

Investigation of antiaging and skin rejuvenation potential of phytoconstituents from *Pyrus communis* loaded topical emulgel

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Abstract: *Pyrus communis* fruit is traditionally used for improving the skin color and texture. The current study was designed to investigate *Pyrus communis* fruit phytoconstituents and their *in-vivo* rejuvenation effects on human skin by developing a stable emulgel formulation. Hydro-alcoholic extract of *Pyrus communis* was subjected to phytochemical analysis (TPC, TFC, antioxidant activity and anti-tyrosinase activity). A stable emulgel formulation loaded with 5% (w/w) *Pyrus communis* fruit extract was developed. Afterwards, this stable emulgel formulation was tested for effects on skin parameters and compared these with placebo (without fruit extract) by employing them on healthy human volunteers (n=13) for 3 months. Investigated *in-vivo* skin parameters were skin erythema, melanin, moisture, sebum and elasticity. *Pyrus communis* fruit extract showed excellent antioxidant and anti-tyrosinase activities. The developed formulation was stable in varying conditions of temperature and humidity for a period of 12 weeks. The active formulation showed statistically significant ($p<0.05$) decrease in skin melanin, erythema and sebum level while increase in skin elasticity and moisture content when compared with placebo. From findings it is concluded that *Pyrus communis* fruit extract loaded emulgel possesses antiaging potential with improvement in skin tone and elasticity, ameliorated skin moisture and showed skin whitening potential.

Keywords: *Pyrus communis*, phytochemical analysis, emulgel, *in vivo* evaluation, skin rejuvenation.

INTRODUCTION

Natural products have been traditionally used for cosmetic purposes all over the world. Plants' secondary metabolites play an important role in protecting skin diseases due to their strong antioxidant and anti-inflammatory potential (Ribeiro *et al* 2015).

Rosaceae family is famous for its fruits. Fruits have been reported to be evaluated for the presence of phenolics compounds, antioxidant activity and secondary metabolites (Kirti *et al.*, 2015). One of the fruit of Rosaceae family is *Pyrus communis*. *Pyrus communis* is a native to central and south west Asia and Eastern Europe. It is usually known as European pear, in Urdu nashpati and common pear. The *Pyrus communis* fresh fruit is a delicious, full of good source of dietary fiber and other healthful constituent particularly vitamin C (Kaur and Arya 2012). Due to its strong antioxidant potential it has a great healing ability (Cinnasamy and Bhargava 2014). In Asia, particularly Pakistan and India, *Pyrus communis* has been used in cosmetics formulations. It has been reported to involve in the presence of Arbutin (phenolicglycoside) which acts as a whitening source of human skin (Petkou *et al.*, 2002). *Pyrus communis* is commonly used as skin scrubber. Facial scrubber work by the process of exfoliation. It is a process by which dead cells and unwanted cells are removed from skin by rubbing a gritty lotion on skin. After few minutes of application skin

becomes soft and glows. In facial cleanser treatment dead cells are removed by gentle rubbing and create way for the new cells. Skin is the largest part of our body. Skin is affected by various factors which cause changing in the structure of skin. Skin is divided into epidermis, dermis and subcutaneous tissues. The epidermis is the outer layer of the skin and sub layer of epidermis is known as stratum corneum. Skin scrubbers works by altering the permeability of stratum corneum so makes skin soft and glowing.

Emulgel is a mixture of emulsion and gel which acts as a topically drug delivery system (TDDS). It is widely used for the healthy as well as diseased skin. Advantages of emulgel with fruit extract are ease in spreadability, greaseless, thixotropic, emollient, long shelf life, bio-friendly and transparent (Kumar *et al.*, 2010, Kshirsagar 2000). Drug absorption through skin enhanced in emulgel dosage form as it decreases the drug skin partition coefficient. That's why in the present study emulgel dosage form is selected (Shailendra *et al.*, 2017).

Purpose of the present study was to investigate phytoconstituents present in the *Pyrus communis* fruit extract and formulate an emulgel containing *Pyrus communis* fruit extract. Furthermore formulation *in-vivo* evaluation was done on human subjects. Antiaging and skin rejuvenation potential was checked by observing different skin parameters like melanin content, erythema, sebum level, elasticity, moisture content.

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MATERIALS AND METHODS

Materials and apparatus

Propylene glycol, liquid paraffin, Analytical grade methanol, triethanolamine and ascorbic acid (Merck, Germany), Span 20, tween 20, methyl paraben, carbopol 940, DPPH, Gallic acid, Kojic acid and Quercetin (Sigma, USA). Rotary evaporator (Heidolph, Co. Ltd., Japan), Refrigerator (Dawlance, Pakistan), UV spectrophotometer (UV 4000 ORI, Germany), Hot Incubator (Sanyo MIR-162, Japan), Digital Humidity Meter (TES Electronic Corp, Taiwan), Microplate reader Synergy HT (BioTek Instrument, USA), pH meter (WTW pH-197i, Germany), Optical microscope (Eclipse E200, Nikon, Japan), Homogenizer (Germany), Mexameter, Corneometer, Sebometer and Elastometer (Courage + Khazaka Electronic GmbH, Germany)

Plant material and identification

The fresh fruit were obtained from Azad Kashmir; Pakistan. Identification of the fruit was performed by the Department of Plant Sciences, Herbarium of Quaid-i-Azam University, Islamabad, Pakistan. The sample was assigned a voucher number (V# 805).

