

## Development, characterization and antioxidant activity of polysorbate based O/W emulsion containing polyphenols derived from *Hippophae rhamnoides* and *Cassia fistula*

Barkat Ali Khan<sup>1,\*</sup>, Naveed Akhtar<sup>1</sup>, Haroon Khan<sup>2</sup>, Valdir de Andrade Braga<sup>3,\*</sup>

<sup>1</sup>Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan, <sup>2</sup>Department of Pharmaceutical Chemistry, Gomal University, Pakistan, <sup>3</sup>Department of Biotechnology, Biotechnology Center, Federal University of Paraíba, Brazil

The objective of this study was to develop a pharmaceutical O/W emulsion containing plant-derived polyphenol extracts and evaluate its stability and antioxidant activity. O/W emulsions were prepared using ionic surfactant polysorbate 80 (Tween 80<sup>®</sup>). The odor was adjusted with few drops of blue sea fragrance. DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to evaluate the antioxidant activity of the plant extracts alone and emulsions containing these extracts. Physical stability was assessed by submitting the emulsions to storage at 8 °C, 25 °C, 40 °C and 40 °C + 70% RH (relative humidity) for two months. Various physical characteristics of emulsions monitored, include color, creaming, liquefaction, centrifugation and pH. Brookfield rotational rheometer was used to determine viscosities and rheological behavior of emulsions. Different types of emulsion were determined microscopically, while pH values of emulsions were measured by a pH meter. Electrical conductivity data confirmed that the outer phase was water. Samples presented an acceptable pH value for an external topical use. Shear thinning behaviour was observed for all emulsions. The polyphenol-rich-plant-derived extracts alone and the extract containing emulsions showed good antioxidant activities. This research confirmed that the method used was suitable for preparing emulsions with *Hippophae rhamnoides* and *Cassia fistula* extracts, suggesting that those emulsions are suitable for topical use.

**Uniterms:** *Hippophae rhamnoides*/pharmacognosy. *Cassia fistula*/pharmacognosy. Emulsion O/W/topical use. Emulsion O/W/conductivity. Emulsion O/W/rheology.

O presente estudo objetivou o desenvolvimento de uma emulsão farmacêutica óleo-água contendo extratos de plantas ricos em polifenóis, a comparação à sua formulação-controle e a avaliação de sua estabilidade, assim como de sua capacidade antioxidante. Extrato concentrado de *Hippophae rhamnoides* e *Cassia fistula* foi encapsulado no interior da fase oleosa da emulsão O/W. As emulsões foram preparadas usando o tensoativo iônico monooleato sorbital de polioxietileno (Tween 80<sup>®</sup>). O odor foi ajustado pela adição de algumas gotas de fragrância azul do mar. O ensaio do DPPH (1,1-difenil-2-picrilidrazil) foi utilizado para avaliar a atividade antioxidante dos extratos de plantas sozinhos e nas emulsões contendo os extratos. A estabilidade física foi avaliada submetendo os cremes a diferentes temperaturas de estocagem, como a 8 °C, 25 °C e 40 °C e a 40% + 70% de umidade relativa por um período de 2 meses. As características físicas das emulsões foram monitoradas por 2 meses incluindo cor, cremosidade, liquefação, centrifugação e pH. O reômetro rotacional de Brookfield foi utilizado para determinar a viscosidade e o comportamento reológico das emulsões. O programa Rheocalc Brookfield foi utilizado para análise dos dados. As características organolépticas também foram avaliadas. O tipo de emulsão foi determinado microscopicamente, enquanto o pH das emulsões foi avaliado por meio de um pHmetro. A estabilidade farmacêutica esperada das emulsões foi alcançada dentro dos dois meses de estudo. Os resultados da condutividade elétrica confirmaram que a fase externa da emulsão era composta de água. O pH das amostras estava dentro da normalidade para uso tópico. A emulsão apresentou boa fragrância

\*Correspondence: V. A. Braga. Departamento de Biotecnologia, Centro de Biotecnologia, Universidade Federal da Paraíba, 58051-900 – João Pessoa – PB, Brasil. Email: valdir@cbiotec.ufpb.br

e pode ser retirada da pele com água após a aplicação, características desejáveis em emulsões O/W. Os extratos vegetais ricos em polifenóis isolados ou nas emulsões apresentaram boa atividade antioxidante. Nossos estudos confirmaram que o método utilizado foi adequado para preparar a emulsão semi-sólida contendo extratos de *Hippophae rhamnoides* e *Cassia fistula*. Nossos achados sugerem que emulsões contendo extratos de *Hippophae rhamnoides* e *Cassia fistula* são adequados para o uso tópico.

**Unitermos:** *Hippophae rhamnoides*/farmacognosia. *Cassia fistula*/farmacognosia. Emulsão óleo-água/uso tópico. Emulsão óleo-água/condutividade. Emulsão óleo-água/reologia.

