according to Blois *et al* (1958),[26] in which the tendency of giving hydrogen atom was evaluated by their decolorizing ability of DPPH solution (the colour change from purple to yellow shades if there is antioxidant activity). However, to proceed the reaction, a mixture of the tested sample and DPPH solution was incubated in dark at room temperature and spectrophotometrically the absorbance had been recorded at 517 nm. The scavenging percentage activity of DPPH was measured by the following equation:

$$\% \text{ DPPH radical scavenging activity} = (A_0 - A_1)/A_0 - \times 100$$

In which A_0 is the control absorbance, and A_1 is the absorbance of the tested formula. After that IC_{50} (the sample concentration that needs to suppress 50% of radical) was concluded using the graph of calibration curve of inhibition percentage versus concentration.[25,27]

Preparing DPPH solution

A 0.2 mg/ml stock concentration of DPPH solution was performed by dissolving 0.02g of DPPH powder in 100ml of methanol.

Preparing ascorbic acid

Ascorbic acid 0.1mg/ml stock solution of ascorbic acid was used as a positive control by adding 10 mg of ascorbic acid powder to 100 ml of methanol. The final solution was stored at 25˚C.

Sample preparation

A stock solution of each formula (P-1 to P-6) was prepared by dissolving 1g of the prepared gel in 100ml of methanol with continuous shaking and heating at 40˚C for complete dissolving, then stored in a refrigerator.

Antioxidant procedure

To prepare different concentrations of each formula, different volumes from their stock solutions (0.1, 0.5, 1.5, 2.0, 2.5, 3.0, 4.0, and 4.5 ml) were taken, mixed with 0.5 ml of DPPH solution, then the volume was completed with methanol to 5 ml to get final concentrations (0.2, 1.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 9.0 mg/ml respectively). Finally, the mixed solutions were left in a dark environment for 30 minutes to measure the absorbance at 517nm by the spectrometer and make calculations.[28]

Choosing the accepted gel formula

Various propolis gel formulae were prepared that contained the different polymers and propolis concentrations as can be illustrated in table 1. Choosing the accepted formula was carried out depending on their evaluation results.

Stability study of the chosen gel formula

The chosen formula was evaluated for its stability during keeping it in a closed container at room temperature for 4 months. This included studying its visual characteristics, spreadability, viscosity and *p*H value.[18]

Statistical analysis

For statistical analysis, Microsoft Excel 2016 using ANOVA (one-way analysis of the variance) was used, the difference was statistically non-significant when ($P > 0.05$) and it was significant when $P < 0.05$. Mean and standard deviation was used to express the values in the data.

RESULTS AND DISCUSSION

Physical characterization

Visual evaluation: All the prepared gel formulae (P-1 to P-6) showed an acceptable appearance and consistency as can be seen in figure 2. However, some bubbles were still in some formulae (especially P-5 and P-6). Also, there were differences in colour and consistency between the CARB-934-based formulae and the POLO-407-based ones as the colour in the latter was brighter and honey-like, also they were greasier than the first that contained CARB-934 as seen in the figure 2.

***p*H evaluation:** The *p*H testing is important in predicting both formula stability and its influence on skin condition. All the *p*H readings for the prepared formulae were within the range of 5-6 as presented in table 2. They were near the normal *p*H of healthy people's skin and buccal mucosa (5-6). Therefore, no unwanted effects like irritation may occur at the application site.[29,30]

Skin irritation test: After close observations on the skin for 24 hours following the application of all prepared gel formulae; none of the volunteers showed any undesirable side effects such as redness, irritation, oedema, scratching, and itching of the skin.

Viscosity Determination: The viscosity of all the formulae prepared was approximate (there are no statistically significant differences, ($p > 0.05$) as shown in table2. However, the viscosity of CARB-934-based formulae (P-1, P-2, P-3) was less than those with POLO-407 (P-4, P-5, and P-6), and it was the highest in P-5 (248.54±1.04 Pa.s) which contained 1% (m/v) propolis extract and 25% (m/v) POLO-407, while the lowest one was P-3 (128.99±0.07Pa.s) in which propolis was 1.5% (m/v) and CARB-934 was 0.5% (m/v), this can be attributed to the presence of propylene glycol which was used as a co-solvent during preparation and where there were differences in fluidity behaviour between the two polymers (CAR-934 and POLO-407) that have little evidence to explain.[31]

