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Original Article

Solid Lipid Nanoparticles of *Platycladus orientalis L.* possessing 5-alpha Reductase Inhibiting Activity for Treating Hair Loss and Hirsutism

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ABSTRACT

Hair loss and hirsutism have been major complaints due to increased concentrations of dihydrotestosterone. The plant *Platycladus orientalis*, with 5-alpha reductase inhibitor properties, has been used to treat these disorders. Its formulation with lipophilic carriers in SLN possesses high loading capacity and greater permeability to hair follicles. The present study aimed to determine the content of active ingredients in the extract of *P. orientalis* L. and to prepare and characterize the solid lipid nanoparticles (SLN) of *Platycladus orientalis* L. extract as a 5-alpha reductase inhibitor. The total methanolic extract was obtained following the maceration technique. This preparation was analyzed by HPLC using Quercetin and Cedrol as standard components. SLNs were prepared by high-shear homogenization and ultrasound. Four Glucire-GMS-Compritol-Precirol lipids and three poloxamer-tween80-Labrasol surfactants were further used in the formulations. Particle size, zeta potential, nanoparticle morphology, encapsulation percentage, crystal structure, physical stability, size, and zeta potential were studied 0, 3, and 6 months after preparation. Within 1-7 days after preparation, formulations containing GMS and compritol lipids became solid and jelly. Meanwhile, the formulations with Precirol as the lipid and Poloxamer as the surfactant with 0.3% extract exhibited desirable properties such as average particle size (192 nm), the encapsulation of the extract inside the nanoparticles was almost 71%, and good zeta potential. This formulation containing precirol as a lipid, poloxamer as a surfactant, and 0.3% plant extract exhibited greater 5-alpha reductase inhibitor activity, and it can be recommended to treat hair loss and hirsutism.

INTRODUCTION

The term *hirsutism* refers to excessive hair growth in the androgen-dependent areas of the body. Although topical treatments such as shaving and electrolysis seem helpful in women with mild hirsutism, laser hair removal offers a more innovative technique[1, 2]. Hyperandrogenism also refers to the higher production of androgen by the adrenal gland or ovary. The most common clinical sign in 60-80% of female patients is hirsutism, i.e., excessive hair growth in androgen-dependent body areas. Other major clinical signs such as acne vulgaris, weight gain, and irregular menstruation are categorized as hyperandrogenism[3,4].

The leading causes of over-androgens can be identified by history and physical examinations (age of onset, time and severity of symptoms, and tests on skin, breasts, pelvis, and abdomen).

Laboratory tests on free testosterone and dihydro epiandrosterone sulfate levels can also be carried out, if history and physical examination are not accurate enough to determine the actual cause. Estimates show that 5-10% of women suffer from hirsutism[5, 6]. A significant feature of androgenic alopecia is the progressive pattern of hair loss, which frequently occurs in humans and certain mammals. Transforming terminal hair into valvular hair is a common secondary physiological sexual characteristic in androgenic alopecia. Also, white men are four times more prone to losing hair than black men[7-9]. The 5-alpha reductase enzyme converts testosterone into its active form, dihydrotestosterone. In androgenic alopecia, excessive secretion of the 5-alpha-reductase enzyme causes hormonal abnormalities such as acne, hirsutism, and hair loss, so its inhibitors are mainly introduced as valuable drugs. This more often occurs in those areas of the head with androgenic alopecia than in areas with no development of androgenic alopecia [10-12]. Cyproterone acetate, used for treating hirsutism and hair loss, acts by blocking androgen receptors and partially inhibiting 5 alpha reductase; however, it needs a high dose and has several side effects such as weight gain, edema, headache, and even hepatotoxicity [13,14]. Spironolactone is a competitive inhibitor of dose-dependent androgen receptors, limiting side effects such as urinary incontinence, chest pain, and gastrointestinal upset [15]. Finasteride can also be used orally and topically to treat hirsutism. In a clinical trial, the effects of 1% finasteride topical gel with its oral form were studied for 6 months. The results showed the similarity between the effects of oral and topical forms [16].

Generally, most drugs available in the market and others under production have negligible water solubility. On the other hand, solid lipid nanoparticles can offer an attractive target, especially for low water-soluble drugs. Since 1990, these nanoparticles have been increasingly used as an auxiliary system for emulsions and liposomes. These colloidal carriers, which are nanometers in size, are usually formed by replacing a liquid lipid (oil) from an oil-in-water emulsion with a solid physiological lipid or a mixture of solid lipids acting as a lipid matrix that can be solid at room and body temperature [17-19]. Since many nanoparticles are known to be suitable carriers for drug delivery to hair follicles, solid lipid nanoparticles were considered for drug delivery in this study. The other reason was their high loading of lipophilic drugs and suitable permeability to hair follicles. In topical treatment, it is essential to use methods involving increased drug transfer and their accumulations in the skin, which can improve therapy effectiveness.