Extraction

The fresh fruit (1kg) of *Pyrus communis* without seeds were grinded with distilled water. Add methanol to make final volume 80% (v/v) hydro-alcoholic extract. This mixture was macerated for 3 days. It was filtered with muslin cloth, and the filtration was repeated with filter paper (whattman no. 20) for 3 times. The resultant filtrate was evaporated through rotary evaporator under vacuum at 40°C fitted with a chiller at 2°C to obtain a solidified extract. This solidified extract was stored in airtight tawny colored containers in refrigerator (8°C) for further use.

DPPH assay for Antioxidant Activities (AOA)

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical was used to determine the antioxidant activity as described with minor modification. The concentration of DPPH 100uM in methanol was used. Total assay volume was 100ul, containing 10ul of the test solution and 90ul of DPPH solution in a 96 well plate. The contents were mixed and incubated at 37°C for 30 minutes. Synergy (HT Bio Tek® USA) microplate reader was used to determine the diminution in absorbance at 517 nm. Standard antioxidant used was ascorbic acid. All experiments were carried out in triplicate. The percent inhibition was determined by the following formula (Ratshilivha *et al.*, 2014).

$$\text{Inhibition (\%)} = \left(\frac{\text{Abs. of control} - \text{Abs. of test solution}}{\text{Abs. of control}} \right) \times 100$$

Where

Absorbance of control=Total radical activity without inhibitor.
Absorbance of Test=Activity in the presence of test compound.

Methodology of tyrosinase inhibitory assay test (TIA Test)

Tyrosinase inhibitory assay was measured according to the Kim with slight modification. 60 units enzyme, 10 ml of test compound and 150ul of buffer (50mM of pH 6.8) in each well were incubated for 15 minutes at 30°C. After incubation pre-read at 480nm was taken and 1mM of substrate per well was added and reincubated at same condition for 30 minutes and after read was taken at 480nm. Kojic acid was taken as positive control. Result was measured by giving formula. IC50 was calculated by making serial dilution of original concentration.

$$\text{Inhibition (\%)} = 100 - \left(\frac{\text{Absorbance of test compound}}{\text{Absorbance of control}} \right) \times 100$$

Methodology of Total phenolics content test (TPC Test)

The total phenolics content (TPC) of each of the above extract was measured by colorimetric FCR method as described by Wolfe *et al* with modifications. 10µl of 10% diluted FCR was mixed with 100µl of sample solution and incubated for 10 minutes followed by the addition of 90µl of 15% w/v sodium carbonate aqueous solution. This mixture was incubated for further 90 minutes at 37°C. Absorbance was measured at 750nm. Both positive (gallic acid) and negative controls were included. TPC was calculated using the calibration curve ranging from (0-100 µg) and data expressed as mg gallic acid equivalent per gram of dry extract (mg of GAE/g of DE). Assays were carried out in triplicates.

Methodology of Total flavonoids content test (TFC Test)

The total flavonoids content test (TFC) was measured by modified colorimetric method. A calibration curve was established using Quercetin as standard, 1mg/ml in methanol, ranging from 0 to 100µl (0-100µg). All solutions were made in methanol. 100µl of sample solution was mixed with 25µl of 1% sodium nitrite solution and allowed to stand for 5 min, followed by the addition of 10µl of 10% Aluminum chloride solution and again allowed to react for 5min. finally, 35µl of 4% sodium hydroxide solution was added and mixture was diluted with 30µl of methanol. Absorbance was measured at 510 nm. TFC was calculated using the calibration curve equation and expressed as mg Quercetin equivalent per gram of dry extract (mg of QE/g of DE) (Wolfe *et al* 2003).

Stability evaluation

The prepared formulations (active and placebo) were exposed to stability studies for 3 months study period under changing the conditions of temperature and humidity. The different conditions were used at 8°C, 25°C, 40°C, and 40°C+75% relative humidity. During this period, formulations were assessed by physical stability (color, odor, phase separation and liquefaction).

