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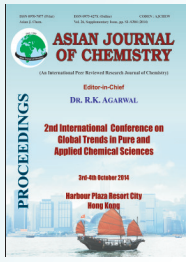

Microemulsion Containing Ellagic Acid Rich Extract and Its Characterization

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Ellagic acid, the active component in *Punica granatum* extract, has been reported to have the antioxidant, anti-tyrosinase and ultra-violet induced pigmentation in the human skin effects. In this study, extract was obtained from the *Punica granatum* fruit rinds which were extracted by 50 % ethanol and analyzed ellagic acid by UV method at 365 nm. The percentage of ellagic acid is about 84.48 %. Microemulsions were formulated using pseudoternary phase diagram. Palm oil and water were chosen as the oil and aqueous phase, respectively. The surfactant (Tween 80) and co-surfactant (PG) were used in preparation of the microemulsion. They were mixed in different ratios (2:1 and 5:1). The results showed the region of microemulsion on the phase diagram was obtained when increase the ratio of Tween 80. Evaluation of microemulsion regarding particle size, zeta potential, conductivity, stability, viscosity, scanning electron microscopy, refractive index and pH. This study established that a stable ellagic acid rich extract containing microemulsion was used for further topical uses.

Keywords: Ellagic acid, *Punica granatum*, UV, Microemulsions.

INTRODUCTION

Pomegranate (*Punica granatum* L) belongs to the family Punicaceae[1]. *Punica granatum* contains richly coloured grains which contain delicious juice[2]. The fresh weight of *Punica granatum* fruit consists of 30-50 %. Fruit of pomegranate is divided into three distinctive parts these are juice having 30 % of total fruit weight, seeds of fruit at the rate of 3 % of the total fruit weight and the peels having a network of interior membranes[3]. Almost 50 % of the total fruit weight is considered to be the edible part in which seeds have 20 % while juice has remaining 80 % shear. Fresh juice of fruit has water (85 %), total sugars (10 %) and pectin, polyphenolic flavonoids and ascorbic acid (3 %). Rich amounts of sugars, pectin and crude fibers are found in the seeds of *Punica granatum* fruits. Dried *Punica granatum* seeds contain, isoflavone phytoestrogens genistein, steroid estrogen, phytoestroge coumestrol and daidzein. Fructose and glucose (in the same similar quantities), calciumis are glutamic acid and aspartic acid (have also been reported in the pomegranate juice (PJ) depending upon the varieties of *Punica granatum* the soluble polyphenol contents is found 0.2-1.0 % in the pomegranate juice[4]. Oxidative stress is effectively encountered by the antioxidants and hence the skin is protected[5]. Important polyphenols are found in the juice of pomegranate which includes ellagitannin, ellagic acid and punicalagin which are considers as the important antioxidants[6].

According to the modern research, the juice of *Punica granatum* has a considerable amount of antioxidants and the topical sunscreens are effectively improved by the *Punica granatum*. Similarly if the juice of *Punica granatum* is compared with the equal amounts of green tea and red wine the antioxidant potential of pomegranate juice is greater than the both. Furthermore pomegranate peel has been reported to have a dermal regeneration and its seed promote epidermal regeneration. *Punica granatum* juice extract is a strong photo-chemo-preventive agent[7]. It also has been reported as an effective skin whitener if it has ellagic acid[8]. It is generally known that microemulsions are a superior system in terms of thermodynamic stability, high solubilizing capabilities and enhanced absorption. Therefore, *Punica granatum* extract was incorporated in microemulsion for better solublization and greater absorption.

The chemical constituents of the *Punica granatum* vary according to variety, cultivation conditions, habitat, environment and ripening of fruit. *Punica granatum* is the potent source of antioxidants elements compounds including flavonoids and hydrolyzable tannins which consider 92 % antioxidant activity[3,9].

EXPERIMENTAL

Extraction process: 100 g shade-dried ground plant material of *Punica granatum* fruit rinds was subjected to extraction three times. Sample was soaked in ethanol with water mixture (50 %) for 2 h at 60 °C. Filtrate was collected and under reduced pressure ethanol was obtained. Then the resultant aqueous mixture was acidified and passed through reflux condenser for 6 h at 70 °C. Mixture was diluted (water) and precipitates were collected and dried in tray driers under reduced pressure[10].

Analysis of *Punica granatum* extract by UV-visible spectrophotometer: A UV-visible spectrophotometer (Shimadzu, Japan) was used. Methanol was used as blank. UV detector wavelength was set at 365 nm.

Preparation of stock solution: The stock standard solution of ellagic acid was diluted to required concentration range of 0.156 to 10 µg/mL. The absorbance obtained from each concentration was plotted against concentration of ellagic acid to give the calibration curve (Fig. 1). The calibration curve obtained was (y = 49.29x + 6.25), (R^2 = 0.998) for the quantitation, the amount of ellagic acid was calculated based on absorbance of sample and using the calibration curve. The percentage calculated was found to be 84.48 %. The correlation coefficient (R^2) was used to determine the linearity of the calibration curve.

