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Formulation and pharmaceutical evaluation of a W/O emulsion of *Hippophae rhamniodes* fruit extract

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ABSTRACT

The aim of this study was to formulate and evaluate a new formulation of organic fruit extract of Hippophae rhmanoides. 25 formulas of w/o emulsions consisting of varuios concentration of oily phase, emulsifyer and aquaous phase were first formulated and then evaluated for various parameters such as colour, smell, type of emulsion, viscosity, electrical conductivity, centrifugation at 5000 rpm, liquefaction and pH using appropriate equipments. The stable formulation consisted of 1% Fruit extract of Hippophae rhmanoides, 16% mineral oil, 5% ABIL EM90, 1% lemon oil and 77% distilled water. All the results derived from this study showed good stability over the 4 week study period.

Keywords: *Hippophae rhamnoides*, w/o Emulsion, fruit extract, rheology.

INTRODUCTION

The formulation of liquid-liquid emulsion is a common practice in food and pharmaceutical industries. Incorporating plant extract in the form of emulsion is currently drawing more attention in the field of research due to their therapeutic importance. [1] The main advantage of emulsions is that they increase the solubility and bioavailability of therapeutic drugs as well as the ability to favor the topical transport of hydrophilic solute. Topical emulsions also avoid gastrointestinal environment and first pass [2]. ABILE EM 90 is a suitable surfactant for the formulation of water-in-oil emulsions, w/o/w and o/w/o multiple emulsions. Preparations with ABIL EM90 are highly stable towards heat. [3] Now a day, herbal extracts are used in the cosmetic preparations for augmenting beauty and attractiveness. [4] The use of cosmetics requires both their efficacy as well as minimal risk of skin irritation/skin sensitization. This is influenced by their formulation, nature of their use and quantity and quality of ingredients. [5] Hippophae rhamnoides is a deciduous, dioecious plant with numerous greenish-yellow flowers and bright orange, globular, ellipsoid fruit belongs to family Elaeagnaceae. [6] Hippophae rhamnoides juice is an important source of some valuable chemicals such as vitamin C, tocopherolmacrotrients, organic acids and polyunsaturated fatty acids. [7] Hippophae rhamnoides has been used for the treatment of radiation damage, inflammation, burns, eczema, psoriasis, lupus erythematosus and dermatosus. [8, 9] The shelf life is the time period for which a drug can be stored before it becomes unfit for use due to chemical decomposition/physical deterioration. [5] In this study we formulated a w/o emulsion of natural extract and investigated its pharmaceutical evaluation over a 4 weeks study period as from commercial point of view it is important that new products should be marketed as quickly as possible however sufficient stability profile at ambient temperature and under varying external influences must be provided [10].

MATERIALS AND METHODS

Materials

Hippophae rhamnoides berries were purchased from Pak Sea Buckthorn International Skardu, Pakistan. ABIL-EM90 was purchased from Franken Chemical (Germany) and Methanol, n. Hexane & paraffin oil were purchased from Merk KGaA Darmstadt (Germany). Ethanol was taken from BDH England.

Apparatus

RV III Disc Spindle Rheometer (Brookfield USA), Centrifuge Machine (Hettich EBA 20, Germany), Cold Incubator (Sanyo MIR-153, Japan), Conductivity-Meter (WTW COND-197i, Germany), Digital Humidity Meter (TES Electronic Corp, Taiwan), Electrical Balance (Precisa BJ-210, Switzerland), Homogenizer (Euro-Star, IKA D 230, Germany), Hot Incubator (Sanyo MIR-162, Japan), PH-Meter (WTW pH-197i, Germany), Refrigerator (Dawlance, Pakistan), Rotary evaporator (Eyela, Co. Ltd. Japan).

Methods:

Preparation of Extract and Emulsions

Hippophae rhamnoides berries, purchased from Pak Sea Buckthorn International Skardu, Pakistan were extracted in methanol: water (1:1) and methanol: n-hexane: water (1:4:1). 25 formulations of W/O emulsion were prepared with various concentration of surfactant (ABIL- EM90), liquid paraffin and distilled water as shown in table 1. All these formulations were observed with respect to color, phase separation and liquefaction for 25 days while keeping them at 25°C in incubator. The formulations BA6, BA16 and BA21 were found stable at 25°C. 4 samples of each of these three formulations were studied further for 21 days while keeping them at 8°C, 25°C, 40°C and 40°C + 75% RH. The sample BA21 was found stable at all 4 storage conditions and selected for further in-vitro study. It was observed for 4 weeks with respect to color, smell, type of emulsion, viscosity, electrical conductivity, centrifugation, liquefaction and pH.