Preparation of *Pyrus communis* emulgel

Table 1 shows the composition of emulgel to be prepared. Emulgel was prepared with the addition of methanolic extract of fresh fruit was known as active emulgel (with extract) and placebo emulgel (without extract). Aqueous phase (tween 20, propylene glycol, methyl parabin and distilled water) of emulsion was formulated by heating to $75\pm 1^\circ\text{C}$. *Pyrus communis* fruit extract was added to aqueous phase. Furthermore, oily phase (paraffin oil and span 20) was heated to the same temperature as in aqueous phase. Then aqueous phase was mixed with oily phase slowly and continuous stirring to form an o/w emulsion. Carbopol 940 (1.5g) was suspended in distilled water to make final volume 100ml. After complete the dispersion, triethanolamine was added drop wise and check the pH after each addition of it until the pH reached within the range of 5.5-6.5. Finally, oil in water emulsion was added in gel under continuous stirring for 15 minutes at 2000 rpm by digital homogenizer, then decrease the speed of digital homogenizer at 1000 rpm for 5 minutes and then the speed was again reduce to 500 rpm for 5 minutes for rational homogenization that emulsion cooled at 25°C . During the mixing process add some drops of fragrance (fruit oil) were added to give pleasant smell to emulgel to attract the female volunteer.

***In vitro* sun protection factor**

Sun protective factor of the emulgel was measured by applying a spectrophotometer as mentioned previously by Dutra *et al.*, About 1gm of sample was prepared to 100ml with methanol in a measuring flask and sonicated it for 05 minutes. After sonication, it is filtered by using cotton plug and first 10 ml of this solution is discarded. Next 5ml of this was again prepared to 50ml with methanol and 5ml of which was prepared to 25 ml methanol. Then by taking methanol as standard blank, the absorption of the sample was calculated in the range of 290-320nm each 5nm.

***Non -invasive in vivo* evaluations**

Study protocol and ethical approval for non-invasive in-vivo applications

A single blinded study was designed for the comparison of the two emulgel i.e. the formulation having *Pyrus communis* extract and other was the placebo. 20 healthy female volunteers were selected randomly. The age of volunteer was within range of 22-30 years. After that patch test was performed, each volunteer was provided two formulations (active formulation and placebo), their application instruction on cheeks as well as its storage. Each volunteer was provided a time table of 2nd week, 4th, 8th, 10th and 12th week of study periods for 3 months. This study was approved by the Pharmacy Research Ethics Committee (PREC) for *in vivo* studies (Ref. No. 46/S-2018/PREC), The Islamia University of Bahawalpur, Pakistan and was directed according to the international guidelines of Helsinki Declaration.

Patch test

Before the start of study on human subjects a test known as patch test was performed to check any skin irritation, for this test, a patch on the forearm marked with an area of 5x4 cm on each subject. Then test formulations (active and placebo) were applied on that marked area of skin of each participant. On right arm the placebo was apply and on the left forearm active emulgel was applied and measured the values for erythema contents of skin. After 48 hours, skin erythema and melanin level was measured from Mexameter.

Skin erythema and melanin contents

Skin erythema and melanin contents were measured by an instrument called Mexameter. This device has a mechanism on the theory of light reflection and emission. An elastic spring on its probe ensures a stable pressure on the skin. It emits light of 3 different wavelengths (green=568nm, red=660nm and infrared=880nm) when probe touch the skin. This emitted light gets reflections from the skin and the quantity of absorbed light is quantified by the receiver. Erythema content is measured from the potency of absorbed and reflected light of wavelength 568 and 660 nm whereas melanin level from 660 and 880 nm respectively (Wan *et al.*, 2017). Three readings were measured on the tested skin area and their mean was measured.

Moisture contents of skin

Moisture content of skin was measured by an instrument known as Corneometer. Its probe contains of gold electrodes overlaid by a thin layered insulation material with minor dielectric constant. On touch with skin, an electric field penetrates into the epidermis and moisture content is quantified as dielectric constant dependent capacitance variations of water. The measured value is shown as arbitrary units (Manosroi *et al.*, 2011). Three consecutive readings were performed and their mean was recorded as accurate reading to decrease the chances of error.

Elasticity of skin

Elastometer was employed to measure the skin elasticity. Its probe was used to measure the elastic characteristics of skin. Its basic theory works on the resilient ability of the stratum corneum when disturbed (suction and stretching). The measured value was shown in the LED display present in the devise in the form of percentage of elasticity. Three consecutive values were performed and finally mean reading was taken to decrease the chances of error.

Sebum contents of skin

The sebum level of skin was measured by an instrument known as Sebumeter. It works on the mechanism of oil or grease spot photometry and uses the difference in light intensity by a plastic strip which is known as Sebumeter tape. When this tape is touched to the surface of skin, it becomes translucent. After putting tape into the

Table 1: Composition of *Pyrus communis* emulgel and control (100g)

Ingredients	<i>Pyrus communis</i> emulgel (gm)	Control emulgel (gm)
Liquid paraffin	7.5	7.5
Span 20	1.0	1.0
Tween 20	0.5	0.5
Propylene glycol	5.0	5.0
Methyl parabin	1.5	1.5
Carbopol 940	1.5	1.5
Active extract	5	-
Distilled water	q.s	q.s

Table 2: Stability evaluation of emulgel formulation and placebo at different temperatures

Observed Parameters	Fresh		After 12 weeks							
	P	F	8°C		25°C		40°C		40°C+75%	
			P	F	P	F	P	F	P	F
Color	W	W	W	W	W	W	W	W	W	W
Liquefaction	N/A	N/A	-	-	-	-	-	-	+	+
Phase separation	N/A	N/A	-	-	-	-	-	-	-	-

W=White, - = no change, N/A= not applicable, +=slight change

instrument aperture, its translucency is taken by photocell by the emission of light. Three consecutive readings were performed to take mean of them and finally mean value use to decrease the chances of error.

