

Research Article



Formulation Design and Evaluation of Anti-Microbial Activity of Emulgel Containing Essential Oil of *Myrtus communis* L.

Hiba Sabah Sabri^{a*}, Wedad Kamal Ali^a, Baydaa Hameed Abdullah^b, Widad M.K. Al-Ani^c

^aDepartment of Pharmaceutics, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.

^bDepartment of Clinical Laboratory Sciences, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.

^cDepartment of Pharmacognosy, College of Pharmacy, Al-Mustansiriya University, Baghdad, Iraq.

*Corresponding author's E-mail: Hiba_sabah86@yahoo.com

Accepted on: 10-09-2016; Finalized on: 30-09-2016.

ABSTRACT

Myrtus communis L (Family Myrtaceae) is an aromatic evergreen perennial shrub or small tree, widespread in the Mediterranean region, have been used widely in the traditional medicine due to variety of its therapeutic properties. The Extraction of myrtle oil from the leaves of Myrtus plant by hydrodistillation method using Clevenger type apparatus, produce a light yellow essential oil at 0.55 % yield, the chemical composition of myrtle oil was analyzed by gas chromatography-mass spectrometry (GC-MASS) revealed that myrtle oil consist of 19 compounds; the major constituents that present in high percentage were: alpha Pinene (32.65.1%), 1,8Cineole (25.1%), Linalool (16.37%), Eugenol (6.56%). The antimicrobial activities of Myrtle oils against *Staphylococcus aureus*, *Streptococcus pyogen*, *Pseudomonas aeruginosa* and *Candida albicans* were evaluated by disk diffusion method, Myrtle oil exhibited varying levels of antimicrobial activity against the investigated pathogens. The objective of this study was to formulate topical antimicrobial agents containing myrtle oil for the treatment of human skin diseases. The emulgel formulas were prepared by using different types and concentration of emulsifying agents (Span 80, Tween 80), gelling agents type (methylcellulose, Carbopol940, Xanthan gum, HPMCK15M) at different ratios and olive oil. The prepared formulas were characterized for the physical appearance, pH, viscosity and *in vitro* drug release study. The results revealed that all the prepared emulgel formulas have acceptable physical attributes. After selection of optimum formula, it was subjected to further tests to confirm its stability, antimicrobial activity, Kinetic Modeling of drug release and skin irritation study on rat skin.

Keywords: myrtle oil, herbal emulgel, Clevenger type apparatus, emulsifying agent, gelling agent.

INTRODUCTION

Topical and transdermal formulations have a long history of use. Over 2000 years ago, Greek physicians used formulations containing salt, vinegar, honey and resins to treat skin lesions and ulcers. Chinese, Egyptian and Roman medical histories describe numerous remedies applied topically as pastes and poultices.^{1,19}

Semisolid preparations represent dosage forms that have properties in between solid and liquid dosage forms and possess characteristic rheological properties such that they can be easily applied on biological membranes and can be retained on the site of application for a prolonged time. Semisolid dosage forms may contain one or more active ingredients in suspended/dissolved forms, in inclusion complexes, or in a solubilized state and are applied topically to the skin or on the surface of the eye, nasally, vaginally, or rectally for local and/or systemic effects.² Emulgel: Emulsion hydrogels, also known as emulgels, are biphasic hydrogel based formulations. Emulgels are usually formed either by dispersing an oil phase in a gel phase or by inducing gelation of the external phase of an oil-in-water emulsions. The concept of emulgels was conceived to eliminate the limitation of the hydrogels in delivering hydrophobic drugs.³ Essential oils are volatile products of aromatic plants secondary metabolism, normally formed in special cell or group of cells found in many leaves and stems. It is concentrated

or stored either in particular region of the plant or in various organs in the same plants.⁴ Biological activity of essential oils depends on their chemical composition, which is determined by the plant genotype and is greatly influenced by several factors such as geographical origin, environmental and agronomic conditions.⁵

External applications of essential oils after mixing with carrier oil involve in massage, lotions, or dressings. Vaporization and inhalation (taking into the body by breathing) both are the part of application. Essential oil may be ingredient of gargles and mouthwashes. The internal use of essential oils is very rare. An external application of the oil is the most common and widely used method as compare to the internal application.⁸

Myrtus communis L Common myrtle belongs to the Myrtaceae family, which comprises approximately 145 genera and over 5500 species. *Myrtus communis* is an important medicinal and aromatic plant, because of their Essential oils are gaining remarkable interest for their potential multipurpose uses as antioxidant, antibacterial, and antiseptic agent.⁶

True Myrtle is characterized by its branches, which form a close full head, thickly covered with ovate or lanceolate evergreen leaves. Their leaves are 3–5 cm long and contain tannins, flavonoids and volatile oils.