Regarding the possible side effects of chemical drugs, which have led to their limited use, most researchers have been attracted to herbal plants that can be used similarly to treat hair loss and hirsutism. *Red reishi*, a fungus, has long been used to inhibit testosterone [20]. Likewise, green tea, especially its epigallocatechin compounds, has shown significant anti-androgenic effects that can control 5 alpha reductase [21]. Moreover, Terpenes and flavonoids in *Platycladus orientalis (L.)* strongly inhibit the 5-alpha reductase enzyme [22,23]. *P. orientalis (L.)*, a single-stemmed and evergreen tree, belongs to the Cupressaceae family and is the only species of its genus native to Korea, Manchuria, northern China, and Iran. In Persian, it is called Noosh, Sarv Khumraei, or Sarv Tabari [24,25]. Since the leaf extract (containing flavonoids) can lower the level of serum uric acid, it is commonly used for treating rheumatism and gout [25,26], colds, coughs, bronchitis, asthma, chronic inflammation of the trachea, improving respiratory function[27], and inhibiting platelet-activating factor, which is related to the pinusolid and pinusolidic acid diterpenoids [23, 28]. In this study, cedrol, acting as a terpene found in *P. orientalis (L.)*, was used as an active ingredient in the total extract in SLN.

MATERIALS and METHODS

All reagents used were purchased commercially and were of the highest grade available: Precirol (Gattefose, France), Glyceryl Monostearate (BDA Laboratory, United Kingdom), Compritol (Gattefose, France), Glucire (Gattefose, France), Poloxamer (Uniqema, Belgium), Tween 80 (Croda-international PLC, England), Labrasol (Gattefose, France), Ethanol 96 degrees (Noor Zakaria Razi, Iran), Methanol (Merck, Germany), Ethyl acetate (Merck, Germany), Etherdopterol (Merck, Germany).

Plant Collection

At first, new shoots of *Platycladus orientalis*, sampled from the Ferdowsi University Campus, Iran in June 2021, and subsequently verified by Dr. Sirousmehr in the botany department, University of Zabol, Iran (Fig. 1), were dried at laboratory temperature and away from sunlight. Then, being identified by Mr. Joharchi, it was kept and numbered 37063in the herbarium of Mashhad School of Pharmacy.

Fig. 1 Appearance of *Platycladus orientalis*

Preparation of Total Methanolic Extract

After pulverizing the dried branches, the total methanolic extract was extracted using the maceration technique. Next, 250 g of the powder was poured into large balloons, and the extract-containing solvent was obtained 24 hours after adding distilled methanol in the appropriate amount. This iteration was done 5 times. The solvent was then removed by rotary to receive a total methanolic extract, which looked dark green and had a strong odor. Finally, after getting dried in a freezer, the extract was weighed and prepared for the subsequent phases [29,30].

Biofilm Formation Assay in the Presence of the Biocides

Pseudomonas aeruginosa (ATCC 27853), *Proteus mirabilis* (ATCC 1611), *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 1189), *Enterococcus fecalis* (ATCC 1787) and *Streptococus pneumonia* (ATCC 1234) were the six antibiotic-resistant pathogenic bacteria employed in this study.

Following the aforementioned method, the microplate was covered and incubated aerobically for 24 hours at the appropriate temperature. Using an automated ELISA counter, the OD (Optical Density) was first measured at 600 nm. After that, the contents of each microplate well were aspirated, and each well was then washed three times with 250 L of sterile physiological saline. 200 L of 99% methanol was used to fix any residual adherent bacteria in each well, and after 15 minutes, each well was discarded and allowed to dry. Then, 0.2 mL of 2% crystal violet was added to each well and allowed to stain for 5 minutes. The dish was progressively washed in distilled water to remove extra discoloration. After the plate had air dried, 160 mL of 33% (v/v) glacial acetic acid was added to each well to dissolve the dye that had been attached to the adhering cells. Using an automated ELISA counter, the OD of each well was determined at 492 nm.

Preparation of Different Fractions

Various plant fractions (*P. orientalis*) were prepared in different solvents. First, 10 g of total methanolic extract was weighed and transferred to a beaker. Then, it was dissolved in some methanol. Ether diopter was added to the extract, which was transferred to a decanter funnel. After stirring the mixture, it was allowed to form two phases. The upper phase, the ether diopter fraction, was isolated. The separation step was performed 3 times, and the resulting ether diopter fraction was discarded for later stages. Similarly, the lower phase, methanolic fraction, was mixed with some ethyl acetate in the decanter funnel. After two phases, the upper phase, ethyl acetate fraction, was separated with three iterations. The resulting ethyl acetate fraction was discarded for later steps. For these two fractions, the solvent was removed by rotary to get dried in the freezer [29,31].

Determining Active Substance Content in total Extracts and Fractions

HPLC Method

The washing solvent was methanol water made by the gradient (Table 1). The column was 250 × 4.6 mm C18, 5 µm, the solvent flow rate was 1 ml/min, the injection volume was 20 µl, and the UV detector was 365 nm. Initially, to draw the standard curve, some solutions with concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml were made from Quercetin, and the standard curve was then drawn. A solution of 0.5 mg/ml of Quercetin was also injected into the device to identify the desired peak. Next, a 20 mg/ml solution of total methanolic

extract and ethyl acetate and ether diopter fractions were injected into the injection device, and finally.

Gas Chromatography-Mass Spectrometry (GCMS)

To determine the content of plant extract in nanoparticles based on the previous literature and regarding the fact that detection of active substance in the fractions is impossible by the HPLC method, it was concluded that its amount was higher in the total extract. Since only the total extract was used to identify and determine the amount, the extract was first examined in terms of the presence of anethole substance. Yet, no total anethole extract was recorded, so this material was used as an internal standard. Thus, a Methanol solution with a concentration of 600 µg / ml of total extract and 5 µg / ml of anethole was prepared. A 4 µLs of this solution was injected into the device to determine the content of anethole and cedrol [32].