Statistical and mathematical analysis

The percentage changes in the individual values of different parameters of skin measured by the following formula:

$$\text{Percentage change (\%)} = \left[\frac{(A - B)}{B} \right] \times 100$$

Where,

A= Individual value on any parameter at 2nd, 4th, 6th, 8th, 10th & 12th week.

B= Zero hour value of that parameter

The values obtained for different skin parameters (melanin, erythema, skin moisture, skin sebum and elasticity) were determined statistically using (SPSS, V 17.0) software. The ANOVA (Analysis of variance) was applied to assess eventual changes between various time intervals and paired sample t-test to determine any difference between two emulgel (active and placebo), and post-hoc analysis through LSD (Least Significant Difference), which computes "pairwise comparisons", i.e. the smallest significant difference between two means variation. A difference was measured significantly at a P-value inferior to 5% (P<0.05).

RESULTS

Total phenolics contents (TPC)

According to Wolfe K's method using gallic acid as standard, total phenolic content in the fruit extract was calculated from the calibration curve. It found to be

43.62± 1.76mg GAE/g (Mean ±SEM, n=3) as shown in fig. 1.

Total flavonoid contents (TFC)

According to Wolfe K's method and using Quercetin reagent as standard, a hydro-alcoholic fruit extract of *Pyrus communis* showed the total flavonoids content in the current studied samples. It was found to be 15.85±0.87mg QE/g (Mean ±SEM, n=3) as indicated in the fig. 1.

Antioxidant Activity

Fig. 2 shows antioxidant potential of *Pyrus communis* fruit extract calculated according to the Ratshilivha's method by using ascorbic acid as standard. The calculated DPPH activity was 78.29%±0.4 (Mean ±SEM, n=3).

Tyrosinase inhibitor activity (TIA)

Fig. 2 indicates tyrosinase inhibitory potential of *Pyrus Communis* fruit extract measured according to Kim's method by using the Kojic acid as standard. It showed tyrosinase inhibitory potential up to 79%±0.73 (Mean ±SEM, n=3).

Stability studies

During 12 weeks study period physical stability parameters (color, odor, phase separation and liquefaction) of both formulations (active & placebo) showed no change but a minor change was only observed at 40°C and 40°C+75%RH as shown in table 2.

In-vitro Sun Protective Factor (SPF)

In present study, the developed emulgel formulation showed a good SPF value of 4.0.

Evaluation of non invasive in vivo studies

Evaluation of primary irritation test of skin (Patch Test)

Results revealed that there was no any type of itching/irritation observed in any participant of the study after application of both placebo and active formulation.

Assessment of Erythema content

Results revealed a sequential decrease in erythema after the application of both placebo and active formulation as shown in fig. 4. But the decrease made by active formulation was more prominent and steady whereas placebo showed non steady decrease in erythema content. By applying the statistical measures of ANOVA with 5% level of significance, the formulation revealed significant difference in the average percentage changes of skin erythema with passage of time. Pair sample t-test exhibited a significant difference between the effects of both placebo and formulation during the study.

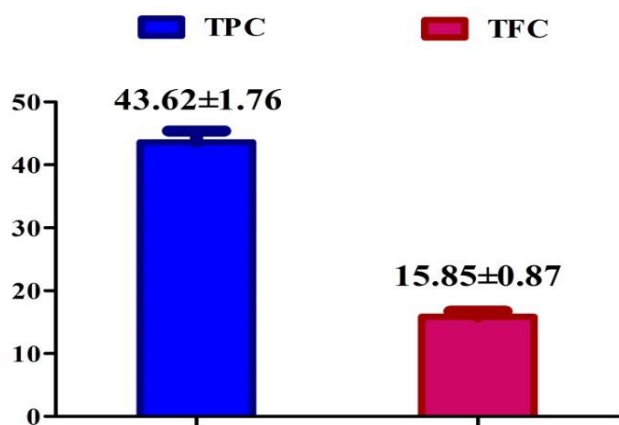


Fig. 1: Total Phenolic and flavonoid content of *Pyrus communis* fruit extract.