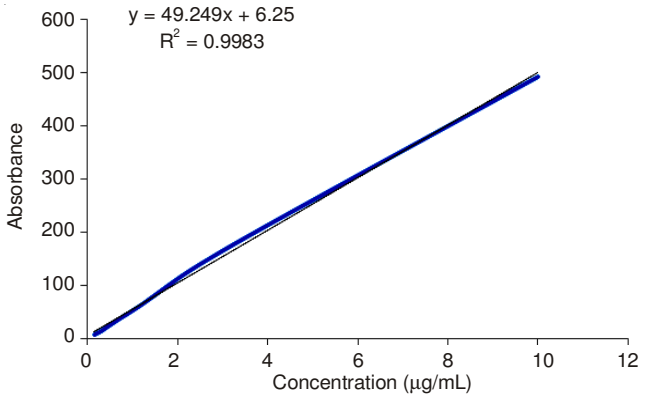

Fig. 1. Calibration curve of ellagic acid

Determination of ellagic acid in pomegranate extract: Briefly, 1 mL concentrated botanical sample and ten millilitres 80 % ethanol were mixed, further diluted to10 µg/mL. The sample solution was mixed well by using Vortex (Scientific industries, USA) and filtered through 0.45 µm cellulose acetate membrane before analysis. The amount of ellagic acid in extract was back-calculated from the calibration curve equation.

Pseudoternary phase diagram construction: All microemulsions were formulated with full pseudoternary phase diagram. In this study, palm oil and water were chosen as one of the oil and aqueous phase, respectively. They were mixed in different ratios (2:1 and 5:1) of surfactant and cosurfactant mixtures (Fig. 2a and 2b). For construction of the phase diagram, oil, surfactant mixture and water were weighed and mixed together at room temperature. After each mixing, the sample was allowed to equilibrate for 24 h. The microemulsion regions in the pseudoternary phase diagram were determined.

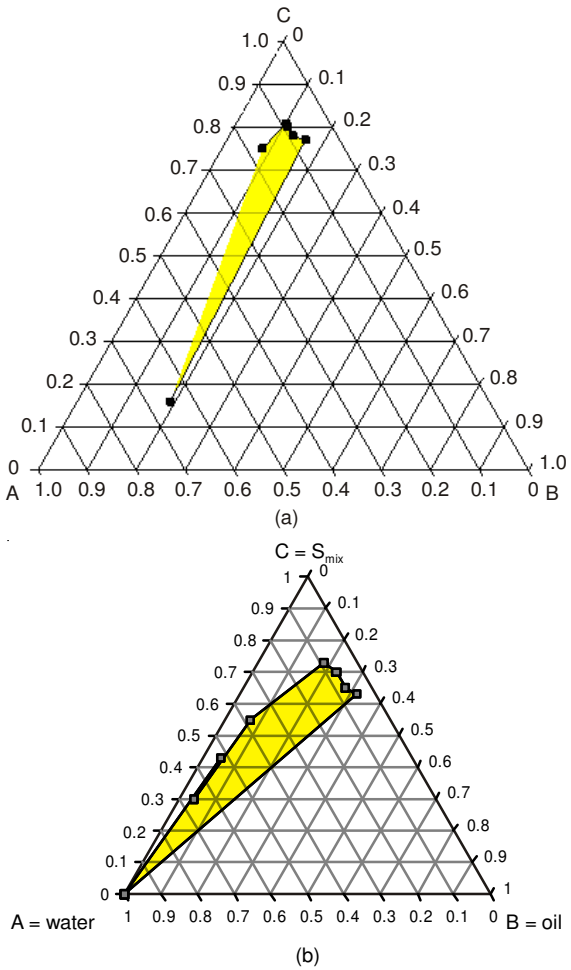

Fig. 2. Pseudoternary phase diagram of surfactant mixture, oil and water; (a) S_{mix}2:1 and b) S_{mix}5:1

The results showed the region of microemulsion on the phase diagram was obtained with increased ratio of Tween 80.

Characterization of microemulsion containing *Punica granatum* extract

By the help of Zeta sizer (Malvern instrument Ltd. ZEN3600, UK) zeta potential was measured by putting the samples in the disposable zeta cells and results were recorded accordingly. Microstructure of microemulsions was characterized by using the scanning electron microscopy (SEM). Model of the SEM was 19800 Hitachi S-3400N VP-SEM and it was Japan made. Droplet size of samples on the average basis was measured by Zeta sizer (Malvern instrument 1td. ZEN3600, UK) at 25 °C and their refractive indices (RI) were also calculated Abbe refractometer (HEDAO, China).