Table 1 Characteristics of washing solvent in the HPLC method

Methanol (%)	Water (%)	Time(Minute)
20	80	0
100	00	12
100	0	17
20	80	20
20	80	22

Preparation of Solid Lipid Nanoparticles

Preparation of Initial Formulations of Solid Lipid Nanoparticles

SLN was prepared by high-shear homogenization and ultrasound method. Initially, to get an optimal formula, 4 different lipids (Table 2), including Precirol, GMS, Compritol, and Glucire, and 3 different surfactants, including Labrasol, Poloxamer, and Tween 80, were considered. Lipid concentration (5%) and surfactant concentration (1.25%) were also selected according to previous studies. After developing these formulas, it was observed that the formulas containing presirol and poloxamer had an appropriate particle size and size distribution. Yet, other formulas with glycerol monostearate, camperitol, and glucire alone had no suitable size, so it was decided that the formulas combine different proportions of two lipids, Glycerol Mono Stearate (GMS) and Precirol, with varying ratios of poloxamer surfactant. This time, the percentage of total extract gradually raised from 0.1 to 0.3 total, and 9 formulas were developed and reviewed in this phase (Table 4). In each formulation, particle size and zeta and PDI potential measurements were performed, and finally, the formulation containing precirol + poloxamer + 0.3% total extract was considered the best. Moreover, it was observed that 3 and 6 months after preparation, it remained stable in terms of particle size, PDI, and zeta potential. No significant difference was observed between the values and the prototype, so it was reported to be suitable in terms of stability over time.

Determining SLN Properties

Determining particle size and zeta potential

A particle size analyzer was also used to evaluate particle size and zeta potential. This way, 10 µLs of the freshly prepared and cooled sample was poured into a 1.5 cc microtube and diluted with 990 µl of the outer phase, i.e., deionized water (1:100)[33].

Determination of melting point of components and investigation of nanoparticle lipid network by Differential Scanning Calorimetry (DSC)

For the present study, a DSC device was used, which is equipped with Stare software. The samples and references were compared in the temperature range of 0 to 300 °C at a rate of 5 °C/min and under nitrogen gas at a flow rate of 20 ml/min[34]. For this testing purpose, the final formulation was used in powder resulting from a freeze dryer after 24 h of incubation in the freezer at -70 ºC. The formulation was placed in the freeze dryer for 24 hours to get dried thoroughly. Moreover, total extract, precirol lipid, vacant SLN, and SLN containing a whole extract were analyzed by DSC.

Investigation of morphology of Solid Lipid Nanoparticles by Electron microscopy

Transmission electron microscopy (TEM) imaging was used to study the morphology of solid lipid nanoparticles. The sample was diluted about 50 times with distilled water. Then, 20 µl of the sample was placed on carbon-coated grids and dried after 30 seconds by a paper filter. 20 µl of 2% uranyl acetate in water was placed on the grids and, after 30 seconds, was dried with a dry paper filter. After drying, the sample was analyzed under an electron microscope [35,36].

Determination of the Content of Encapsulation Extract in Nanoparticles

A formula including lipid (5%), poloxamer surfactant (1.025%), and methanolic extract with an internal standard of anethole was prepared to determine the percentage of encapsulation of the extract in SLN. In addition, 0.1 cc of cedrol was added to 1 cc of a methanolic solution containing 100 mg of the whole extract. To increase the volume, 0.33 cc of cedrol was added to the formula. The formula was then passed through a 0.45 filter, and 100 µl was mixed with 900 µl of a 1: 2 methanol-chloroform mixture. The mixture was vortexed for 30 seconds. Then 4 µL were injected into the device for testing, so the percentages of Anethole and Cedrol were measured.

Investigation of stability of solid lipid nanoparticles over time

Nanoparticles were evaluated in terms of color, odor, appearance, and other properties, including size and zeta potential in 0, 3, and 6 months after preparation.

Table 2 Melting point and Log P of lipids

Substances	Melting point (°C)	Log P
Precirol	57-63	8.21
Glycerol monostearate (GMS)	58	5.97
Compritol	67	9.6
Glucire	55-57	3.4

All formulas were initially made with 0.3% extract. Once more, they were made with 0.2% extract. In total, 24 formulas were developed and tested at this stage (Table 3).

Table 3 Initial formulations of 4 lipids and 3 surfactants with 0.2 and 0.3% extract concentrations

Formula	Lipid phase		Water phase	
	Lipid (5%)	Extract	Surfactant (1.25%)	Water
PP1	Precirol	0.2	Poloxamer	To 100%
PP2	Precirol	0.3	Poloxamer	
PT1	Precirol	0.2	Tween80	
PT2	Precirol	0.3	Tween80	
PL1	Precirol	0.2	Labrasol	
PL2	Precirol	0.3	Labrasol	
GP1	GMS	0.2	Poloxamer	
GP2	GMS	0.3	Poloxamer	
GT1	GMS	0.2	Tween80	
GT2	GMS	0.3	Tween80	
GL1	GMS	0.2	Labrasol	
GL2	GMS	0.3	Labrasol	
CP1	Compritol	0.2	Poloxamer	
CP2	Compritol	0.3	Poloxamer	
CT1	Compritol	0.2	Tween80	
CT2	Compritol	0.3	Tween80	
CL1	Compritol	0.2	Labrasol	
CL2	Compritol	0.3	Labrasol	
GLP1	Glucire	0.2	Poloxamer	
GLP2	Glucire	0.3	Poloxamer	
GLT1	Glucire	0.2	Tween80	
GLT2	Glucire	0.3	Tween80	
GLL1	Glucire	0.2	Labrasol	
GLL2	Glucire	0.3	Labrasol	