## INTRODUCTION

Emulsions show potential applications in paint, food, cosmetic and pharmaceutical industries. There has been given special attention to the emulsion as a vehicle for carrying drugs to the body as they increase the bioavailability of those compounds (Herbert *et al.*, 1988). Oil-in-water or O/W emulsions are commonly used as water-washable drug bases and for general cosmetic purposes (Magdy, 2004). Additional value can be given to those formulations by including active ingredients with specific cosmetic effects. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients (Bleckmann *et al.*, 2006). There is a growing attention in natural antioxidants present in plants. Many antioxidant compounds are isolated from natural herbs and extracts and used as potential antioxidants in cosmetics (Naveed, 2001). Owing to their beneficial and therapeutic properties, natural plant extracts and their derived products have frequently been included in the form of emulsions in current pharmaceutical and cosmetics formulations and preparations.

The applications of topical emulsions need both their efficacy as well as nominal risk of skin irritation/skin sensitization. This is prejudiced by their formulation, nature of their use and quantity and quality of ingredients. The shelf life is the time period for which a drug can be hoard before it turns out to be unfit for use due to chemical decay/physical deterioration. *Hippophae rhamnoides* juice is a significant source of several precious chemicals such as ascorbic acid, toco-pherolmacrotrients, organic acids and polyunsaturated fatty acids (Akhtar *et al.*, 2010). Polyphenols (flavonoids) are present in *Hippophae rhamnoides*. The chief flavonoids in *Hippophae rhamnoides* are isorhamnetin, quercetin, myricetin and kaempferol. *Hippophae rhamnoides* has been used for the treatment of photo damage, inflammation and burns in Chinese folk medicines as well as in the treatment of skin ailments such as psoriasis, eczema, lupus erythematosus and dermatosis (Barkat *et al.*, 2011). *Cassia fistula* usually well-known as Indian Laburnum, is scattered in Asia, Mauritius, South Africa, Mexico,

China, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers and it is recognized by the British pharmacopoeia. The key constituents present in *Cassia fistula* are tannins, fatty acids, isoflavonoids, flavonoids, glycosides, anthraquinones and phenolic compounds (Barkat, Naveed, 2012).

Numerous plant polyphenols are value-adding chemicals and are used as constituents in cosmetics, foods and pharmaceuticals. The prospective use of phenolic compounds for the introduction of new skin care cosmetics (creams/emulsions) has been underlined (Kiken, Cohen, 2002). Plant polyphenols can be used as sunscreen, whitening and anti-aging agents (González *et al.*, 2008).

Emulsion stability is an important factor governing the shelf life of products. Predominantly, emulsions are thermodynamically unstable systems and they are predisposed to break over time due to various physicochemical mechanisms such as creaming, flocculation, coalescence, phase inversion and/or Ostwald ripening. Stability in emulsions must be considered. The present research involved the formulation development of emulsions, systematic study of the antioxidant properties of extracts alone and in emulsions and stabilization of emulsions.

## MATERIAL AND METHODS

### Chemicals

Folin Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co., Ltd (Saint Louis, USA). Polysorbate 80, Span 20, Liquid paraffin, Stearic acid, Bees wax and Cetomacrogol were taken from Merck Germany.

### Plant Materials

*Cassia fistula* samples were collected from old campus (Abbasia campus), the Islamia University of Bahawalpur, Pakistan, while *Hippophae rhamnoides* berries were purchased from Pak Sea Buckthorn International, Skardo, Pakistan. The identification was performed at

Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. The voucher specimens were deposited at the herbarium of Pharmacognosy Section, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur Pakistan.

## Apparatus

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 the Plant Extracts

Extraction of plants is described by Barkat and Naveed (2012) and shortly describe here. 150 mg of *Cassia fistula* leaves was extracted by 70% methanol for 72 h at room temperature in a 5 liters beaker in a dark room. 320 mg of *Hippophae rhamnoides* berries were compressed and successively macerated in a mixture of 2 liters of methanol and distilled water in a ratio of 1:1. The macerated plant material was filtered through 16 layers of muslin cloth for coarse filtration. The coarse filtrate was then filtered through a Whatman No. 1 filter paper in order to get particle free extracts. The filtrate was evaporated under reduced pressure at 40 °C in a rotary vacuum evaporator and stored in amber container and under refrigeration (-18 °C) until used for further analyses.

### Total Phenolics determination

Yafang *et al.* (2011) method was adopted with slight modification. 200 µL of plant extract were oxidized with Folin-Ciocalteu reagent (1 mL; 0.5 N) and the reaction was then neutralized with 1 mL of the saturated sodium carbonate (75 g/L). The absorbance of the resulting blue color solution was measured at 760 nm with spectrophotometer after incubation for 2 h at room temperature. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligram of gallic acid equivalent (mg GAE/g).