Viscosity of samples was mea-sured at 25 °C. For this purpose spindle number 41 was used at the average rate of 50 rpm. All the measurements were in three replications. A conductivity meter (Conductometer WTW, Cond 197i and Germany) was used to measure the electrical conductivity of MEs. For this purpose conductivity cells with a cell constant of 1 were used. At the temperature of 25 °C the pH values of microemulsion by using pH meter (Inolab, Germany). Three replications were used for this study.

Physical stability study: Regarding temperature stability and centrifugation, the physical stability of microemulsions

TABLE-1
CHARACTERISTICS OF BLANK MICROEMULSION AND MICROEMULSION CONTAINING *Punica granatum* EXTRACT (n = 3)

Formulations	Zeta potential (mv)	Viscosity (cP)	Particle size (μm)	Conductivity (μS/cm)	pH	*RI	Phase separation
Blank microemulsion	-03	5.23 ± 0.88	9.28 ± 0.4	0.4	5.97	1.41	No
Microemulsion with extract	-5.2	5.23 ± 0.66	8.08 ± 0.2	1.5	5.39	1.45	No

*Refractive index

was studied. For phase separation, flocculation or precipitation the microemulsions were kept at the different temperatures of 8, 25 and 40 °C Changes in the homogeneity of microemulsions were studied by the centrifugation at the rate of 6000 rpm for 0.5 h, this experiment was conducted at the temperature of 25 °C. Instrument used for this purpose was Helttich of Germany.

Statistical methods: Data of three replications was analyzed statistically. P value less than 0.05 and 95 % of confidence interval was significant in this regard.

RESULTS AND DISCUSSION

Scanning electron microscopy (SEM): At the ambient temperature microemulsions formation was recorded. Increase in the weight ratio of surfactant/co surfactant (S_{mix} = 5:1) caused an increase in existence of regions of microemulsion.

Particle sizes of base microemulsion containing *Punica granatum* extract were investigated and average particle size after loading the drug no significant difference was observed. The microemulsion containing *Punica granatum* extract was found to have minimum particle size on the average basis and that was 8.08 ± 0.2 nm. The refractive index of the base microemulsion and microemulsion containing *Punica granatum* extract was found 1.41 and 1.45, respectively that represents a transparent medium which indicates microemulsions are transparent structures[11].

A strong correlation was observed between the electrical conductivity and specific structure of microemulsion systems. The base microemulsion and microemulsion containing *Punica granatum* extract had the average conductivity in the range of 6.7 to 7.1 μs/cm which showed o/w structure of microemulsion. Also, o/w structure of microemulsion is confirmed with colour tests[12]. prepared and evaluated nanoemulsion based aceclofenac gel for topical delivery. The conductivity of the resultant formulations was greater than range of 0.0867 to 0.149 μS/cm, which showed the microemulsion system is of oil in water (o/w).

The base microemulsion and microemulsion containing *Punica granatum* extract had appropriate observed pH value (5.39 to 5.97) for the case of topical application it proved to be the best of all. Incorporation of *Punica granatum* extract did not affect the observed pH value of the microemulsions significantly (Table-1). As reported in literature with a broad range of pH 4 to 7 skin pH value was found variable[13]. The pH of our formulations is well within the reported range of skin pH.

The mean viscosity of formulations was from 5.23 ± 0.88 cps to 5.93 ± 0.66 cps. There was no significant difference found between the viscosities of base microemulsion and microemulsion containing *Punica granatum* extract ($p > 0.05$).

For three months an experiment was conducted visually in this experiment. Flocculation was found not in the visual observations. Moreover there was no sign of phase separation observed under the stress condition at the rate of 6000 rpm for 0.5 h. The centrifugation tests showed in the absence of any phase separation during the whole experimentation the homogeneity of the microemulsions ensures an encouraging physical stability of the both separations[14].

In our microemulsion preparations, the viscosity of base microemulsion and microemulsion containing pomegranate extract were 5.2 ± 0.31 and 5.3 ± 0.21, respectively.

The structure of micro globules of base microemulsion and microemulsion containing *Punica granatum* extract were also tested with SEM image and it was found that size and shape of micro globules of both formulations has been changed in terms of globules size and shape *i.e*. increased size and irregular shaped globules due to agglomeration of micro globules. This is because of sample preparation procedure in which each sample was spread on a glass slide and dried at 100 °C due to which micro globules coalesce and by heating, micro globules ruptured. The images showed the residual of *Punica granatum* extract as well as excipients.

Conclusions

• It is possible to formulate topical microemulsions containing 50 % hydro-alcoholic extract of *Punica granatum* having thermodynamically stability with three months storage.

• Further studies such as irritability to skin and acceptance from the volunteers/consumers have evaluated the value of our prepared microemulsions.

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