This species is a very aromatic plant because of the high essential oil content in its leaf, flower and fruit glands.⁹



The isolation of essential oils from *Myrtus communis L.* leaves is usually obtained by hydro distillation method with a Clevenger-type apparatus, according to the Italian Official Pharmacopoeia⁷. Myrtle oil consists of different types of terpenes, phenols and oxides. The main terpenes found are monoterpenes, sesquiterpenes, and long chain terpenes.¹⁰

MATERIALS AND METHODS

Liquid paraffin and Olive oil were purchased from Solvochem, UK. Carpobol 940, Xanthan gum, Methyl cellulose, HPMC K15m, Brij35 and Sabouraud Dextrose Agar Medium were purchased from HIMEDIA, India. Ethanol was purchased from Sigma Aldrich, USA. Span 80 and Tween80 were purchased from Merck, Germany. Methyl paraben and Propyl paraben were purchased from Interchimiques SA-France. Triethanolamine was purchased from Alpha Chemika, India. Propylene glycol was purchased from Avonchem, UK. Sodium sulfate anhydrous was purchased from BDH-England.

Extraction of Myrtle Oil

until extraction Harvested leaves of *Myrtus communis L.* were obtained from local Baghdad gardens,, and dried to at room temperature then the dried leaves were grinded, and the essential oils of the plant were extracted by hydro-distillation method of the plant material, the most common method for volatile oil extraction, using a Clevenger type apparatus. In this process the plant material being boiled in water, using a fire source from below the vessel. The oils were separated from the aqueous layer by using a separatory funnel and dehydrated with anhydrous sodium sulphate and stored in clean, dark brown bottles at 4°C. This process was repeated until almost all the essential oil was extracted.¹¹

Identification of Myrtle Oil Components

Myrtle oil was analyzed by Gas chromatography/Mass spectrometry (Shimadzu GCMS-QP2010Ultra), using a capillary column (HP-5MS, phenyl methyl silicon, 25 m × 0.25 mm, 1 µL injection). Helium was used as the carrier gas (1.53 mL/min).

The GC oven program was as follows: initial temperature 70°C for 3 min, programmed rate 12°C/min up to 240°C. The injector temperature was 240°C. For MS detection, an electron ionization system with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 240°C. The identification of the compounds was based on the comparison of their relative retention time and mass spectra with those from (Wiley/ChemStation data system).^{12,13,29}

Determination of Antimicrobial Activity of Myrtle Oil

Microbial Strains

The essential oil was tested against four clinical isolated strains provided by the Laboratory of clinical Laboratory sciences department, Al mustansiriah Pharmacy College. The identity of the microorganisms used in this study

(*Staphylococcus aureus*, *Streptococcus pyogen*, *Pseudomonas aeruginosa*, and *Candida albicans*) was confirmed by standard biochemical tests and morphological studies. *Staphylococcus aureus*, *Pseudomonas aeruginosa* were grown on nutrient agar, *Streptococcus pyogen* was grown on brain heart infusion agar, and *Candida albicans* were cultured on yeast malt agar plates. Microbial suspensions were then made from the agar plates using sterile relevant broths at a concentration was adjusted to 0.5McFarland standards, of approximately 10⁶CFU/mL.^{14,15}

Disc Diffusion Method

The fresh oil was tested for its antimicrobial activities. The disc diffusion method was used for antimicrobial screening as follows:

A Sterile Mueller-Hinton agar medium was used for the antimicrobial assay of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogen*, and Sabouraud agar for the *Candida albicans*. The media were prepared and allowed to solidify in the plates and then 0.1 mL of the microbial suspension (10⁶CFU/mL) was streaked over the surface of the medium using a sterile glass spreader.³¹ The well were made by using cork borer (6mm) under aseptic conditions and then 50 µL from each of the oil dilutions were put on each well. The plates were then incubated for 24-48 hours at 37°C in order to get reliable microbial growth. Microbial inhibition zones were measured using ruler.^{18,30}