### DPPH free radical scavenging activity

Sharma *et al.* (2011) method with slight modification was adopted for free radical scavenging activity of

both, extracts alone and emulsions containing extracts. Stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493. 5 mL. Stock solutions were added to 1 mL of sample solution at different dilutions (250-1500 µg/mL). After 30 min, absorbance was measured at 517 nm spectrophotometrically. Percentage scavenging activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where  $A_0$  = absorbance of the control and  $A_1$  = absorbance of the sample. Scavenging activity was compared with ascorbic acid.

### Preparation of emulsions

O/W emulsions (control formulation and formulation containing the extracts) were prepared by the addition of the aqueous phase to the oily phase with continuous agitation. The emulsions were stabilized by Polysorbate 80 (Tween 80®) (Table I). Oily phase was heated to 70±1 °C. At the same time, aqueous phase was heated to 75±1 °C and extracts were added to the preparation. Next, aqueous phase was added to the oil phase in a drop-to-drop basis. Mixing was carried out at 41.9 g by the mechanical mixer for 20 minutes until complete aqueous phase was added. Finally, 2 to 3 drops of fragrance were added during this stirring time to give good odor to the formulations. Control formulation was also prepared by the same above method but without plant extracts (the active ingredient) and served as control.

### Characterization of emulsions

Emulsions were examined organoleptically (odor, color, thickness, look, feel) and physically (creaming/sedimentation and phase separation). Type of emulsions was determined microscopically. pH and electrical conductivity values of freshly prepared emulsions and emulsions kept at different storage conditions were determined by a digital pH-meter and digital conductivity-meter respectively. Centrifugal tests were performed for emulsions immediately after preparation. The centrifugal tests were repeated for emulsions after 24 hours, 7, 14, 21, 28 and 60 days of preparation. The centrifugal tests were performed at 25 °C and at 4192 g for 10 minutes by placing 5g of sample in disposable stoppered centrifugal tubes. Rheological parameters and viscosity of the emulsions was determined using Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories) using spindle # CPE40 at 25 ± 0.5 °C. Rheogram curves

**TABLE I** - Composition of emulsions (% w/w)

Composition	Base	Active Formulation with <i>H. rhamnoides</i>	Active Formulation with <i>C. fistula</i>
Liquid Paraffin	24	24	24
Stearic Acid	5	5	5
Span 20	2	2	2
Bees wax	7	7	7
Cetomacrogol	5	5	5
Polysorbate 80	6	6	6
Plant Extract	Nil	5	5
Mix of methyl and propyl paraben	1	1	1
D/W	50	45	45

constructed with ascendant and descendant segments were obtained with rotation speeds increasing progressively (0.1 to 0.4 g) and gradually decreasing (0.4 to 0.1 g).

#### Pharmaceutical stability tests

Stability tests were performed on samples kept at  $8 \pm 0.1^\circ\text{C}$  (in refrigerator),  $25 \pm 0.1^\circ\text{C}$ ,  $40 \pm 0.1^\circ\text{C}$  and  $40 \pm 0.1^\circ\text{C}$  (in incubator) with 75% relative humidity (RH) for 2 months.

#### Mathematical analysis

The percentage changes for the individual values of pH and conductivity, taken every week, were calculated by the following formula;

$$\text{Percentage Change} = [(A - B) / B] * 100$$

Here; **A** = Individual value of any parameter of 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> or 8<sup>th</sup> week

**B** = Zero hour value of that parameter (freshly prepared emulsions)

#### Statistical analysis

Statistical analysis was performed by using SPSS 12.0 on the PC computer. The ANOVA (analysis of variance) test was applied to determine difference of all the parameters studied at the initial and different time intervals at all storage conditions. Statistically, a significant difference was considered at a p value of less than 0.05 (5%).

#### Rheocalc software

Rheological parameters (flow index and % confidence of fit) of emulsions were analyzed by using the Brookfield software, Rheocalc.

## RESULTS

### Total phenolics contents

Total phenolic contents (TPC) determined spectrophotometrically showed adequate amount of these important contents present in extracts of *Hippophae rhamnoides* and *Cassia fistula*. Total phenolic content is directly linked with antioxidant activity. Total phenolic content, articulated in mg GAE/g of *Hippophae rhamnoides* and *Cassia fistula* are shown in Table II.

**TABLE II** - Total phenolic content (mg GAE/g) of *Hippophae rhamnoides*

Total Phenolic Content (mg GAE/g)	
<i>Hippophae rhamnoides</i>	<i>Cassia fistula</i>
51.23±1.38b	64.39±1.21a

Data are expressed as means  $\pm$  standard deviations ( $n = 3$ ); values marked by the same letter in same column of same class are not significantly different ( $P > 0.05$ ).

