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# *PRUNUS DOMESTICA*, *PRUNUS PERSICA* AND *PRUNUS AVIUM* EXTRACTS: DETERMINATION OF RADICAL SCAVENGING ACTIVITIES AND DEVELOPMENT OF NATURAL ANTIOXIDANT EMULSIONS

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## Abstract

**Background:** Nowadays antioxidants from plants origin are considered as a promising source of biologically active substances; as synthetic agents are accompanied with a number of side effects.
**Materials and Methods:** Three fruit extracts *Prunus domestica* (plum), *Prunus persica* (peach) and *Prunus avium* (cherry) of family *Rosaceae* and genus *Prunus* were prepared and characterized. These natural antioxidant extracts were used for the development of stable oil/water emulsions. DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was used to evaluate antioxidant activities of various fruit extracts alone and in emulsions containing these extracts. Novel o/w emulsions were developed by using a suitable combination of oil phase and aqueous phase loaded with natural fruit extracts. Physicochemical parameters i.e. organoleptic evaluation (color, liquefaction and phase separation), pH and conductivity were monitored at 8°C, 25°C, 40°C, 40°C + 75% relative humidity and 50°C for 3 months as per stability studies guidelines.
**Results:** Various fruit extracts and emulsions containing these extracts showed good and comparable antioxidant activities. Test formulations containing fruit extracts (6%) showed good stability compared to control formulations and pH and conductivity were found as desired. Formulations were studied for patch test on 33 healthy human (female) volunteers and observed for any skin reaction. No skin irritation was observed.
**Conclusion:** It can be concluded that the final formula was suitable for preparing new antioxidant emulsions loaded with pleasant fruity extracts which remain economical, effective and completely safe for human skin therefore, enhancing patient compliance.

**Key words:** *Prunus domestica; Prunus persica; Prunus avium*; *Rosaceae*, antioxidant activity; novel formulations.

## Introduction

Antioxidants in a cosmetic emulsion formulation may be incorporated as active ingredients as they protect human skin from free radicals produced by UV radiations (Zaman et al., 2011). Primary sources of naturally occurring antioxidants are fruits and vegetables (Akhtar et al., 2008).
*Rosaceae* (the rose family) family of flowering plants having the largest genus *Prunus* (plums, peaches, cherries and almonds) is one of the six most economically important families (Maatta-Riihinen et al., 2004). Plum (*Prunus domestica*) is a stone/drupe fruit like cherry and peach (Okie, 2008). Plums are good sources of fibres, potassium, vitamin A, calcium, iron, vitamin C and anthocyanins, are low in calories (Kim, 2003). However, there is a limitation on the antioxidant activity of plums. Phytonutrients in plums can help keep wrinkles at bay, so can be used as an anti-aging agent (Dae-ok et al., 2003). Peach (*Prunus-persica*) is rich in potassium, magnesium and zinc. Many properties of peach are in its peel, so it is interesting to eat this fruit without peeling (Daymi et al., 2010). Peach has beneficial properties for the skin, especially in cosmetics when used externally to cure dry and dull skins (Athar and Nasir, 2005). Cherry (*Prunus-avium*) is counted amongst the richest fruits in phenolic compounds with an important antioxidant activity. Cherry is rich in water, sugars, vitamins A, B and C, pectin, phenolics and anthocyanins. It is a skin cleanser and reduces inflammation (Usenik et al., 2008).

Emulsions are good vehicle for carrying drugs to body as they increase their bioavailability (Khan et al., 2013). Vanishing creams get their name from the fact that they disappear when spread onto the skin and have a very attractive pearl-like appearance (Kuntal et al., 2012).

Plum, peach and cherry are delicious edible fruits enriched in natural antioxidants. Masks are carried out with pulp of these fruits and applied on face; scanty reports on these fruits in the form of stable o/w emulsion preparations were forced to direct towards their skin applications. A few topical formulations are if available in the market those are very expensive. Hence, attempt was made first time to establish the role of *Prunus domestica*, *Prunus persica* and *Prunus avium* extracts by newly prepared, stable and economical emulsions. Current study also provided a comparison of antioxidant activities and stability parameters of three fruit extracts (of same family *Rosaceae*) and their formulation.

## Materials and Methods

### Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co., Ltd (Saint Louis, USA). Methanol and hydrochloric acid (Merck KGaA Darmstadt, Germany). Distilled water was prepared in the Laboratory of Natural Products and Cosmetics, Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, IUB, Pakistan. Stearic acid from Merck (Germany), Liquid paraffin Merck (Germany), Propyl paraben from Acros Organics (USA), Methyl paraben from Acros Organics (USA), Sodium Hydroxide from Sigma Aldrich Lab. GmbH (USA) and KOH from Riedel (USA).