Determination of λ max of Myrtle Oil

The ultraviolet (UV) absorbance for myrtle oil was examined in phosphate citrate buffer solution (pH5.5) and in absolute ethanol between 200 and 400 nm by UV-Visible spectrophotometer and maximum absorption (λmax) values were determined and further used for plotting calibration curve of the oil in these two media.¹⁷

Formulation of Myrtle oil Emulgel Formulas

The detailed composition for the emulgel formulas is given in Table 1. The formulations F1 and F2 were prepared by dispersing methyl cellulose in heated purified water (80°C), and the dispersion was cooled to room temperature and left overnight to ensure hydration of the gel While F3 was prepared by dispersing carbomer 940 in purified water with continuous stirring, F4, F5, F7 and F8 were prepared by dispersing xanthan gum in purified water with continuous stirring. F6 was prepared by dispersing HPMCK15M powered in heated distilled water (75°C) and the dispersion was cooled and left overnight.²⁰ In case of carbopol 940, triethanol amine should added drop by drop to adjust the pH of the gel.²¹

The oil and aqueous phases of o/w emulsion were prepared separately as follows, the oil phase was prepared by mixing myrtle oil with olive oil with certain amount of span 80, whereas the aqueous phase was prepared by dissolving the needed quantity of tween80 in the Purified water, the quantity of surfactants used



according to HLB theory. Methyl paraben and propyl paraben (preservatives) were dissolved in propylene glycol and mixed with aqueous phase, then Sodium meta bisulfate (antioxidant) added to the aqueous phase.

Oily phase was heated to 70 ± 1 °C. At the same time, aqueous phase was heated to 75 ± 1 °C.

Next, the aqueous phase was added to the oil phase in a drop-to-drop basis with continuous stirring until cooled to room temperature.

Finally Incorporation of emulsion into gel base by mixing the both gel and emulsion in 1:1 ratio with continuous stirring.¹⁶

Characterization and Evaluation of Myrtle oil Emulgel Formulas

Physical Appearance

All the prepared emulgel formulations were inspected visually for their color, homogeneity, consistency, grittiness and phase separation.²⁴

pH Determination

The pH of all emulgel formulations was determined using pH-meter by putting the tip of the electrode into the emulgel without dilution and after (2 min) the result was recorded.^{2,23}

In vitro Dissolution Test of Myrtle Oil Emulgel

The *in vitro* release of myrtle oil from emulgel formulas was performed by modified method using dissolution apparatus (paddle type) and dialysis membrane (M.WT 12000).

The membrane was soaked in phosphate citrate buffer of pH 5.5 for 24 hours and opened from both sides and then one of the parties was tightly closed by elastic rubber.

A 2 gm of emulgel (that contain 80mg of myrtle oil) was placed inside the membrane and then the free end of membrane was closed by another rubber.

The membrane was fixed around the paddle of the USP dissolution test apparatus and immersed in the dissolution jar (previously filled with 500ml of phosphate citrate buffer (pH 5.5) at 32 ± 0.5 °C with stirring rate of 50 rpm.^{25,33}

Samples of 5 ml were taken at intervals of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min.

The samples were filtered through a filter (0.45 µm, millipore) and analyzed at λ_{max} of myrtle oil using UV-visible spectrophotometer.

The drug release experiments were conducted in triplicate (n = 3).

Skin Irritation Study

After selection the best formula which gave the highest percentage of release. The selected formula was applied on the properly shaven skin of three rats and its adverse

effects like alteration in color, alteration in skin morphology should be checked after 24 hrs then the procedure repeated after one week to ensure that there is no sensitivity to the emulgel formula.^{17,32}

Viscosity Study

The viscosity of the selected formula F5 was carried out with Brookfield Digital viscometer (LV DVE model) using S-64 spindle number.

100gm of the sample was placed in a glass container and the viscosity measured at different rates 2, 2.5, 5, 10, 12, 20, 30, 50, 100 rpm, the temperature was maintained at 37° C. The viscosity was read directly after 30 seconds.³⁴

In vitro Antimicrobial Activity

The optimum selected formula was tested for its antimicrobial activities.

The disc diffusion method was used for antimicrobial screening as follows: A Sterile Mueller Hinton agar medium was used for the antimicrobial assay of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogen*, and Sabouraud agar for the *C. albicans*.