### DPPH free radical scavenging activity

The change in absorbency produced by reduced DPPH was used to evaluate the antioxidant ability of the plant extract and emulsions containing plant extracts. The antioxidant activity of plant extract and after addition of plant extract to the emulsions is shown in Table III.

### Type of emulsions

The amaranth red dye was mixed with the emulsions and a drop of the emulsion was placed on the microscopic slide, covered it with a cover slip, and examined it under

**TABLE III** - *In vitro* antioxidant activity ( $IC_{50}$   $\mu\text{g/mL}$ ) of plant extracts alone and emulsions containing plant extracts

DPPH radical scavenging assay			
<i>H. rhamnoides</i>	<i>Cassia fistula</i>	<i>H. rhamnoides emulsion</i>	<i>Cassia fistula emulsion</i>
107.26 $\pm$ 1.34	89.07 $\pm$ 1.31	102.49 $\pm$ 1.117	88.95 $\pm$ 1.49

Data are expressed as means  $\pm$  standard deviations ( $n = 3$ ); values marked by the same letter in same column of same class are not significantly different ( $P > 0.05$ ).

a microscope. The disperse globules appeared red and the ground colorless, indicated O/W emulsions.

### Stability studies

#### Organoleptic tests

The emulsions (control and active formulations) were divided into four samples, which were kept at 8 °C in refrigerator, at 25 °C, 40 °C and at 40 °C + 75% RH (Relative Humidity) in thermal incubators. They were organoleptically evaluated to note alteration in odor, color, liquefaction and phase separation for a period of 2 months at definite time intervals. No differences were observed in the organoleptic properties among emulsions for the two months of the experiments under different conditions as mentioned above.

#### Centrifugation

Centrifugation tests were performed at 4192 g for 10 minutes to check phase separation for 2 months at different time intervals. There was no phase separation found after centrifugation in any of the samples of emulsions.

#### Electrical conductivity

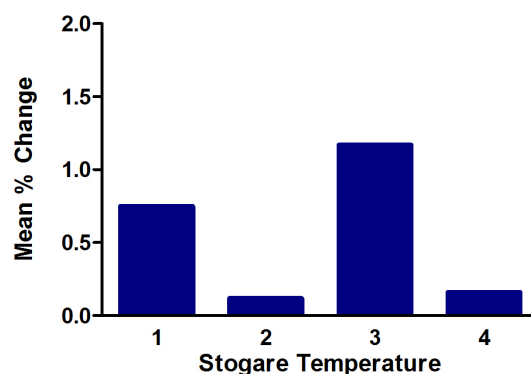
The conductivity of the prepared emulsions were measured for all the samples kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH immediately after preparation and then repeated after 12, 24, 36, 48, 72 hours and 7, 14, 21, 28 and 60 days. The test was performed in triplicate for each sample. The percentage changes in the conductivity values are presented in the Figures 1, 2 and 3.

#### pH Tests of emulsions

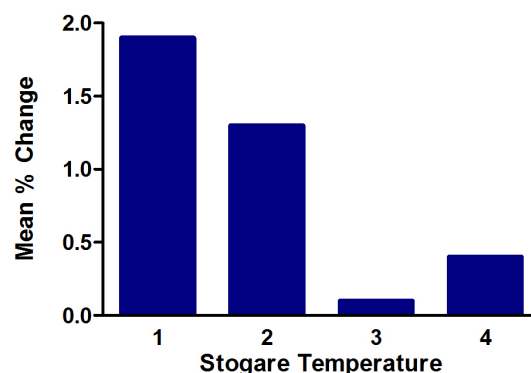
There were no significant changes in pH values of emulsions at all storage temperatures as a function of time. Minor changes of pH values as a function of time and storage temperatures indicated that the examined emulsions were stable. pH values of the emulsions kept at different storage conditions for 2 months have been determined and reported in Table IV as mean and SEM.

### Rheological parameters

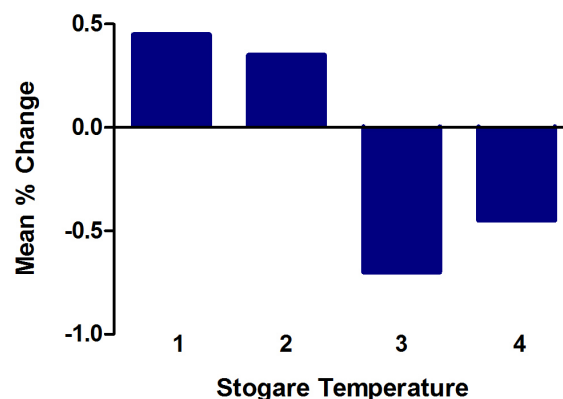
Rheological parameters (flow index and %



**FIGURE 1** - Mean % changes in the conductivity of base from zero time to 60 days. 1= 8 °C, 2= 25 °C, 3= 40 °C, 4= 40 °C + 75% RH.