The oily phase consisting of paraffin oil and emulsifying agent (ABIL- EM 90) was heated up to 70±1ºC. The aqueous phase, distilled water was heated to the same temperature and then H.rhamnoides extract was added to it. The aqueous phase was then added to the oily phase drop by drop and stirred until all aqueous phase was added; lemon oil was added during this stirring time to give

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Table 1: The composition of 25 formulations of w/o emulsions.

Formulation code	ABILE EM90 (%)	Liquid paraffin (%)	D/W (%)	Active ingredient (%)	Lemon oil (%)
BA1	3	16	79	1	1
BA2	3	18	77	1	1
BA3	3	20	75	1	1
BA4	3	22	73	1	1
BA5	3	24	71	1	1
BA6	3.5	16	78.5	1	1
BA7	3.5	18	76.5	1	1
BA8	3.5	20	74.5	1	1
BA9	3.5	22	72.5	1	1
BA10	3.5	24	70.5	1	1
BA11	4	16	78	1	1
BA12	4	18	76	1	1
BA13	4	20	74	1	1
BA14	4	22	72	1	1
BA15	4	24	70	1	1
BA16	4.5	16	77.5	1	1
BA17	4.5	18	75.5	1	1
BA18	4.5	20	73.5	1	1
BA19	4.5	22	71.5	1	1
BA20	4.5	24	69.5	1	1
BA21	5	16	77	1	1
BA22	5	18	75	1	1
BA23	5	20	73	1	1
BA24	5	22	71	1	1
BA25	5	24	69	1	1

Table 2: Physical Charactristics of emulsion Kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH

good fragrance. Base was also prepared by the same method and with same ingredients but without H.rhamnoides extracts i.e. the active ingredient.

RESULTS AND DISCUSSION

Physical characteristics

Color and Smell

All the 4 samples of emulsion kept at 8°C, 25°C, 40°C and 40°C + 75% RH were studied by visualization and physical characteristics regarding the stability of emulsion is presented in Table 2. The emulsion presented a homogenous feature with characteristics yellowish color as the active ingredients contain caretenoids.No change in color may be attributed to different factors contributing to the emulsion stability such as component of oil phase; paraffin oil is a transparent, colorless, viscous oily liquid and As the active ingredient i.e. H.Rhamniodes fruit extract contains polyphenols which are well documented to have microbicide activities against huge number of bacteria.[11,12], thus it may protect the formulation components from microbial growth of those organisms which might produce such substances which are able to change

Table 3: pH Values of emulsion kept at 8°C, 25°C, 40°C and 40°C + 75% RH for a period of 28 days.

Time	8°C	25°C	40°C	40°C+ 75% RH
0 Hour	5.18	5.18	5.18	5.18
12 Hours	5.15	4.80	4.75	4.91
24 Hours	4.18	4.14	4.22	4.65
36 Hours	4.16	4.43	4.10	4.58
48 Hours	4.10	4.16	4.08	4.25
72 Hours	4.49	4.47	4.05	4.18
7 Days	4.40	4.01	4.45	4.10
14 Days	4.25	4.17	4.30	4.22
21 Days	4.35	4.23	4.11	4.15
28 Days	4.40	4.30	4.05	4.25

the color of the formulation during the storage time. The smell reduced slightly at the end of study period. This may be due to the volatilization of Lemon oil.

Liquefaction

A slight liquefaction was observed in the sample kept at 40°C on 21st and 28th days of the study period of 28 days while no liquefaction was observed in any of the sample kept at 8°C, 25°C, and 40°C + 75% RH during whole study period of 28 days. The slight liquefaction may be because as soon as an emulsion is prepared time and temperature dependent process occurred leading to decreased viscosity which results in increased liquefaction. [13]

Phase Separation

All the samples were stable at all storage conditions i.e. 8°C and 25°C and 40°C and 40°C+ 75% RH throughout the study period of 28 days. No oily phase separation was observed even at higher temperatures during the study period. No phase separation may be attributed to a number of stability factors like using ABIL EM 90, a heat stable emulsifying agent. [14]

Determination of type of emulsion

Type of the emulsion was determined to confirm whether the type of emulsion prepared is based on the number of w: o phase ratio. A certain amount of emulsion was taken in test tube and diluted with certain amount of water. The emulsion was immiscible and did not dilute with water confirming this emulsion was w/o type.