The media were prepared and allowed to solidify in plates and then 0.1 mL of the microbial suspension (10^6 CFU/mL) was streaked over the surface of the medium using a sterile glass spreader.

The well were made and the size used was 6mm under aseptic conditions and then sufficient amount of the formula was added into the pore.

The plates were then incubated for 24- 48 hours at 37°C in order to get reliable microbial growth. Microbial inhibition zones were measured measured in millimeters using a ruler. All tests were performed in triplicate.²⁶

RESULTS AND DISCUSSION

Identification of Myrtle Oil Components

Extraction of the essential oil from the *Myrtus communis* L. leaves produced a light yellow essential oil at 0.55 % yield. The components of myrtle oil can be identified by The GC/MS instrument. Approximately 19 compounds, representing 99.487% area of the essential oil, were identified by GC-MS analysis of myrtle oil revealed that the major constituents of the oil were: alpha Pinene (32.65.1%), 1,8Cineole (25.1%), Linalool (16.37%), Eugenol (6.56%). The components of myrtle oil were illustrated in Table 2.

Antimicrobial activity of myrtle oil

In vitro antibacterial activity of M. communis EO was assessed by the disc diffusion method against three strains of bacteria: *Staphylococcus aureus*, *Streptococcus pyogen*, *Pseudomonas aeruginosa* and against *candida albican* was expressed as diameter of the inhibition zones Table 3. Myrtle oil has a good antibacterial and antifungal activities as documented in many published papers which



conclude that the antimicrobial activity of myrtle oil is related to its components, each one of these components has a specific mechanism contribute to its antimicrobial activity.^{18,27}

Determination Myrtle Oil λ max

The λ max of myrtle oil in phosphate citrate buffer solution (pH5.5) and in absolute ethanol was 280 nm which is closely related to the λ max of Eugenol.³⁵

Characterization of Myrtle Oil Emulgel Formulas

Physical Appearance

It was obtained that liquid paraffin emulgel prepared formulas were generally white creamy appearance with good consistency while olive oil emulgel prepared formulas has light yellow appearance with smooth and excellent homogenous consistency. Carbopol 940 based emulgel formulas gave formulations very thick than other formulas.

pH Determination

The pH values of all prepared formulas ranged from 5.17 to 6.62 and this matched the requirements for topical preparations to avoid skin irritation.

Table 1: Main Compositions of Myrtle Oil Emulgel Formulas

Emulgel formulas	Myrtle oil	Methyl cellulose	Carbopol 940	Xanthan gum gumgum	HPMCK15M	Propylene glycol	Span 80	Tween 80	Olive oil	Methyl paraben	Propyl paraben	Sodium meta bisulphate	Water
F1	4	3.5				5	0.5	1.5	8	0.03	0.01	0.1	100
F2	4	3.5				5	1	3	8	0.03	0.01	0.1	100
F3	4		1			5	1	3	8	0.03	0.01	0.1	100
F4	4			1		5	0.5	1	8	0.03	0.01	0.1	100
F5	4			1		5	1	3	8	0.03	0.01	0.1	100
F6	4				2	5	1	3	8	0.03	0.01	0.1	100
F7	4			2		5	1	3	8	0.03	0.01	0.1	100
F8	4			1		5	1	3	16	0.03	0.01	0.1	100

Table 2: The Components of Myrtle Oil.

Peak	Ret Index	R. time	Components	Area %
1	920	4.892	Butyl isobutyrate	1.83
2	948	5.407	alpha-Pinene	32.65
3	943	6.050	beta-Pinene	0.45
4	958	6.227	beta.-Myrcene	0.38
5	969	6.508	alpha.-Phellandrene	0.12
6	919	6.629	4-Carene	0.20
7	1059	7.017	Cineole	25.1
8	976	7.223	Ocimene	0.33
9	1018	7.442	D-Limonene	0.47
10	1052	7.955	Terpinolene	0.51
11	1082	8.183	Linalool	16.37
12	1123	8.3	cis-Linaloloxide	0.35
13	1392	8.66	Eugenol	6.56
14	1143	10.188	alpha.-Terpineol	0.21
15	1270	10.583	Lavandulol	5.09
16	1386	11.586	Aromadendrene	1.90
17	1352	12.042	Geraniol acetate	2.73
18	1579	12.136	alpha.-Humulene	2.14
19	1507	12.411	Caryophyllene oxide	2.08