**FIGURE 2** - Mean % changes in the conductivity of *Hippophae rhamnoides* emulsion from zero time to 60 days. 1= 8 °C, 2= 25 °C, 3= 40 °C, 4= 40 °C + 75% RH.



**FIGURE 3** - Mean % changes in the conductivity of *Cassia fistula* emulsion from zero time to 60 days. 1= 8 °C, 2= 25 °C, 3= 40 °C, 4= 40 °C +75% RH.



**TABLE IV** - Average pH Values of Base and Emulsions kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH for a period of 2 months

	pH (Mean ± SEM) under different storage conditions							
	8 °C		25 °C		40 °C		40 °C + 75% RH	
Base	5.20±0.08		5.4±0.55		4.83±0.89		5.35±0.11	
HR Emulsion	5.25±0.76		5.36±0.35		5.17±0.48		5.43±0.80	
CF Emulsion	5.13±0.10		5.45±0.72		5.32±0.58		5.75±0.11	

HR= *Hippophae rhamnoides* Emulsion, CF= *Cassia fistula* Emulsion

**TABLE V** - Different rheological properties of base

	Temperature															
	8 °C				25 °C				40 °C				40 °C +75%RH			
	Flow Index		C.F.		Flow Index		C.F.		Flow Index		C.F.		Flow Index		C.F.	
	HR	CF	HR	CF	HR	CF	HR	CF	HR	CF	HR	CF	HR	CF	HR	CF
Freshly prepared	0.43	0.48	99.5	99.9	0.43	0.48	99.5	99.9	0.43	0.48	99.5	99.9	0.43	0.48	99.5	99.9
2 months	0.51	0.41	99.7	99.2	0.50	0.62	99.9	99.4	0.52	0.55	99.5	99.7	0.52	0.48	98.5	98.8

C.F. = Confidence of Fit, HR= *Hippophae rhamnoides* Emulsion, CF= *Cassia fistula* Emulsion, C.F. = Confidence of Fit.

confidence of fit) of emulsions reserved at various storage conditions up to two months were observed and have been given in Tables V while Rheograms of the emulsions have been given in Figures 4, 5, 6, 7, 8 and 9.

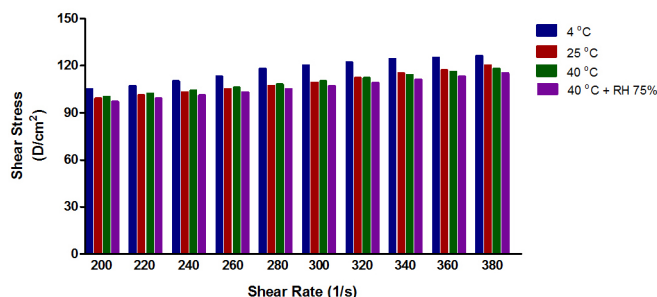
*Viscosity studies*

Mean Viscosity (cP) ± standard deviations (SD) of the emulsions studied for 2 months stored at various storage conditions are presented in Table VI.

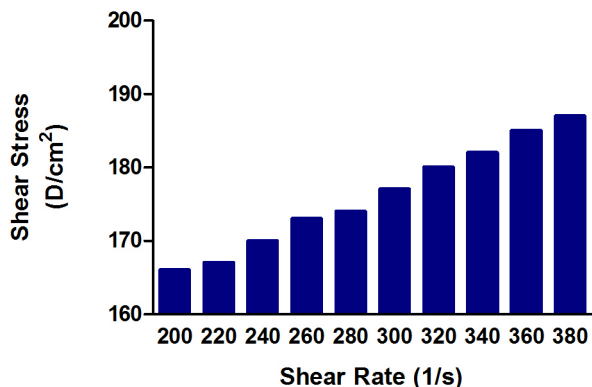
**DISCUSSION**

Topical emulsions (cosmeceuticals) are prepared to reduce wrinkles (skin aging), fight acne/melasma and to control/normalize skin sebum secretion. For various types of skin complaints, topical formulations like sunscreen,

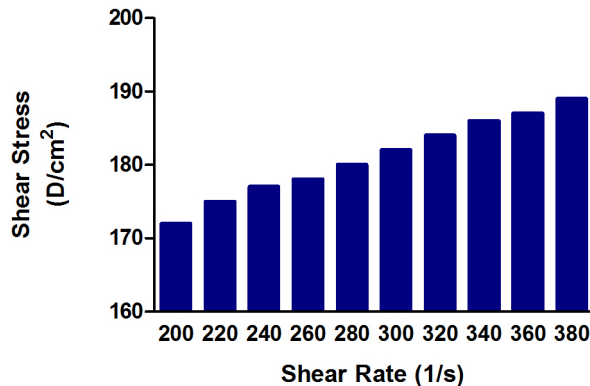
skin protective, anti-acne, anti-melasma, anti-wrinkle and anti-aging are developed using either natural or synthetic