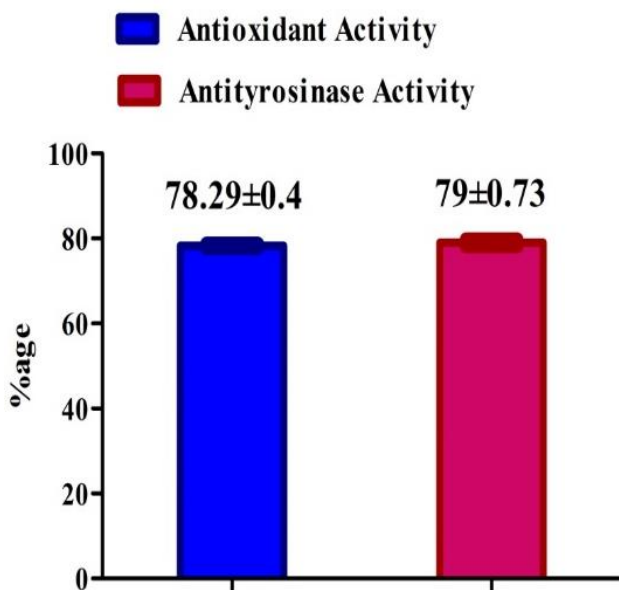


Fig. 2: Anti-oxidant and Anti-tyrosinase activities of *Pyrus communis* fruit.

Assessment of melanin contents

Results showed no effect on melanin content after the application of placebo until 2nd week, but after that there was a minor increase in the melanin content. While, active formulation showed a marked decrease in melanin level in a steady manner. Two-way ANOVA showed that the decrease in melanin level was significant as compared with the placebo. Formulation LSD testing showed significant difference at 4th, 6th, 8th, 10th and 12th week as compared with placebo. Moreover, pair sample t-test also showed significant variance observed in the effects of both placebo and active formulation.

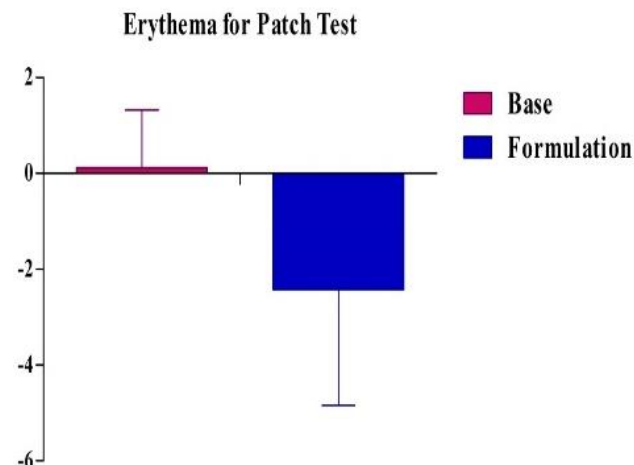


Fig. 3: Change in skin erythema in Patch test formulation

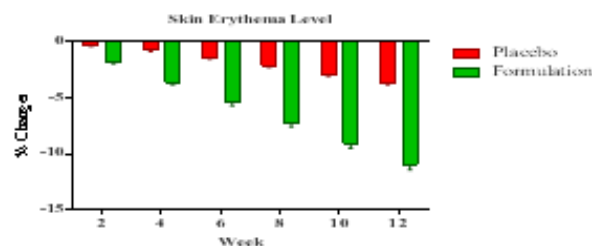


Fig. 4: Percentage change in skin erythema after the application of placebo and active.

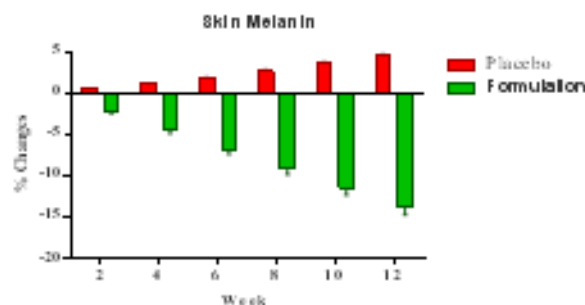


Fig. 5: Percentage change of skin melanin after application of placebo and active formulation.

Assessment of moisture contents of skin

It was evident from the fig. 6 that both placebo and active formulation made an increase in the skin moisture content during the study. Active formulation showed for increase

in moisture content as compared to placebo. Statistically active formulation showed significant difference from the placebo. Pair sample t-test described a significant variation between the effects of placebo and active formulations during the whole study period.

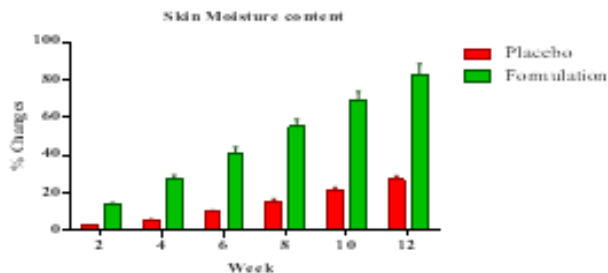


Fig. 6: Percentage change in skin moisture content after application of placebo and active formulation.