Centrifugation Test

Centrifugation is based on the principle of centrifugal force to sepa-

Table 2: Physical Charactristics of emulsion Kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH

		Fresh	After 12 hrs	After 24 hrs	After 36 hrs	After 48 hrs	After 72 hrs	After 7days	After 14days	After 21days	After 28days
Color	**A**	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	B	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	C	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	D	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Smell	**A**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Liquefaction											
	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Phase Separation	**A**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Y=Yellow, -ve =No change, +ve = Slight change;
A=At 8°C; B=At 25°C; C= At 40°C; D= At 40°C+75%RH (Relative Humidity).

Table 4: Viscosity Values (cP) of emulsion kept at 8°C, 25°C, 40°C and 40°C + 75% RH for a period of 28 days.

Time	8°C	25°C	40°C	40°C+75% RH
0 Hour	480.15	480.15	480.15	480.15
12 Hours	478.92	480.03	480.15	479.26
24 Hours	480.05	480.50	479.95	481.02
36 Hours	480.20	481.25	480.08	479.56
48 Hours	479.85	481.00	477.62	478.48
72 Hours	480.05	480.98	473.20	480.25
7 Days	478.55	481.92	473.55	477.51
14 Days	478.69	482.07	471.25	480.94
21 Days	479.15	479.68	473.42	480.94
28 Days	479.15	480.05	472.80	479.05

rate two liquids on the basis of their densities. It is a useful tool for evaluating and predicting the shelf life of emulsions. [13] In this study centrifugation test AT 5000 RPM was performed (Hettich EBA 20, Germany) for all the 4 samples kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH up to a period of 28 days immediately after preparation and then repeated after 12, 24, 36, 48, 72 hours and 7, 14, 21 and 28 days. No phase separation on centrifugation was observed in any of the samples kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75%RH up to 28th day of observation. This indicated that the emulsions were stable at all the storage conditions for 28 days. It may be due the proper homogenization speed during emulsion formulation prevented the base and the formulation breakage during stress testing. [15]

pH Tests

The pH of the prepared emulsion was measured for all the 4 samples kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH by using digital PH-Meter (WTW pH-197i, Germany) immediately after preparation and then repeated after 12, 24, 36, 48, 72 hours and 7, 14, 21 and 28 days. Each sample was triplicately done. The pH of freshly prepared emulsion and after storage for 28 days showed some decline as shown in the Table 3.The decline in pH may be because of the production of acidic species as the juice of H.rhamnoides is very acidic and has high concentration (30-36 mg/1000 gm) of organic acids such as quinic acid (18-19 gm/1000 gm). [16]

Conductivity

Conductivity is mostly used to determine the nature of an emulsion and to control its stability with the passage of time as it allows the detection of creaming, sedimentation and phase inversion The conductivity of the prepared emulsion was measured by Conductivity-Meter (WTW COND-197i, Germany) for all the 4 samples kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH immediately after preparation and then repeated after 12, 24, 36, 48, 72 hours and 7, 14, 21 and 28 days. Each sample was triplicately done. No electrical conductivity was seen in any of the samples kept at 8°C, 25°C and 40°C+ 75% RH. No conductivity is attributed to the use of non ionic emulsifying agent, ABIL EM90 as well as the non-conductive nature of the oily phase. However slight conductivity (Average 0.005 µS/cm) was noted at the end of study period in the sample kept at 40°C that may be due the slight liquefaction, appearance of aqueous phase and production of acidic species.

Viscosity

Viscosity plays very important role in the flow properties of emulsions. [17] Viscosity was determined with the help of RV III Disc Spindle Rheometer (Brookfield USA) for all the 4 samples kept at 8°C, 25 °C, 40 °C and 40 °C +75%RH immediately after preparation and then repeated after 12, 24, 36, 48, 72 hours and 7, 14, 21 and 28 days. Each sample was triplicately done. The viscosity of freshly prepared emulsion was not different from the viscosity of emulsion after 28 days storage at 8°C, 25 °C and 40 °C +75%RH however slight reduction was observed in the viscosity of emulsion kept at 40 °C as shown in the table 4.Stable viscosity indiates no flocculation thuogh slight liquefaction (reduced viscosity) was observed in the sample kept at 40 °C.

CONCLUSION

From the present findings we concluded that;the w/o emulsion with the extract of Hippophae rhamnoides in concentration of 1% showed good physical characteristics and pharmaceutical stability providing a novel emulsion delivery system for various skin diseases. However further in-vivo study has to be performed to evaluate this emulsion for the cosmetic market. Our investigations have been proved to be promising in terms of future potential applications of Hippophae rhamnoides extract, as skin-care products, cosmetics and/or pharmaceutical preparations owing to these properties.

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