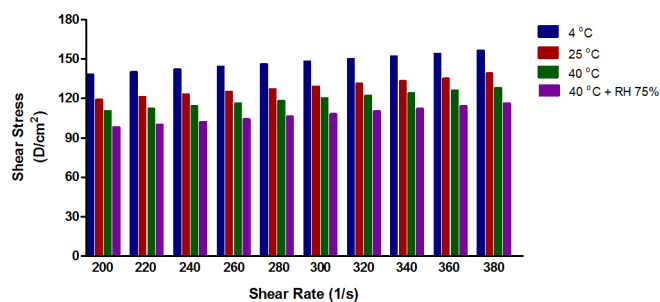
**FIGURE 5** - Rheogram of control formulation at different temperature after 2 months. Temperatures tested were 8 °C (blue bars), 25 °C (red bars), 40 °C (green bars), and 40 °C + 75% RH (violet bars).



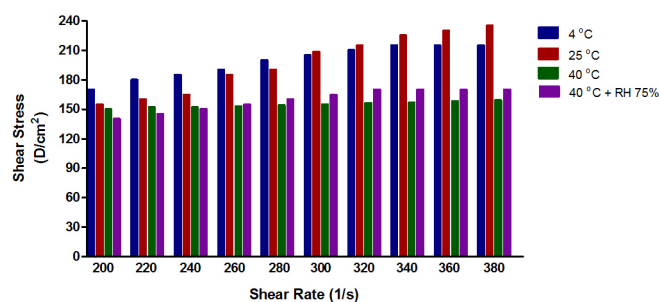
**FIGURE 4** - Rheogram of freshly prepared control formulation.



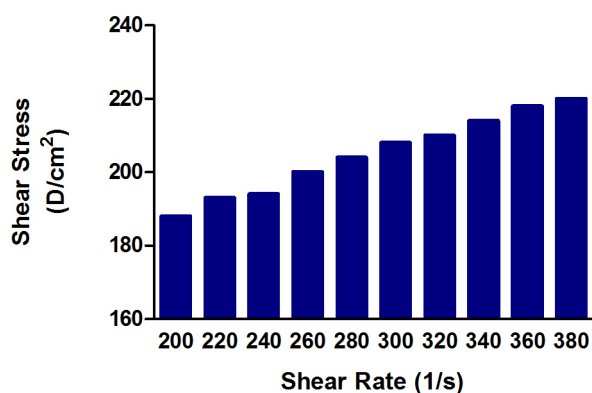
**FIGURE 6** - Rheogram of freshly prepared HR Emulsion.



**FIGURE 7** - Rheogram of HR Emulsion at different temperature after 2 months. Temperatures tested were 8 °C (blue bars), 25 °C (red bars), 40 °C (green bars), and 40 °C + 75% RH (violet bars).



**FIGURE 9** - Rheogram of CF Emulsion at different temperature after 2 months. Temperatures tested were 8 °C (blue bars), 25 °C (red bars), 40 °C (green bars), and 40 °C + 75% RH (violet bars).



**FIGURE 8** - Rheogram of freshly prepared CF emulsion.

materials. For example, Mahmood and Akhtar have documented that a combination of lotus and green tea emulsions had improved skin parameters (Mahmood and Akhtar, 2013). In addition, Casagrande *et al.* (2006) have described that topical formulations containing quercetin present protective effect against UVB-induced oxidative stress in hairless mice. The designing procedure for topical formulation needs quality attributes. The eminence of a formulation must satisfy the consumer's need in terms of its out-put. The plant extracts used in topical preparation have diversity of properties like antioxidant, antiseptic, anti-inflammatory, emollient, anti-seborrhetic and anti-kerolytic activity. Topical products with plant extracts

**TABLE VI** - Viscosity of emulsions during storage for 2 months

Time (months)	Temperature (°C)	Mean Viscosity (cP) ± SD of control (n=3)	Mean Viscosity (cP) ± SD of HR (n=3)	Mean Viscosity (cP) ± SD of CF (n=3)
0	8.0 ± 0.5	128.45 ± 1.02	194.32 ± 1.05	150.56 ± 1.14
0.5	8.0 ± 0.5	128.37 ± 1.01	188.22 ± 1.12	148.89 ± 1.02
1	8.0 ± 0.5	128.34 ± 1.03	186.22 ± 1.07	148.43 ± 1.09
2	8.0 ± 0.5	128.09 ± 1.01	181.22 ± 1.03	146.23 ± 1.12
0	25 ± 0.5	128.45 ± 1.02	194.32 ± 1.05	150.56 ± 1.14
0.5	25 ± 0.5	128.41 ± 1.11	184.68 ± 1.12	145.29 ± 1.32
1	25 ± 0.5	128.27 ± 1.39	182.32 ± 1.14	145.11 ± 1.22
2	25 ± 0.5	128.03 ± 1.07	168.91 ± 1.12	143.38 ± 1.07
0	40 ± 0.5	128.45 ± 1.02	194.32 ± 1.05	150.56 ± 1.09
0.5	40 ± 0.5	128.21 ± 1.21	159.62 ± 1.08	144.52 ± 1.08
1	40 ± 0.5	118.17 ± 1.09	151.22 ± 1.01	142.55 ± 1.09
2	40 ± 0.5	107.89 ± 1.30	149.39 ± 1.22	140.46 ± 1.12
0	40 ± 0.5/75% RH	128.45 ± 1.02	194.32 ± 1.05	150.56 ± 1.09
0.5	40 ± 0.5/75% RH	128.21 ± 1.21	145.35 ± 1.01	141.52 ± 1.09
1	40 ± 0.5/75% RH	128.17 ± 1.09	143.30 ± 1.13	142.55 ± 1.07
2	40 ± 0.5/75% RH	127.89 ± 1.30	131.22 ± 1.09	134.46 ± 1.03