Spreadability test: The CARB-934 containing formulae (P-1, P-2 and, P-3) exhibited a good spreadability (1.495±0.075, 5.15±0.35, and 3.23±0.17mg.cm/s respectively), ($p > 0.05$) when applied between the slides as can be seen in table 2, in which they needed seconds to pass the required distance (7.5cm). While the other formulae (P-4, P-5 and P-6) in which POLO-407 was used as a gelling agent stayed in their position between slides and showed no movement even after 10 minutes' period

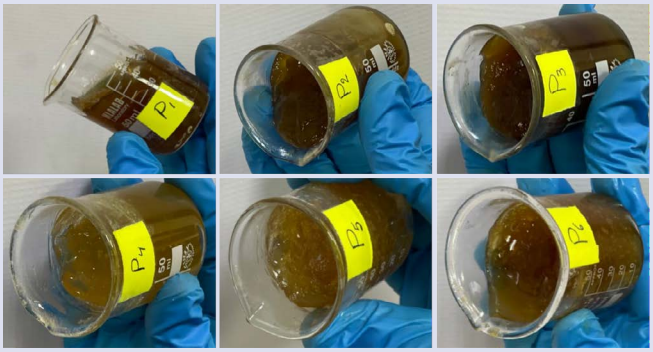

Figure 2: A representative image of the prepared propolis gel formulae assigned as (P-1 to P-6).

Table 2: Physical evaluation results of the prepared formulae.

Formula no.	pH (n=3)	Viscosity (n=3) (pa.s)	Spreadability (n=3) (mg.cm/s)
P-1	5.46±0.088	139.37±0.41	1.495±0.075
P-2	5.66±0.088	132.88±0.24	5.15±0.35
P-3	5.72±0.02	128.99±0.07	3.23±0.17
P-4	5.52±0.05	181.1±0.59	------
P-5	5.36±0.08	248.54±1.04	------
P-6	5.33±0.08	159.84±0.76	------

due to their high viscosity. The results were reasonable and compatible with the achievement of Bonacucina G *et al.* (2006) who studied the rheological, adhesive, and release characteristics of semisolid Carbopol/tetraglycol systems, and concluded that the adhesiveness and the spreadability of the polymer gels are directly proportional with their viscosity.[32]

Antimicrobial activity

The antimicrobial activity of the prepared formulae was studied toward different bacterial species (*Staphylococcus aureus, Pseudomonas aeruginosa*, and *Enterococcus fecalis*) and fungi (*Candida albican*) as illustrated in table 3, figure 3. All propolis formulae inhibited the growth of *C. Albicans* and the larger inhibition zone was in the P-3 formula (16.1mm). On the other hand, the bacterial behaviours were controversial towards the propolis formulae, *pseudomonas aeruginosa* was resistant to all prepared formulae except P-2, P-5 and P-6 (12mm in all), whereas, in the case of *enterococcus fecalis,* only CARB-934 containing formula inhibited its growth with the largest inhibition zone in P-2 (13.2 mm). Finally, the growth of *staphylococcus aureus* was inhibited in all formulae except in P-6, with the largest inhibition zone in P-2 and P-5 (15.5 and 14.6 mm respectively). So, CARB-934 -based formulae exhibited better antimicrobial activity which could be attributed to their low viscosity that facilitates the release of propolis and consequently delivers their antimicrobial activity. In contrast, POLO-407-based formulae had high viscosity that hinders the release of propolis. Furthermore, POLO-407 is a triblock copolymer with a hydrophilic centre and two polyethylene oxide (PEO) hydrophobic tails. Due to its structure, POLO-407 has the ability to form micelles around propolis molecules reducing the amount of freely available propolis and consequently its antimicrobial activity.[14,33]

Antioxidant activity

All propolis formulae had antioxidant activity, CARB-934 containing formulae exhibited higher antioxidant activity than those with poloxamer specifically in P-2 in different diluted concentrations. P-3 also exhibited a good scavenging activity especial at a concentration greater than 1mg/ml. On the other hand, POLO-407 containing formulae showed lower electron reducing activity, therefore, the best calculated IC50 belonged to P-2 (1.19 mg/ml) which also showed a good percentage of scavenger activity over different concentrations as shown in figure 4, table 4. However, this outcome could be attributed to the entrapment of the propolis and declining its release from poloxamer polymer due to the high viscosity as well as micelles formation as it was illustrated above.[14,33]

Choosing the accepted gel formula

The Choice was based on the different previous evaluations. In table 2, it is clear that the physical evaluations of the CARB-934- based formulae were more acceptable, especially regarding their spreadability and viscosity unlike those which were high in the POLO-407 concentration. On the other hand, the antimicrobial and antioxidant activity showed that the P-2 formula inhibited the growth of all the tested microorganisms as illustrated in table 3, as well as exhibited

Table 3: Susceptibility tests of prepared propolis gel formulae toward various bacterial and fungal species.