Table 4 Mixed formulations including two lipids, Precirol, GMS, with Poloxamer surfactant each with variable lipid contents

Formula	Lipid Phase	Water Phase
1	GMS (30%) +Precirol (70%) +extract (0.1%)	Poloxamer (1.25%) + Water to 100% (for 10cc)
2	GMS (30%) +Precirol (70%) +extract (0.2%)	
3	GMS (30%) +Precirol (70%) +extract (0.3%)	
4	GMS (50%) +Precirol (50%) +extract (0.1%)	
5	GMS (50%) +Precirol (50%) +extract (0.2%)	
6	GMS (50%) +Precirol (50%) +extract (0.3%)	
7	GMS (70%) +Precirol (30%) +extract (0.1%)	
8	GMS (70%) +Precirol (30%) +extract (0.2%)	
9	GMS (70%) +Precirol (30%) +extract (0.3%)	

Table 5 Characteristics of formulas containing GMS lipid and poloxamar surfactant with 0.2 and 0.3% extracts

Formula	Z-Average	PDI	Z-Potential
GP1	104.1±10.8	0.187±0.012	-11.88±2.5
GP2	103.4±11.2	0.214±0.015	-12.5±5.7

Table 6 Characteristics of formulas containing Glucire lipid with 3 surfactants of poloxamer, Labrasol and Tween 80 with 0.2% and 0.3% extracts

Formula	Z-Average	PDI	Z-Potential
GLP1	98.86±11.4	0.561±0.048	-7.80±1.2
GLP2	34.32±9.6	0.430±0.039	-9.88±1.3
GLL1	1420±62.1	0.921±0.062	-9.06±2.1
GLL2	1368±59.1	0.858±0.051	-10.07±2.4
GLT1	113.7±12.4	0.195±0.027	-4.35±0.5
GLT2	156.2±14.3	0.225±0.036	-651±0.2

Table 7 Characteristics of formulas containing precirol lipid and two surfactants, ploxamer and twin 80 with 0.2 and 0.3% extracts

Formula	Z-Average	PDI	Z.Potential
PP1	228.1±17.3	0.256±0.025	-19.8±3.2
PP2	192.5±14.8	0.233±0.021	-21.9±3.8
PT1	280.8±23.1	0.288±0.031	-16.0±3.7
PT2	280.8±22.4	0.245±0.028	-12.2±2.9

Table 8 Properties of hybrid formulas including GMS and Precirol lipids and Poloxamer surfactant with 0.1, 0.2 and 0.3% extracts

Formula	Z-Average	PDI	Z-Potential
1	183.6±18.3	0.196±0.023	-12.6±3.5
2	220.4±21.2	0.176±0.014	-11.5±4.2
3	339.2±28.3	0.585±0.045	-10.2±4.8
4	187.6±17.4	0.159±0.029	-17.1±7.6
5	187.9±29.5	0.322±0.038	-15.8±6.8
6	243.8±37.6	0.254±0.032	-13.9±5.9
7	280.6±34.4	0.209±0.028	-11.3±4.6
8	257.1±29.7	0.374±0.072	-10.7±3.8

Table 9 Evaluation of particle size, PDI, and zeta potential of formula containing Precirol lipid and poloxamar surfactant and 0.3% extract during 0, 3, and 6 months after preparation

Time	Z-Average	PDI	Z-Potential
Primitive	192.5±14.8	0.233±0.021	-21.9±3.8
After 3 months	189.2±12.4	0.255±0.032	-20.8±3.4
After 6 months	187.5±13.6	0.210±0.025	-20.7±4.1

RESULTS and DISCUSSION

Determination of active ingredient content in total extract

From 10g of total methanolic extract, 0.8g of ether diopterol fraction and 0.45g of ethyl acetate fraction were obtained. In the HPLC method, chromatograms of neither the total extracts nor fractions showed any significant peak for Quercetin detection.

In gas chromatography, the percentages of cedrol and anethole were 1.17% and 1.53%, respectively (Fig. 2). Proportionally, the amount of cedrol in the total extract is 3.82 µg / ml, so the percentage of cedrol in the total extract was 0.63%.