HR= *Hippophae rhamnoides* Emulsion, CF= *Cassia fistula* Emulsion, SD = Standard Deviation.

argue to have fewer side effects as compared to products containing synthetic agents. In this study, we formulated an O/W emulsion for facial use. Therefore, the water content in formula contains 50% by weight of the emulsion.

Generally, the type of emulsions is determined by the phase ratio of oil and water if these numbers are high (>3). For example, in this study with 40.0% of oil and 60.0% of water (an o:w phase ratio of 2:3), the emulsion was expected to be O/W (Friberg, 1988). Microscopic examinations exposed that all emulsions were O/W. When color amarant was used, the emulsion background was pinkish with colorless globules. However, with oil soluble color, Sudan red globules were reddish against a colorless background. Confirmation of emulsion type was carried out by the electrical conductivity test, where all emulsions conducted electricity and thus confirmed to be O/W.

The freshly prepared control was white while the active formulations were pale yellow in color. There was no change occurred in color up to the observation period of 2 months. This showed that the emulsions were stable at different storage conditions i.e. 8 °C, 25 °C, 40 °C and 40 °C + 75% RH throughout 2 months study period. No change in the color may be attributed to different factors contributing the emulsions stability. As plant extracts contain anti-bacterial components which protect the emulsion components from microbial growth and thus might produce such substances which are able to change the color of the formulation during the storage time. In addition, since emulsions and other water containing dosage forms should be preserved from microbial contamination, a preservative mixture of methyl and propyl paraben was used.

It is important to highlight that both total phenolic content and the antioxidant activity measured by DPPH were preserved in our emulsions. This is desired for the emulsions preparation, since most of the desired effects are mediated by antioxidants. Our data is in accordance to what is reported by Kumar *et al* (2005), in which a methanol extract of *Bauhinia racemosa* preserved its antioxidant and total phenolic content after processing.

After formulation of emulsions, time and temperature-dependent phenomenon occur to effect its separation leading to decline in viscosity which results in increased liquefaction (Herbert *et al.*, 1988). No liquefaction was observed in any of the sample of control and active formulation of both plant extract stored at 8 °C and 25 °C during 2 months study period. However, liquefaction was observed in the sample from 21<sup>st</sup> day to 60<sup>th</sup> day kept at 40 °C and 28<sup>th</sup> day to 60 day kept at 40 °C + 75% RH.

Creaming occurs because of the differences in density of two phases under the influence of gravity which

results in phase separation (Derrick, 2000). There was no phase separation observed in any of the samples kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH up to observation period of 2 months with naked eye. It showed that the emulsions were stable at all storage conditions considering phase separation as a parameter of stability.

Centrifugation is an extremely helpful means for assessing and predicting the shelf life of emulsions (Herbert *et al.*, 1988). In this study centrifugation test was performed for emulsions stored at different storage conditions up to a period of 2 months at definite time intervals. No phase separation on centrifugation was seen in any of the samples. It showed the proper homogenization speed during emulsion formulation prevented them from breakage during stress testing as described by Abdurrahman and Rosli (2006).

The electrical conductivity test was carried out as a confirmatory test of emulsion type as well as a stability test. According to James *et al.* (2000), conductivity differences occur when an emulsion creams and the oil proportion increases in the upper part of emulsion and the water proportion increases in the lower part of emulsion. In addition, according to Garti *et al.* (1982), subjection of emulsions to conductivity test before and after stress will indicate their stability. The extent of the conductivity differential between the two interpretations will show the degree of instability.

In this study, conductivity test was performed for all the samples of emulsions kept at different storage conditions up to a period of 2 months at definite time intervals. The conductivity of emulsions stored at 8 °C, 25 °C showed insignificant difference from zero hour to 2 months study period which indicate that the emulsions were stable as well as the two phases had suitable oil to water ratios which has been generally observed with stable emulsions whereas the conductivity of emulsions stored at 40 °C and 40 °C + 75% RH showed decline (as shown in figure 1-3) which indicated instability according to Martin (2007).