Microorganisms	Zone of inhibition (mm)							
	P-1	P-2	P-3	P-4	P-5	P-6	Positive Control*	Negative Control
S. aureus	12.8	15.5	13.8	10.7	14.6	R	22.5	R
p. aeruginosa	R	12	R	R	12	12	34	R
E. fecalis	10.5	13.2	12.2	R	R	R	21.3	R
C. Albicans	14.1	13.7	16.1	13.6	11.1	12.8	18.1	R

*The antimicrobial discs were streptomycin 25µg, ciprofloxacin, levofloxacin 5 µg, and econazole 18.1 µg for *staphylococcus aureus, pseudomonas aeruginosa*, *enterococcus fecalis* and *candida albican* respectively.

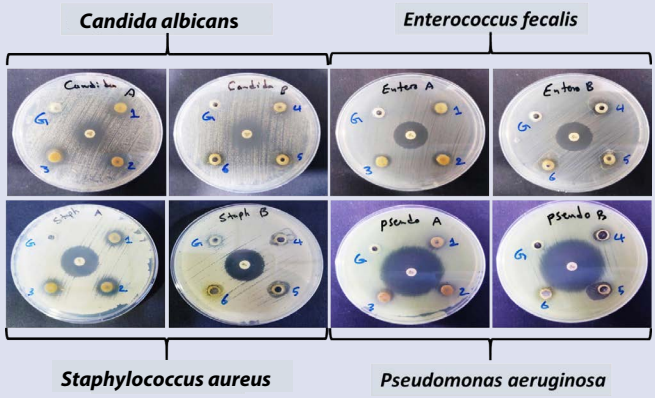

Figure 3: Inhibition zones of propolis gel against different microorganism (*Staphylococcus aureus, Enterococcus fecalis, Candida Albicans, Pseudomonas aeruginosa).*

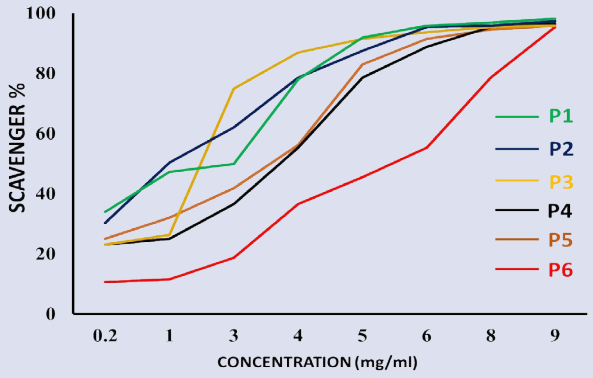

Figure 4: The scavenging effect of different propolis gel formulations using the DPPH assay method.

Table 4: IC50 of the prepared formulae.

Formula Type	IC50
P-1	1.43
P-2	1.19
P-3	1.8
P-4	3.2
P-5	2.86
P-6	5.13

a good scavenging activity and the best calculated IC50 as shown in figure 4, and table 4 respectively. As a result, the choice of the best-prepared formula fell on P-2.

Stability study of the chosen gel formula

The chosen formula (P-2) was clear from any changes in its appearance, and consistency after keeping it in a closed container at room temperature for four months. Moreover, there were no noticeable changes in *p*H, spreadability, and viscosity evaluation results.