Table 3: Antibacterial inhibition Zone Diameter of Myrtle Oil Including the Size of the Borer

Sample Code	Conc. mg/ml	Zone of Inhibition (mm)			
		<i>Staphylococcus aureus</i>	<i>Streptococcus pyogen</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albican</i>
1	500	31mm ± 0.6	29mm ± 1.6	28mm ± 0.6	47mm ± 1.8
2	250	29mm ± 0.9	27mm ± 1.9	26mm ± 0.7	36 mm ± 1.4
3	125	27mm ± 1.2	25mm ± 1.5	23mm ± 0.9	31 mm ± 1.9
4	62.5	24mm ± 1	22mm ± 2	21mm ± 0.4	25mm ± 1.5
5	31.25	21mm ± 0.9	17mm ± 1.6	–	22mm ± 1.2
6	15.625	16mm ± 0.5	–	–	*
DMSO		–	–	–	–

(values are mean ± SD) (n=3); _ mean no inhibition; * Mean not tested; All the result including the diameter of the disc

Table 4: Antimicrobial Inhibition Zone Diameter of F5

	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogen</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albican</i>
F5	21mm ± 1.4	20mm ± 2	17mm ± 1.7	25mm ± 0.9
C	-	-	-	-

(Values are mean ±SD) (n=3); C (negative control) =Formula (F5) without myrtle oil; (-) Mean no inhibition zone.

All the result including the diameter of the disc (6mm)

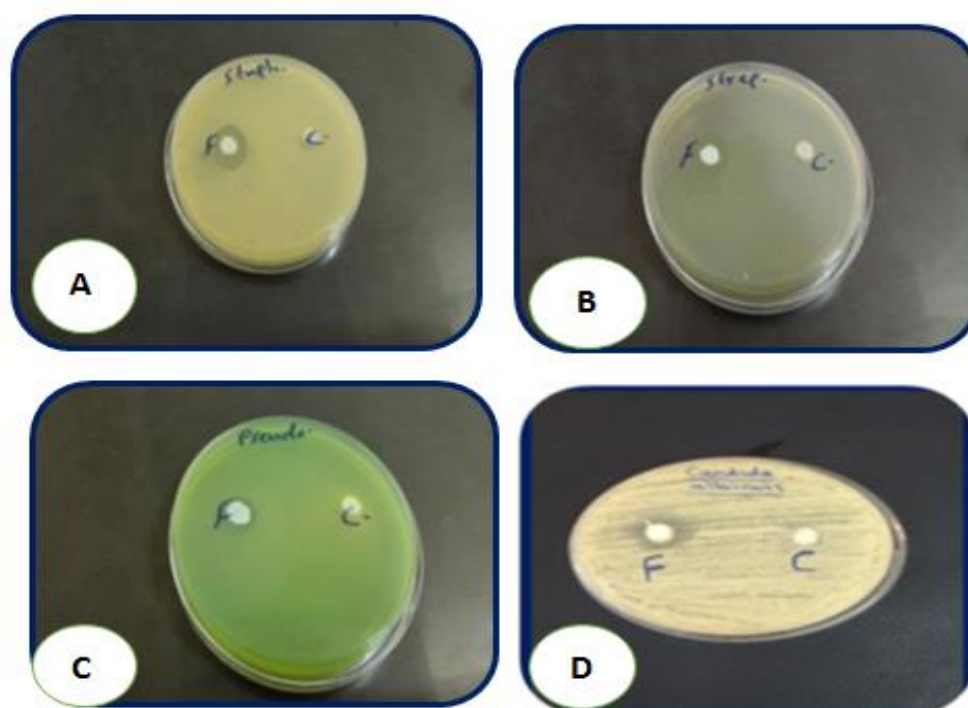


Figure 3: Photographs of zones of inhibition of F18 against (A) *Staphylococcus aureus*, (B) *Streptococcus pyogen*, (C) *Pseudomonas aeruginosa*, (D) *Candida albican*

In vitro Dissolution Test of Myrtle Oil Emulgel

Effect of Concentration of the Oil used in the Preparation of the Emulgel

It was observed that by increasing the concentration of olive oil from 8% in F5 to 16% in F8 resulted in significant decrease in the release of myrtle oil after 6 hrs ($p < 0.05$)

as shown in Figure 1, these results may be explained according to the concept of escaping tendency of drugs, i.e., that increase the concentration of the oil result in increased the entrapment of the drug within the formula with subsequent reduction in drug release rate and extent.^{22,28}