Determination of Particle Size and Zeta Potential

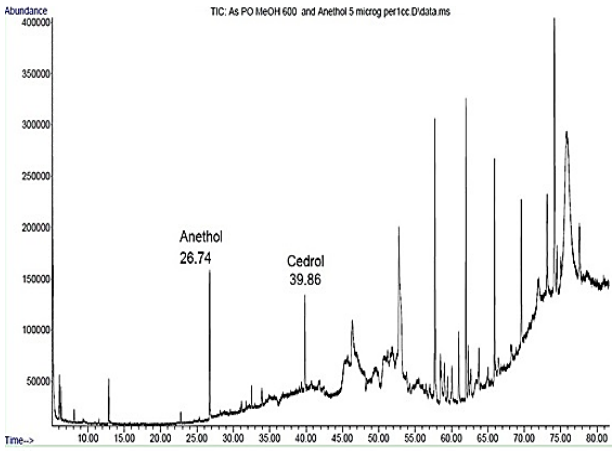

Fig. 2 Chromatography of total extract with a concentration of 600 µg / ml and standard Anatole with a concentration of
5 µg / ml

After half an hour, the formulations containing GMS, i.e., GT1, GT2 (GMS lipid and Tween80 surfactant with 0.1 and 0.2% extract) and GL1, GL2 (GMS lipid and Labrasol surfactant with 0.1 and 0.2% extract), were solidified, so they were unable to measure size, but next day, the formulations of GP1, GP2 (GMS lipid and Precirol surfactant with 0.1% and 0.2% extract) were suitable for size measurement. However, a week after construction, they were pretty solidified (Table 5).

After manufacture, the formulations containing Compritol became solid. On the other hand, during the Diax stage, as the machine speed increased, formulations with Glucir were foamed significantly and poured out of the container (Table 6). Also, the halving percentage of surfactant in the formula made no changes, so the circumference of the Diax device for the formulation was not raised at all, yet, the result was nearly satisfactory.

In less than a month, the PL1 and PL2 formula (Precirol lipid, Labrasol surfactant, and 0.1% and 0.2% extracts) was completely solidified (Table 7). Since the formula containing persirol and poloxamer was suitable in terms of appearance and size, it was made with 0.4% and 0.5% extracts; however, it was found that increasing the percentage of formula extract by more than 0.3% could not achieve the desired results in terms of stability and appearance. At this phase, the PP2 formula (Precirol lipid, Poloxamer surfactant, and 0.3% extract) was chosen as the best formula, mainly because of its desirable appearance, size, and zeta results.

Combination formulation No. 9 (GMS 30% + Precirol 70% + extract 0.3%) was solidified a day after manufacture, so it was not able to measure size. Since formulas 2, 5, and 6 were appropriate in appearance, they were re-examined in size and zeta potential after a month. Still, their size increased after 3 months without having a proper appearance (Table 8). Finally, PP2 was chosen as the final formula. The results of size (Fig. 3A), zeta potential (Fig. 3B), and PDI (Fig. 3C) at times 0, 3, and 6 months after preparation (Table 9) represented that the values did not differ significantly from time 0. Still, the stability formula had a good value (P value: Ns).

Determination of Melting point of Components and Investigation of Lipid Network of Solid lipid Nanoparticles by Calorimetry (DSC)

The thermal analysis of materials (pure lipid (Fig. 4A), blank SLN (Fig. 4B), SLN containing total extract (Fig. 4C), and total extract (Fig. 4D) were performed using DSC. The pure Precirol diagram shows a melting point similar to the reference. As expected, a lipid melting point was observed in blank SLN.

Based on DSC results, in the blank SLN diagram (Fig. 4B), the main peak was formed at about 62 °C. Compared with the melting point of Precirol, it was expectedly desirable (57-63). In the diagram of the plant extract (Fig. 4D), there are two main peaks (~50 and ~100), which are related to the extract components

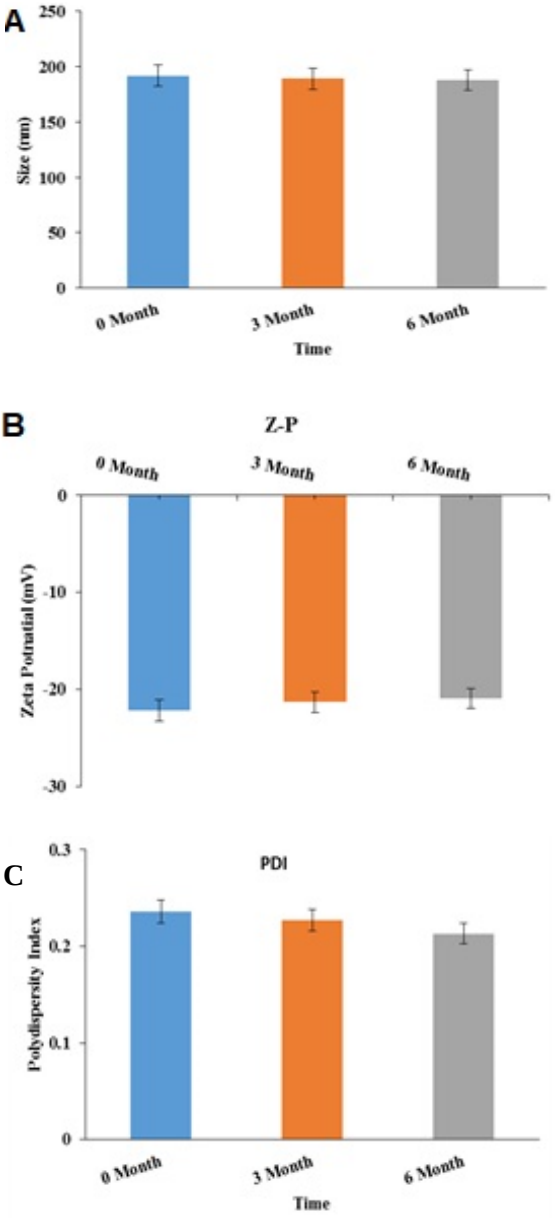

Fig. 3 Comparison of average particle size of formulation containing Precirol+ poloxamar + 0.3% extract during 0, 3 and 6 months (A); Comparison of zeta potential of final formulation containing precirol + poloxamar + 0.3% extract during 0, 3 and 6 months (B); Comparison of PDI final formulation containing precirol + poloxamar + 0.3% extract during 0, 3 and 6 months (C)