CONCLUSION

This study demonstrated a simple and successful method of extraction of Babylon propolis. The polymer type and the concentration of propolis extract were important parameters to design and formulate an effective antimicrobial, the antioxidant formula of propolis. After many trials, a gel formula (P2) which contained 1.0% (m/v) of propolis extraction and 0.5% (m/v) of CARB-934 was proposed as an acceptable one according to the physical characteristics and was effective against *S. aureus*, *P.aerogenosae*, *E.fecalis* and *C. Albicans*. It has a lower IC50 value and has therefore been considered the best antioxidant formula. During preparation, there were some difficulties in dissolving the propolis extract in the two types of polymer dispersions despite using propylene glycol as a co-solvent in large quantities and it was difficult to use higher concentrations of the extract, this may be due to the limited solubility of the waxy propolis extract in any aqueous media and this would increase the cost of the formula, so other types of co-solvents may be recommended in future studies. However, this study was very helpful and beneficial for researchers interested in formulating natural pharmaceutical products containing such ancient materials as propolis to determine their antimicrobial and antioxidant activities.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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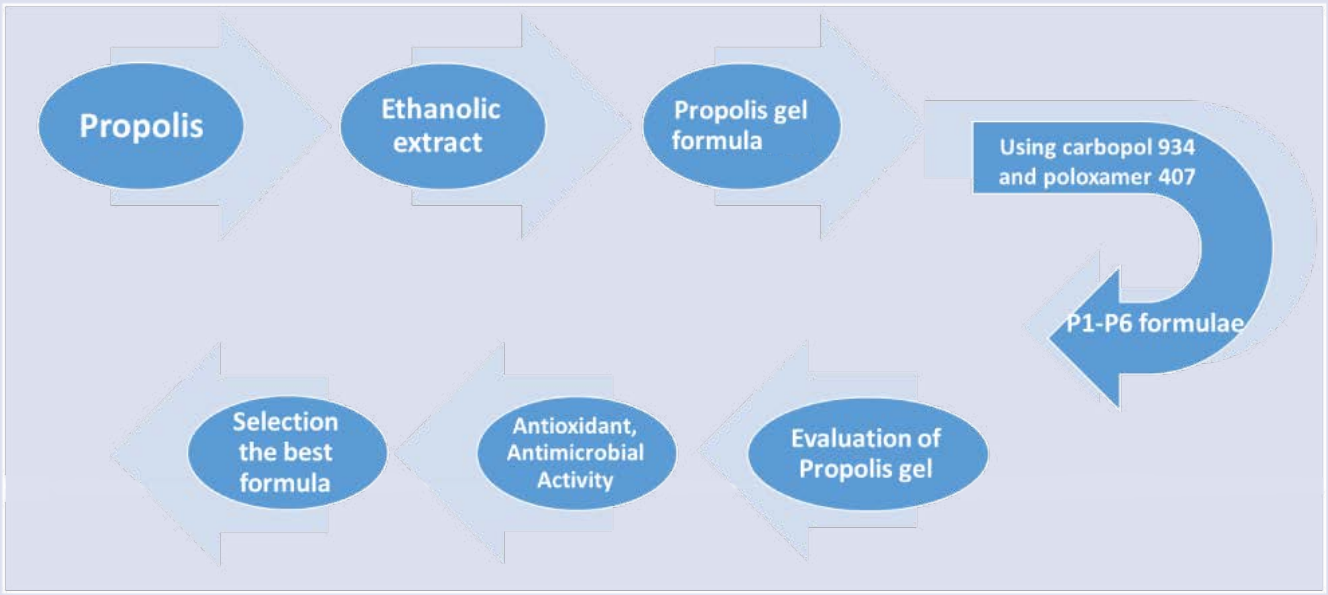
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Shahad Myasar Alfaris is currently a lecturer of industrial pharmacy at department of pharmaceutics, College of Pharmacy at the University of Mosul, Mosul, Iraq. She has a MSc degree of Medical Biotechnology at University of Portsmouth Faculty of Science with a strong interest in design and formulate better drug delivery systems, drug targeting, controlled release systems, and a broad background that includes organic synthesis, physicochemical characterisation techniques, and biochemical assays.

Rasha Khalid Dhahir has a BSc degree from the College of Pharmacy, University of Mosul since 2006, where she has been an Assistant Lecturer since 2019, in the field of pharmaceutics, and has taught pharmaceutical technology, pharmaceutical calculations of undergraduate courses in addition to that she has contributed to the publication of research papers that focused on the formulation of fast-dissolving tablets through various methods such as direct compression and freeze-drying, as well as on other topics such as nano emulsions, dry powder inhalers and *in situ* gels.

Amina Mudhafar Al-Nima obtained her BSc and worked at the college of Pharmacy/ University of Mosul, Iraq since 2004, and received her MSc in pharmaceutics from the same college in 2017. She was promoted to Lecturer in 2020. To date, she serves as a member of the department of pharmaceutics/ College of Pharmacy/ University of Mosul, Iraq and has published many research articles on fast dissolving films, semi-solid preparations, nano emulsions, dry powder inhalers and floating *in-situ* gels.

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