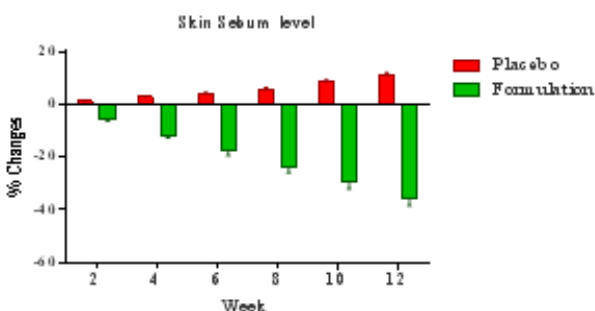


Fig. 7: Percentage change in skin sebum content after application of placebo and active formulation.

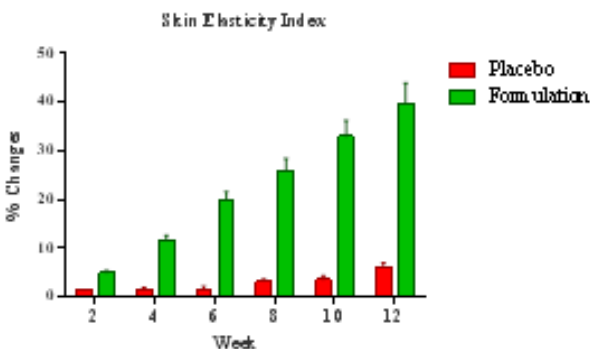


Fig. 8: Percentage change in skin elasticity after application of placebo and active formulation.

Assessment of sebum level in skin

Statistically significant decrease was observed with active formulation during the study period of 12 week as shown in the fig. 7. Whereas, placebo abruptly increased the skin sebum content, which was also statistically insignificant. Pair sample t-test indicated a significant disagreement between the effects of placebo and formulation during the whole study period.

Assessment of elasticity of skin

In the current study, formulation showed articulated increase in the skin elasticity measured by Elastometer®. Both placebo and active formulation showed an increase in skin elasticity as depicted in fig. 8. But the effect exhibited by formulation was more pronounced and regular than the placebo. The ANOVA analysis showed the increase in skin elasticity was significant by the application of active formulation as compared with placebo. Pair sample t-test revealed that the effects on skin elasticity with placebo and active formulation were significantly different to each other.

DISCUSSION

Rosaceae family is rich source of phytoconstituents like phenolic acids, flavonoids, vitamin C with a range of diverse and potent physiological functions (Marja *et al.*, 2001). This is also evident from the present study that *Pyrus communis* fruit extract possess ample amount of total phenolic and flavonoid content as described by (Singh *et al.*, 2017). The *Pyrus communis* fruit extract exhibited good antioxidant and anti-tyrosinase activities which may be attributed to the presence of phenolic and flavonoid contents present in its extract (Kriti *et al.*, 2015; Muddathir *et al.*, 2017).

Emulgel containing fruit extract of *Pyrus communis* did not showed any irritation after its application on skin. Patch test with both placebo and active formulation is cleared. It clearly indicates that formulation is safe for the human volunteers use (Gasper *et al.*, 2008).

Sun safety in terms of sunscreens that absorbs harmful UV rays is the prime strategy to prevent photo-damage of the skin (Helfrich *et al* 2008). In the present study the dosage form is Emulgel containing *Pyrus communis* fruit extract showed good Sun protection factor. Prior studies showed that the fruit extract rich in phenolic and flavonoid compounds have the remarkable ability to absorb the UV-radiations and may evidence of prospective natural sunscreens in cosmetic formulations and prevent from photo-induced skin damage (Costa *et al.*, 2015).

Emulgel in the current study showed good anti-erythematic effect which is also supported by a previous study in which it was noted by Chinnasamy *et al* 2014 that *Pyrus communis* extract helps in the wound healing process as it has flavonoid and tannins. Tannins help in the construction of wound. As wound healing process consists of four phases like homeostasis, inflammatory, proliferative and maturation phase. Presence of flavonoid helps in the regeneration of collagen tissues. *Pyrus communis* extract showed a scar less wound healing as shown in a study of its effects on rats (Suntar *et al.*, 2011).

Reduction in melanin content after the application of emulgel containing extract of *Pyrus communis* might be due to the presence of ingredient (Arbutin). Arbutin is natural derivative of hydroquinone. Arbutin has a role in tyrosinase inhibiting effects that's why it is used in cosmetic preparations as a natural skin whitening agent. It also has antioxidant and free radical scavenging activity which also has a role in melanin content reduction (Bulduk *et al.*, 2015). Due to these all properties emulgel containing *Pyrus communis* showed very good whitening effects on human skin. In humans, melanin is produced by melanosomes present in melanocytes in the skin from L-Tyrosine, a phenolic amino acid. This torrent of biochemical reactions is organized by the activity of three enzymes; Tyrosinase, Tyrosinase Related Proteins-1 (TRP-1) and Tyrosinase Related Proteins-2 (TRP-2). Flavonoids and phenolics compounds not only behaves as anti-Tyrosinase effect but also its effect was shown by TRP-1 and TRP-2 (Lee *et al.*, 2017).