Although the melting point of cedrol is 87 ºC, its content in the plant extract was low. Thus, the corresponding peak in the SLN diagram with the plant extract (Fig. 4C) was insignificant. Yet, in the DSC diagram of SLN with plant extract (Fig. 4C), there was a lipid peak; however, the peaks in the plant extract diagram disappeared, representing 71% loading of the extract inside the nanoparticles. Thus, it can be inferred that the extract was greatly located within the lipid network in an amorphous form. If the extract was not placed in the lipid network, it should remain on the surface of the nanoparticles. Moreover, since the lyophilized powder was used for testing, it was expected to represent its peaks, but it did not occur.

Investigation of Morphology of Solid Lipid Nanoparticles by Electron Microscopy

The electron microscope graph showed that the nanoparticles are spherical and about 100 nm in size (Fig. 5). The nanoparticles exhibited a spherical shape, and the particle sizes obtained in this method were close to the particle size analysis with DLS results.

Investigation of Extract's Encapsulation Percentage in Nanoparticles

Based on the gas chromatographic results obtained from the SLN sample, the percentages of anethole and cedrol were 0.0089% and 0.079%, respectively. The ratio of anethole to cedrol in this sample was 0.11 (Fig. 6). The initial standard extract concentration was 600 µg/ml. The anethole concentration was 5 µg / ml (1:120), while in the SLN sample, the ratio of anethole to cedrol was 1:1000, so it can be concluded that the ratio of anethole to cedrol in the standard is 8.3 times more than that of the sample. If the percentage of encapsulation was 100%, the ratio should be similar to the standard ratio, i.e., 0.15 ((1.53/1.17)/8.3=0.15). Then, (0.11/0.15)*100=71%. Finally, the percentage of encapsulation of the extract in nanoparticles was 71%.

Biofilm Formation Aassay

According to the study's findings, biofilm development in essential oil concentrations was lower than it was in the control sample (Fig. 6). The greatest rates of biofilm development were seen in *Enterococcus fecalis*, *Proteus mirabilis*, and *Streptococcus pneumonia* (Fig. 7).

Nanostructured Lipid Carriers (NLCs) are a new generation of colloidal drug carriers used for topical applications. They seek to overcome SLN problems. The main difference between SLN and NLC is that SLN is composed of solid lipids, whereas NLC includes a mixture of solid lipids and liquid lipids (70:30 - 99.9: 0.1) [37].

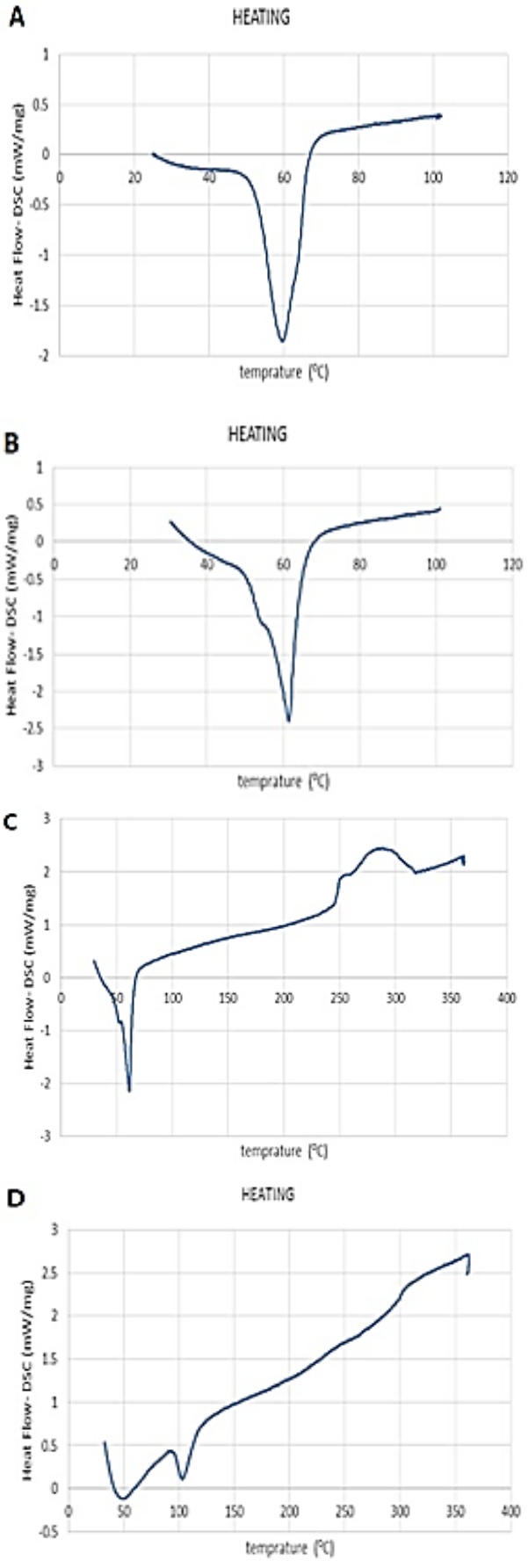

Fig. 4 Evaluation of Precirol Lipid Thermal Analysis (A); Evaluation of blank SLN thermal analysis (B); Evaluation of SLN thermal analysis containing plant extract of *Platycladus orientalis* (C); Thermal analysis of total extract of *Platycladus orientalis* (D)