Effect of the Type of Gelling Agents with each of the Utilized Oil

The effect of the gelling agents on the release of myrtle oil was shown in the Figures 1. It was observed that there was a significant increase ($p < 0.05$) in the amount of Myrtle oil released after 6 hrs. from F5 as compared with F2, F6 and F3. The order of the release in the olive oil formulas was F5 ($97.3 \pm 0.3\%$) > F2 ($85.1 \pm 0.2\%$) > F6 ($78.6 \pm 0.1\%$) > F3 ($46.9 \pm 0.5\%$).

Effect of Concentration of Gelling Agent

F5 and F7 were exploited to study the significance of increasing the amount of xanthan gum, in which 1% and 2% were used respectively. The result showed a significant ($p < 0.05$) decreasing in the release of Myrtle oil due to increasing the viscosity of the formula with increasing the amount of xanthan gum as shown in Figure 1.

Effect of the Total Amount of Surfactant

The effect of increasing the concentration of surfactants (span80 and tween80) from 2% in (F4) to 4% in (F5) lead to significant increasing ($p < 0.05$) in the amount of myrtle oil released after 6hrs as shown in Figure 1. This effect may be referred to the ability of these emulsifying agents to lower the interfacial tension between oil and aqueous layer in the dispersion medium indicating an increasing the hydrophilicity of emulgel which in turn increase penetration of dissolution medium into the emulgel structure and then increasing the amount of myrtle oil released.²²

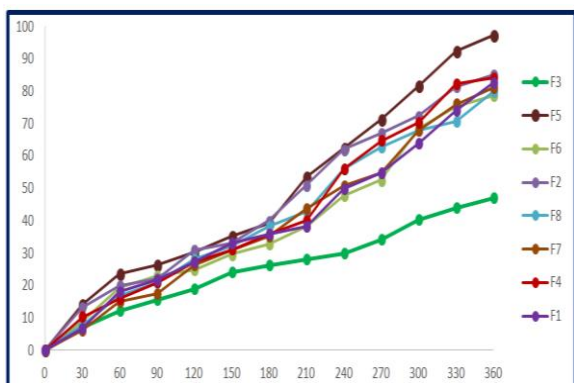


Figure 1: Release Profile of Myrtle Oil from the Formulas

Skin Irritation Study

The results of skin irritation test of the optimized selected emulgel formula (F5), there is no irritation signs on the rat skin like erythema, edema and ulceration after application of the F5 and monitoring of the irritation signs for 24hrs and after repeated the application of the formula after one week that mean there is no sensitivity reactions to the formula.

Viscosity Study

Viscosity of the optimum formula was measured at different speed rates and rheological profile was

generated as shown in Figure 2. The rheological behavior of formula (5) indicated that the systems were non-newtonian in nature showing shear thinning and decrease in the viscosity with increasing speed rates. This pseudo plastic rheology behavior of formulation is useful for topical application of emulgels.

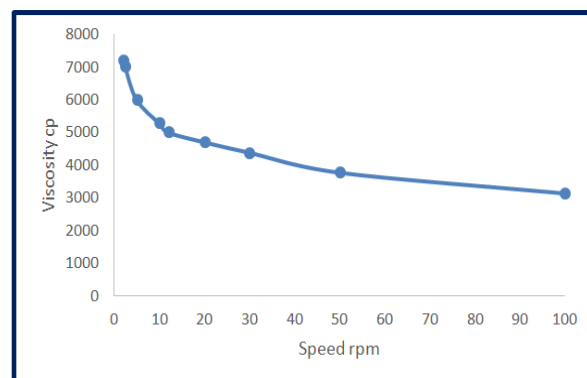


Figure 2: Rheological Profile of Formula 18.

In vitro Anti-Bacterial and Antifungal Activity

Formula (5) exhibited good antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* and good antifungal activity against *Candida albicans*, the diameter of inhibition zones excluding the size of the borer are illustrated in Table (4).

Figure 3(A, B, C, D) show the inhibition zones of F5 against the previous mentioned bacteria and fungi respectively.

CONCLUSION

Herbal emulgel formulations containing myrtle oil were prepared by two steps: emulsion preparation and then incorporation into gel.