This increase in moisture content was related with the presence of vitamin C in the fruit. *Pyrus communis* is a richest source of vitamin C which served to better the hydration level of skin (Ozturk *et al.*, 2015, Kriti *et al.*, 2015). Pear is not only good for oily skin, but is also good for dry and flaky skin. They contain natural humectants, which help balance and preserve the normal water content of the skin and retain its natural moisture also give lubricant effect on skin. Moisture content of skin has a primary role in its physiological working, development and desquamation. High trans-epidermal water loss due to the disturbance in enzymatic activity which results into rough, dry, aged and wrinkled skin (Long *et al.*, 1985). There are many reasons to contribute the water loss and ultimate dryness of the skin and sun produced inflammation is one of the reason of water loss (Kim *et al.* 2007). Hence, the extract of this fruit is widely used in making moisturizing lotion. Regular application of extract can also make your skin even and smooth. Moisture content of skin is maintained by outer layer stratum corneum. So the active formulation help in the maintenance of stratum corneum layer healthy so skin moisture level is maintained. It also neutralizes free radicals and smoothed wrinkles. The current study also showed a marked decrease in skin sebum level which acts as a lubricant and gives water proofing characteristics to the skin. Photo-oxidative stress also contributes to the excessive sebum secretion that involves facial acne development. These results due to the anti 5 α -reductase 1 and anti-oxidant activity polyphenols like Gallic acid present in *Pyrus communis* fruit extract (Koseki *et al.*, 2015).

Decrease in the sebum contents after the application of active formulation may depicted to the presence of polyphenol in fruit extract. It was might be due to the presence of sebaceous glands enzymes may cause

enlargement of sebaceous glands; by changed the testosterone to dihydro-testosterone. As a result of it enlarged sebaceous gland secret high level of sebum secretions in skin. Different phytochemical compounds like polyphenols and sterols may inhibit the production of sebum by preventing the sebaceous gland enzymes which results in the reduction of sebum level. Fruit is also used as household remedy for acne. As it reduces the sebum so helps in the treatment of acne (Dobrev 2007, Ehab *et al.*, 2013).

Collagen is a protein which is responsible for skin elasticity. Vitamin C helps in the construction of collagen and *Pyrus communis* is rich in vitamin C that's why the emulgel containing *Pyrus communis* extract also showed improvement in skin elasticity of volunteers. As the age increases, collagen starts deteriorating by MMPs which results in appearance of skin wrinkles (Kriti *et al.*, 2015). The improvement in skin elasticity in current study may be attributed to the presence of vitamin C and ample amount of phenolic and flavonoids in fruit extracts of *Pyrus communis*.

CONCLUSION

It is concluded from the present study that *Pyrus communis* fruit extract constituted with polyphenolic compounds that show good antioxidant activity. Moreover, it also showed good anti tyrosinase activity which is also evident from its in vivo studies. *Pyrus communis* extract loaded emulgel showed good antiaging potential with improvement in skin tone and elasticity. Also, it ameliorated skin moisture without any complaint of skin irritation and itching. The current study confirms the *Pyrus communis* folklore use as an anti-aging and skin rejuvenation agent. Emulgel containing *Pyrus communis* would be the promising source of skin whitener, moisturizer, toner and antiaging agent used for skin rejuvenation. It is a good addition in cosmetic formulation.

REFERENCES

- Bulduk I and Saglam IA (2015). Optimization of ultrasound-Assisted extraction of Arbutin from *Pyrus communis* leaves by response surface methodology. *J. Biol. & Chem.*, **43**: 167-178.
- Chinnasamy VL and Bhargava A (2014). Wound healing activity of various extracts of fruit of *Pyrus communis* in normal rats. *J. Pharm. Sci. Innov.*, **3**: 148-153.
- Costa SC, Detoni CB, Branco CR, Botura MB, Branco A (2015). *In vitro* photoprotective effects of *Marcetia taxifolia* ethanolic extract and its potential for sunscreen formulations. *Rev. Bras. Farmacogn.*, **25**: 413-418.