Moreover, Precirol is chosen as a solid lipid in nanoparticles because it is a diglyceride with two different fatty acids (C16 and C18), which forms an irregular lipid network, so better loading can be predicted [38,39]. This irregular lipid network makes both controlled drug release and increased encapsulation [40,41]. Based on the findings, SLNs made of Precirol were stable for up to 1 year in terms of particle size and distribution [42,43]. Similarly, in the present study, size stability and zeta potential analyses during 3 months and 6 months after construction were stable and suitable for topical application.

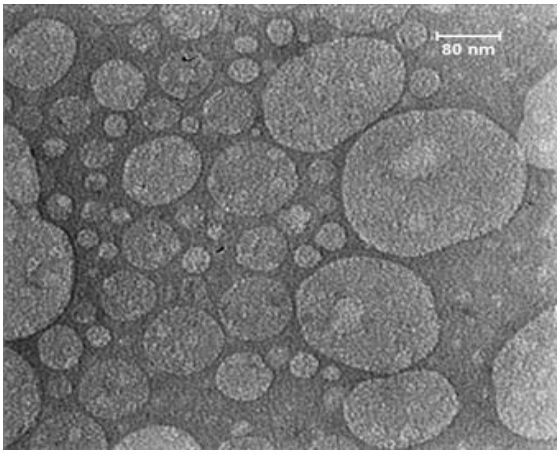

Fig. 5 TEM electron microscope results prepared from SLN containing precirol lipid and poloxamar surfactant and 0.3% extract

Applying nanoscale particles can increase the effectiveness of compounds at lower concentrations so that they can be used for supplying nutrition, monitoring diseases, and reducing the environmental impact of aquaculture-related activities. The small size of these particles can lead to fundamental changes in the structure and properties of these elements. Therefore, numerous new nanotechnology products have been made for various purposes[44]. The sphericity of solid lipid nanoparticles delivers the most significant possible potential for controlled release and protection from the retained drug because such a spherical shape has the longest path for the drug to be trapped in the nanoparticles. Moreover, it has minimum contact with the aqueous medium of the dispersed phase rather than other forms of nanoparticles [45,46]. It was also found in the present study that not only the particle size of nanoparticles was about 16 nm, but also the solid lipid nanoparticles containing plant extract had a smooth and uniform surface and a spherical shape.

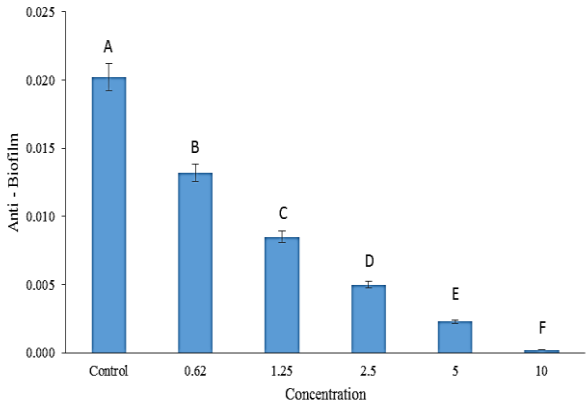

Fig. 6 The anti-biofilm effect of some concentrations of *P. orientalis L.* extract

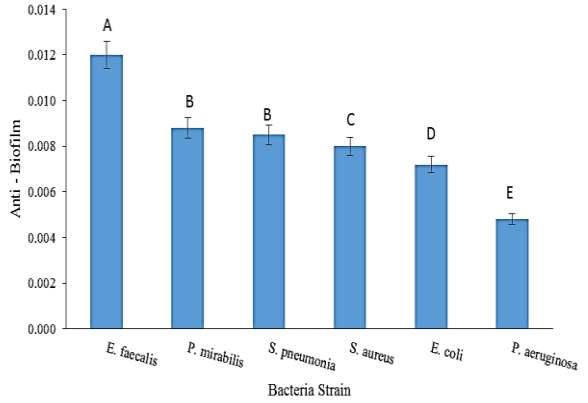

Fig. 7 The anti-biofilm effect of *P. orientalis L.* extract on some bacteria strains

Analysis of follicular penetration of nanoparticles showed that the lipid carrier causes better absorption of the drug by hair follicles. Lipid nanodots are found at the site of the hair follicle even after 24 hours of use, indicating a slow-release system in the hair follicle. The penetration depth depends on particle size. Particle systems can deliver drugs to specific areas of the follicle [47, 48]. Some advantages of using these carriers are slow drug release, blocking, and film-forming properties[37,49]. Moreover, their priority over liposomes is that they take advantage of the benefits of liposomes, protect their inside drug from destruction, and water-in-oil systems have more stability than liposomes [50].