The pH is an important parameter for the effectiveness of topical emulsions. The human has a pH range of 4.5 to 6.0 as defined by Jennifer *et al.* (2003) and 5.5 is believed to be the average pH of the skin. Therefore, the formulations proposed for application to human skin should have pH in this range.

In this study, the pH of freshly prepared control was 5.7 whereas the pH of *Hippophae rhamnoides* and *Cassia fistula* was 5.2 and 5.53 respectively, which is within the range of skin pH. The pH values of control kept at different storage conditions i.e. 8, 25, 40 and 40 °C + 75% RH was found to be increasing gradually in the 1<sup>st</sup> week and then



it started to decline continuously till 60<sup>th</sup> day with some variations. At the end of study, pH of control at 8, 25, 40 and 40 °C+ 75% RH was 5.4, 5.9, 5.3 and 5.8 respectively. Whereas pH of *Hippophae rhamnoides* and *Cassia fistula* emulsions, kept at 8, 25, 40 and 40 °C + 75% RH showed gradual reduction with slight variations with time. The pH values of *Hippophae rhamnoides* emulsions were 5.34, 5.50, 5.25 and 5.4 whereas the pH values of *Cassia fistula* emulsions were 5.5, 5.43, 5.35 and 5.25 at 60<sup>th</sup> day respectively.

By using statistical technique ANOVA at 5% level of significance, it was found that the change in pH of control was insignificant at different time levels and temperature but there was significant difference in pH change of active formulations at different levels of time and temperature. When LSD (least significant difference) test was applied to check the individual average effects of pH of control at different temperatures with the passage of time by taking average pH values of zero hour at different temperatures as standard, it showed insignificant changes except 3<sup>rd</sup> and 4<sup>th</sup> week where differences were significant. Again when LSD test was applied to check the individual average effect of the pH of active formulations at different temperatures with the passage of time by taking average pH values of zero hour at different temperatures as standard, it showed significant changes from 48<sup>th</sup> hour till the end of study period except the 7<sup>th</sup> day.

LSD test showed that there was insignificant change in pH of control at different storage conditions but significant changes were noted in pH of active formulations at different storage conditions with the passage of time. The reduction in pH of the formulations at different storage conditions might be due to the oxidation of paraffin oil which generates aldehydes and organic acids. The other reason may be because of the production of acidic species as the plant extracts are very acidic and have high concentration of organic acids such as quinic acid as described by us and others (Barkat, 2010; Raymond *et al.*, 2003).

The flow characteristics of an emulsion are clearly among some of its more important physical features in either technical or aesthetic terms. Hence the ability to measure, adjust and, if possible, forecast such characteristics is very important.

The shear stress causes strain in solids and liquids; the solid deforms and liquid flows. Rearrangement takes place inside the material due to stress application. In a purely viscous material, all the energy required to produce the deformation is dissipated as heat. On the contrary, in a purely elastic material, all the energy required to produce the deformation is stored.

Emulsions dispersed phase affects the rheology of the emulsion by its globule size, volume concentration viscosity and chemical constituents (Naveed, 2000). Rheological analysis allows the characterization of emulsions, to follow changes in emulsion induced by aging shear and temperature and to predict their stability.

Rheological parameters were performed at 25 °C. Changes in viscosity were noted when shear stress were applied. Rheogram of shear stress versus shear rate were obtained of all the formulations. Viscosities were found to decrease in parallel to increase in shear stress. Viscosities were also found to decrease in emulsions kept at different storage conditions especially at 40 °C. It was observed from the different study that when temperature was increased, the flow molecules through interface are also increased. The flow molecule correlated with viscosities. The viscosity is very sensitive to the temperature hence; the increment temperature caused reduction of emulsion viscosity (Lim *et al.*, 2011).

The Flow index values indicated that emulsions act as a non Newtonian fluid (Pseudoplastic fluid). The result were in agreement with Saravacos *et al.* that emulsions show a marked non-newtonian behavior and most fruit and vegetable fluids and pastes are pseudoplastic, where the flow behavior index varies between 0 to 1 (Saravacos, 1995; Pal, 1992). The rheograms of all emulsions showed non-Newtonian behavior, with flow index less than 1 which was an agreeable rheological property reflecting their pseudoplastic tendency. Emulsions with pseudoplastic flow properties cause the formation of a coherent film covering the skin surface. This characteristic is valuable and critical for a better phenolic antioxidant fortification of the skin surface.

## CONCLUSION

In conclusion, we report that the emulsions containing *Hippophae rhamnoides* and *Cassia fistula* extracts present high antioxidant activity and desirable pharmaceutical stability. Those emulsions could be considered for preparing topical formulations for used in skin care.

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