- Dobrev H (2007). Clinical and instrumental study of the efficacy of a new sebum control cream. *J. Cosmet. Dermatol.*, **6**: 113-118.
- Dutra EA, Oliveira DA, Kedor H, Maria RE and Miritello SM (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Rev. Bras. Cienc. Farm.*, **40**: 381-385.
- Ehab MM and Mahdi YM (2013). Evaluation of the effect of topical atorvastatin solution for the treatment of papulopustular acne. *Int. J. Curr. Pharm. Res.*, **5**: 58-60.
- Gasper LR, Camargo FB, Gianeti MD and Campos PM (2008). Evaluation of dermatological effects of cosmetics formulations containing *Saccharomyces cerevisiae* extract and vitamins. *Food. Chem. Toxicol.*, **46**: 3493-3500.
- Helfrich YR, Sachs DL and Voorhees JJ (2008). Overview of skin aging and photoaging. *Dermatol. Nurs.*, **20**: 177-183.
- Kaur R and Arya V (2012). Ethanomedicinal and phytochemical perspectives of *Pyrus communis* (L.). *J. Pharmacogn. Phytochem.*, **1**: 14-19.
- Kim DW, Park JY, Na GY and Lee SJ (2006). Correlation of clinical features and skin barrier function in adolescent and adult patients with atopic dermatitis. *Int. J. Dermatol.*, **45**: 698-701.
- Kim Y (2007). Antimelanogenic and antioxidant properties of gallic acid. *Biol. Pharm. Bull.*, **30**: 1052-1055.
- Kirti S, Vani P, Gouri S and Rajinder G (2015). Evaluation of phytochemical and antioxidant activity of raw *Pyrus communis* (L.) an under exploited fruit. *J. Pharmacogn. Phytochem.*, **3**: 46-50.
- Koseki J, Matsumoto T, Matsubara Y, Tsuchiya K, Mizuhara Y, Sekiguchi K, Nishimura H, Watanabe J, Kaneko A, Hattori T, Maemura K and Kase Y (2015). Inhibition of rat 5 α -reductase activity and testosterone-induced sebum synthesis in hamster sebocytes by an extract of *Quercus acutissima* Cortex. *Evid. Based Complement. Alternat. Med.*, **2015**: 1-9.
- Kshirsagar N A (2000). Drug Delivery Systems. *Indian J. Pharmacol.*, **32**: 54-61.
- Kumar L and Verma R (2010). *In vitro* evaluation of topical gel prepared using natural polymer. *Int. J. Drug. Deliv.*, **2**: 58-63.
- Lee SG, Karadeniz F, Seo Y and Kong CS (2017). Anti-melanogenic effects of flavonoid glycosides from *Limonium tetragonum* (Thunb.) bullock via inhibition of tyrosinase and tyrosinase-related proteins. *Molecules*, **22**: 1480-1489.
- Long S, Wertz PW, Strauss JS and Downing DT (1985). Human stratum corneum polar lipids and desquamation. *Arch. Dermatol. Res.*, **277**: 284-287.
- Manosroi A, Jantrawut P, Akihisa T, Manosroi W and Manosroi J (2011). *In vitro in vivo* skin anti-aging evaluation of gel containing niosomes loaded with a semi-purified fraction containing gallic acid from *Terminalia chebula* galls. *Pharm. Biol.*, **49**: 1190-1203.
- Marja P, Kahkonen, Hopia AI and Heinonen M (2001). Berry phenolic and their antioxidant activity. *J. Agric. Food. Chem.*, **49**: 4076-4082.
- Muddathir AM, Yamauchik K, Batubara J, Mohieldin EA and Mitsunage T (2017). Antityrosinase, Total phenolic content and antioxidant activity of selected Sudanese medicinal plants. *S. Afr. J. Bot.*, **109**: 9-15.
- Ozturk A, Demirsoy L, Demirsoy H, Asan A and Gul O (2015). Phenolic compounds and chemical characteristics of Pears (*Pyrus communis*). *Int. J. Food. Prop.*, **18**: 536-546.
- Petkou D, Diamantidis G and Vasilakakis M (2002). Arbutin oxidation by pear (*Pyrus communis*) per oxidases. *Plant. Sci.*, **162**: 115-119.
- Ratshilivha N, Awouafack MD, Toit ES and Eloff NJ (2014). The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 *Moringa oleifera* (Moringaceae) trees enables the selection of trees with additional uses. *S. Afr. J. Bot.*, **92**: 56-64.
- Ribeiro SA, Estanqueiro M, Oliveira BM & Lobo SM (2015). Main benefits and applicability of plant extracts in skin care products. *Cosmetics.*, **2**: 48-65.
- Shailendra KS, Ashutosh B and Bipin KN (2017). Emulgel: Magnifying the application of topical drug delivery. *Ind. J. Pharm. Biol. Res.*, **5**: 25-33.
- Singh M, Chauhan PK, Kumar V and Kour J (2017). Assessment of phytochemical and antioxidant potential of underutilized pear (*Pyrus pyrifolia*) and plum (*Prunus domestica*) from indigenous Himalayan region of Himachal Pradesh. *Int. J. Pharm. Sci. Res.*, **32**: 2982-2987.
- Suntar I, Koca U, Keles H and Akol EK (2011). Wound healing activity of *Rubus sanctus* Schreber (Rosaceae): Preclinical study in animal models. *Evid. Based Complement. Alternat. Med.*, Article ID 816156, 6 pages.
- Wan M, Hu R, Xie X, Gong Z, Yi J, Chen H, Xie L, Guan X, Guan L and Lai W (2017). Skin erythema, pigmentation and hydration kinetics after ultraviolet radiation induced photodamage in Southern Chinese Women. *Photochem. Photobiol.*, **93**: 1276-1281.
- Wolfe K, Wu X and Liu RH (2003). Antioxidant activity of apple peels. *J. Agric. Food. Chem.*, **51**: 609-614.