Emulgel Formulas containing myrtle oil were successfully prepared with good physical properties, composed of olive oil as oil phase with (span 80 and tween 80) as emulsifying agents in addition to the gel phase which is composed of either Methyl cellulose or Carbopol 940 or Xanthan gum or HPMC as gelling agent.

The emulgel formula (F5), which has xanthan gum as a gelling agent was chosen as an optimized formula among all of the prepared formulas due to the excellent consistency, homogeneity, spreading properties and the highest percentage of drug release after six hours.

REFERENCES

- 1) Aulton M.E., Taylor K.M. *Aulton's Pharmaceutics: The design and Manufacture of Medicines*. 4th edition. London: Churchill Livingstone; 2013.
- 2) Alekha K.D., Somnath S., Justin T. *Pharmaceutics: Basic principle and application to pharmacy practice*. United States of America: Academic Press publications; 2014, 241-252.
- 3) Sagiri S. S., Singh V.K., Mallick S.P., Arfat A., Al-Zahrani S.M., Pradhan D.K., Pal K. Thermal, Mechanical and Electrochemical Characterization of Gelatin-Based Physical Emulgels, *International Journal of Electrochemical Science*, 10, 2014, 1233–1248.

- 4) Gameda N, Urga K, Tadele A, Lemma H, Melaku D, Mudie K. Antimicrobial Activity of Topical Formulation Containing *Eugena Caryophyllata* L. (Krunfud) and *Myrtus communis* L. (Ades) Essential oil on Selected Skin Disease Causing Microorganism, Ethiop J. Health Sci., 18, 2008, 101-107.
- 5) Celikel N, Kavas G. Antimicrobial Properties of Some Essential Oils against Some Pathogenic Microorganisms, Czech J. Food Sci, 26, 2008, 174–181.
- 6) Bajalan I, Akbarzadeh M, Veysanlu F. Chemical Composition of Myrtle Essential Oil (*Myrtus Communis* L.) in Gilane Gharb from Iran, World of Sciences Journal. 1, 2011, 59-65.
- 7) Kanoun K, Benhammou B, Ghembaza, Bekkara A. Comparative studies on antioxidant activities of extracts from the leaf, stem and berry of *Myrtus communis* L. International Food Research Journal, 21, 2014, 1957-1962.
- 8) Kamble R N, Mehta P P, Shind V M. Aromatherapy as Complementary and Alternative Medicine-Systematic Review, World Journal of Pharmaceutical Research, 3, 2014, 144-160.
- 9) Rowshan V, Najafian S, Tarakemeh A. Essential oil chemical composition changes affected by leaf ontogeny stages of myrtle (*Myrtus communis* L.), Int J Med Arom Plants, 2, 2012, 114-117.
- 10) Raymond C, George N. Natural Products Chemistry: Sources, Separations, and Structures, CRC Press Taylor & Francis Group; 2015.
- 11) Zayza foon G, Odeh A, Mahzia Y, Alla f A. Measurements of essential oil extract and antioxidants in Syrian Myrtus communis L leaves using photochemiluminescence assay, 57, 2011, 6-19.
- 12) Touaibia M. Antimicrobial Activity of The Essential Oil of *Myrtus communis* L Berries Growing Wild IN Algeria, Journal of Fundamental and Applied Sciences, 7, 2015, 150-162.
- 13) Zomorodian K, Moein M, Lori Z, Ghasemi Y, Rahimi M, Bandegani A. Chemical Composition and Antimicrobial Activities of the Essential Oil from *Myrtus communis* Leaves, Journal of Essential Oil Bearing Plants, 16, 2013, 76-84.
- 14) Keyhanfar M, Nazeri S, Bayatb M. Evaluation of Antibacterial Activities of Some Medicinal Plants Traditionally Used in Iran, Iranian Journal of Pharmaceutical Sciences, 8, 2011, 353-358.
- 15) Vinita B, Trupti B, Mitesh T, Nitin M. Khandelwal Formulation and Evaluation of Dental Gel Containing Essential Oil of Coriander Against Oral Pathogen, International Research Journal of Pharmacy, 4, 2013, 48-54.
- 16) Ambala R, Vemula S K. Formulation and Characterization of Ketoprofen Emulgels, Journal of Applied Pharmaceutical Science, 5, 2015, 112-117.