The hair follicle is a long-term reservoir since depletion occurs only during the slow process of sebum extraction or infiltration into the substrates. The nanoparticles are well stored in the hair follicles for more than 10 days. In the stratum corneum, non-particle materials and macto nanoparticles are primarily stored in the corneocytes' upper cell layer and intercellular spaces. This storage is rapidly depleted by washing, cloth contact, and scaling [51]. Thus, hair follicles can be a permanent and suitable repository for drug storage compared to the stratum corneum, which offers slow drug release and reduces application frequency[47].

Solid lipid nanoparticles (SLNs), also known as fat carriers, have emerged as versatile carriers of nanoscale drugs. SLNs can contain drugs, vitamins, molecules, and almost all xenobiotics. SLNs are also potential carriers for gene transfer and anticancer drugs [52]. It is necessary to use lipid nanoparticles to protect the bioactive compound against degradation at all processing, storage, transport, and use stages. Moreover, the final nanostructures should control the release rate of bioactive compounds at a specific location or in response to specific environmental stimuli such as changes in pH, ionic strength, and temperature[53]. To prepare solid lipid nanoparticles, 5 - 40% lipid and up to 5% emulsifier were used to stabilize the formulation [54]. The selected surfactant for Precirol lipid was poloxamer [55,56], so in the present study, it was also used to present better results than other surfactants (Tween80 and Labrasol) were obtained. In this study, besides preparation and determination of the properties of solid lipid nanoparticles containing plant extracts, due to the lower percentage of surfactants which offer the desired results, 5% lipid and 1.25% surfactant were suggested to save material consumption.

There is a direct relationship between a decrease in zeta potential and a loss in physical stability. The zeta potential of -60 mV is excellent for strength and slightly higher than -30 mV, which is good for electrostatic stability and leads to physical stability [57,58]. In the present study, it was also observed that formulas with very low zeta potential had poor stability, so even within a few hours or days after preparation, they lost their stability. However, the final formula, containing presirol and poloxamer with 0.3% extract, had a zeta potential (-20), which was expected to have good physical stability. Evaluation of the preparation of solid lipid nanoparticles for topical administration of Kaempferia Parviflora extracts showed that the particle size of 108-82 nm yielded up to 87. In addition, the total amount of KP flavonoids in SLNs and gels that penetrated the skin after 25 hours was

97.57 and 81.04 grams, respectively [59]. In the present study, a large part of the extract, about 71%, is located inside the lipid network and is most likely amorphous.

The stability of bioactive Solid lipid nanoparticles with plant extracts exposed to simulated gastrointestinal conditions was further evaluated. Accordingly, the sage and savory extract phenolic compounds are trapped in the SLN. SLN rich in savory extract represented a higher antioxidant activity. However, all SLNs showed about 100% rosmarinic acid emission in the small intestine. Witepsol SLNs are the most stable carriers used for sage and savory extracts60. *Kaempferia parviflora* (KP) extract is also formulated in SLNs to increase transdermal permeability. The results showed that KP extracts stuck to the molten mixture of oils, surfactants, and PEGylating agents in SLNs to form an oil-in-water microemulsion at high temperatures. Finally, cooling this microemulsion can lead to the formation of SLNs.

Moreover, the formulation with optimal properties uses stearyl alcohol as a nanoparticle matrix. Also, tocopherol polyethylene glycol succinate (TPGS) acts as a surfactant [59]. Solid lipid nanoparticles produced with Witepsol and Carnauba and loaded with rosmarinic acid (RA), using both in vitro and in vivo methods, were dependent on genotoxicity and cytotoxicity. So it was concluded that both nanoparticles, with moderate RA concentrations, without genotoxicity and in vitro cytotoxicity, and with in vivo safety profile, are safe in orally treated mice [60]. The effectiveness of *Platycladus orintalis* compounds (flavonoids and terpenes) in inhibiting 5 alpha-reductase, reducing hair number and hair thickness was also proved in many women with idiopathic hirsutism [22,61]. Cedrol was tested as a major ingredient in Platycladus orientalis for hair loss. The results showed that after 21 days of treatment, increased hair growth in mice treated with 30 mg/ml Cedrol solution was more significant than the negative control and minoxidil (2%) [62].

One of the new methods to solve the problems of plant extract application (such as instability, evaporation, and decomposition in the face of environmental conditions) in food, pharmaceutical, and agricultural industries is Solid Lipid Nanoparticles. Solid lipid nanoparticles containing plant extract have relatively good stability. Nanoparticles from *Platycladus orientalis* and other sources have broader applications in pharmaceutical, food, agricultural, and water treatment industries [63-70]. The increasing significance of NPs compels this extract with nanotechnology, which can facilitate its application and increase its efficiency [67-73]. However, additional *in vivo* studies are recommended to use solid lipid nanoparticles containing the extract of the Platycladus orientalis.

CONCLUSION

The results of this study showed that the SLN formulation made by high-shear homogenization and ultrasound method included Precirol as lipid (5%), Poloxamer as a surfactant (1.25%), and total extract of Platycladus orientalis (0.3%). Moreover, since it is suitable for topical administration in terms of particle size, zeta potential, encapsulation percentage, and appearance, it can be introduced for further *in vivo* studies.

Author Contribution

Conceptualization; J.A. and S.G., Methodology; M.N. and S.D., Formal analysis and validation; N. Sh., writing- original draft; B.FN., J.A.P., and R.Z.S., writing-review and editing; B.FN, J.A.P., and R.Z.S.

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Conflict of Interest

The authors declare no conflict of interest in this study.

Data Availability

All the data is available in the manuscript file

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