- 17) Sundari P, Mullapudi D, Srinivas P. Formulation and Evaluation of Anti-Arthritic Poly herbal Emulgel, International Journal of Pharmacy & Technology, 6, 2014, 6608-6621.
- 18) Soković M, Glamočlija J, Marin P, Brkić D, Griensven L. Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an *In Vitro* Model, Journal *Molecules*, 15, 2010, 7532-7546.
- 19) Maraie N K, Abdul-Jalil T Z, Alhamdany A T, Janabi H. Phytochemical Study of the Iraqi Beta Vulgaris Leaves and Its Clinical Application for the Treatment of Different Dermatological Disease, Word journal of pharmacy and pharmaceutical science, 3, 2014, 5-19.
- 20) Potnis V., Khot M., Kahane M., Mohite S, Bhongale P, Dhamane S. Formulation of gellified emulsions of Clotrimazole using essential oil from *Lavendula angustifolia* Miller, World Journal of Pharmaceutical Sciences, 2, 2014, 1316-1322.
- 21) Basma I A., Nawal A R. Preparation and *in-vitro* Evaluation of Mucoadhesive Clotrimazole Vaginal Hydrogel, Iraqi J Pharm Sci, 1, 2014, 19-25.
- 22) Al-Saraf M.F., Khalil Y. I. Formulation and Evaluation of Topical Itraconazole Emulgel, International Journal of Pharmacy & Therapeutics, 7, 2016, 9-17.
- 23) Bhanu P, Shanmugam V, Lakshmi P. Development and Optimization of Novel Diclofenac for Topical Drug Delivery, International Journal of Comprehensive Pharmacy, 9, 2011, 1-4.
- 24) Patil S S, Phutane K R, Adnaik R S, Mohite K S, Magdum C S. Novel Cosmeceutical Herbal Emulgel for Skin Care, World Journal of Pharmacy and Pharmaceutical Sciences, 3, 2014, 801-811.
- 25) Sahoo C K, Satyanarayana K, Bomma N G, Modugu K R, Nayak P K, Sarangi D. Formulation and Evaluation of Bifonazole organogel for the Application of Topical Drug Delivery System, International Journal of Medicine and Pharmaceutical Research, 1, 2013, 170-178.
- 26) Othman A, Awadh A, Fadhli E, Salama M. Topical Herbal Antimicrobial Formulation Containing *Thymus Laevigatus* Essential Oil. World Journal of Pharmaceutical Research, 3, 2014, 3693-3703.
- 27) Schelz Z., Hohmann J., Molnar J. Recent advances in research of antimicrobial effects of essential oils and plant derived compounds on bacteria, *Ethnomedicine: A Source of Complementary Therapeutics*, 661(2), 2010, 179-201.
- 28) Mohamed M I. Optimization of chlorphenesin emulgel formulation. American Association of Pharmaceutical Scientists, 6, 2004, 81-87.
- 29) Alves S, Luiz L, Paula M, Vieira R F, Ferri P H, Oliveira R. Chemical variability of the essential oils from fruits of *Pterodon emarginatus* in the Brazilian Cerrado, Brazilian Journal of Pharmacognosy, 23, 2013, 224-229.
- 30) Celiktas O.Y., Kocabas E.E., Bedir E., Sukan F V, Ozek T, Baser K. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations, Food Chemistry, 100, 2007, 553–559.
- 31) Ansiya M.A., Krishnakumar K., Anish J., Dineshkumar B. Emulgel with Herbal Essential Oil: A Review, Health sciences: An International Journal, 5, 2015, 1-5.
- 32) Marie K N, Al-Khateeb H E, Al-Waiz M. Topical preparations from the Iraqi plant aloe vera and their efficacy in skin infections, AJP, 2, 2005, 7-15.
- 33) Singh P, Kalia S, Bala R, Gill N. Development and Characterization of clindamycine phosphate emulgel for topical delivery, International Journal of Recent Advances in Pharmaceutical Research, 4, 2014, 47-62.
- 34) Priani S, Humanisya H, Dariusman F. Development of Sunscreen Emulgel Containing Cinnamomum Burmannii Stem Bark Extract, International Journal of Science and Research, 3, 2014, 2338-2341.
- 35) Moffat A, Osselton M., Widdop B. Clarke's Analysis of Drugs and Poisons 4th edition. London: Pharmaceutical Press; 2011, 1381.

Source of Support: Nil, Conflict of Interest: None.