### Plant Collection, Identification and Voucher References

The fresh fruits of *Prunus domestica, Prunus persica, Prunus avium* used for extraction were purchased from local market. Identification of fruits was made by Herbarium of Qaid-e-Azam University (QAU) Islamabad, Pakistan. Their respective voucher numbers were *Prunus domestica* (Plum) ISL-22356, *Prunus Persica* (Peach) ISL-39523 and *Prunus avium* (Cherry) ISL-83235.

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**Preparation of the Fruit extracts**

Fresh, fully ripened delicious fruits of *Prunus demestica, Prunus persica* and *Prunus avium* were extracted whole (without peeling) after carefully removing the stones. 100 g of each fruit sample was crushed and successively macerated (individually) in a mixture of 400ml of 80% methanol and water in a ratio of 80:20 respectively; with 0.5ml HCl (5%); for 24 hours at room temperature in the dark. The macerated fruit materials were filtered from multiple layers of muslin cloth to get coarse filtrates. The coarse filtrates were then filtered through a Whattman No.1 filter paper to get particle free extracts. To obtain concentrated extracts all the three filtrates were evaporated under vacuum at 40°C rotary evaporator (Eyela, Co. Ltd. Japan) to reduce the volume of the extracts to one third (1/3). Very thick; concentrated liquid extracts were stored in amber containers; under refrigeration until used for further analysis.

**Antioxidant activity & $IC_{50}$ µg/ml**

Antioxidant activity (%age inhibition) and $IC_{50}$ µg/ml (minimum inhibitory concentration) of *Prunus domestica, Prunus persica* and *Prunus avium* fruit extracts were determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Vangdal and Slimestad, 2006).

**DPPH radical scavenging assay**

This method was carried out according to (Baylac and Racine, 2003) for free radical scavenging activity of various extracts alone and emulsions containing extracts. 10µl of test solution was added in 96-wells plate followed by the addition of 90 µl of 100 µM methanolic DPPH solution in a total volume of 100 µl. The contents were mixed and incubated at 37°C for 30 minutes. Synergy HT BioTek® USA micro-plate reader was used to measure any decrease in the absorbance (at 517 nm). Ascorbic acid was used as standard antioxidant. All experiments were carried out in triplicates. For the determination of $IC_{50}$ values, test solutions were assayed at various dilutions such as 0.015, 0.0313, 0.0625, 0.125, 0.25, 0.5 mM. The data was computed on Ez-fit software. The decrease in absorbance indicates increased antioxidant activity (See the following formula).

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

Where;

Absorbance of Control = Total enzyme activity without inhibitor
Absorbance of Test = Activity in the presence of test compound

**Preparation of Emulsions**

The O/W emulsions were prepared by the addition of oily phase to the aqueous phase loaded with the *Prunus domestica/Prunus persica/Prunus avium* fruit extract with continuous agitation (Table-I). The aqueous and oily phases were separately heated to 75 ± 1 °C. After heating the oily phase was added slowly to the aqueous phase with continuous stirring at 1500 rpm for about 15minutes.

Three to four drops of fragrance were added during stirring to improve the aesthetic value of the product. After complete addition of the oil phase, the speed of the mechanical mixer was reduced to 1000 rpm for homogenization for 5 min, the speed was further reduced to 500 rpm for 5 min and finally reduced to 275 rpm for another 5 minutes for complete homogenization, until the emulsion cooled to room temperature and a uniform emulsion formed. Respective control formula without any fruit extract was also prepared by the same method.

**Table 1:** Control and Test Emulsions used in current study (%w/w):

| Ingredients | Control | Active Formulation loaded with *Prunus domestica* | Active Formulation loaded with *Prunus persica* | Active Formulation loaded with *Prunus avium* |
|---|---|---|---|---|
| **Oil Phase:** | | | | |
| Stearic Acid | 20% | 20% | 20% | 20% |
| Paraffin Oil | 8% | 8% | 8% | 8% |
| Propyl paraben | 0.02% | 0.02% | 0.02% | 0.02% |
| **Aqueous Phase:** | | | | |
| NaOH | 0.133% | 0.133% | 0.133% | 0.133% |
| KOH | 1% | 1% | 1% | 1% |
| Methyl Paraben | 0.18% | 0.18% | 0.18% | 0.18% |
| Fruit Extract | Nil | 6% | 6% | 6% |
| Water | Q.S. to make 100% (w/w) | Q.S. to make 100 % (w/w) | Q.S. to make 100 % (w/w) | Q.S. to make 100% (w/w) |

Q.S. = Quantity Sufficient

**Statistics**

At 0.05 level of significance, data was compared to assess the significance of difference using Graph Pad Prism (GPP) version 5.01. Two-way ANOVA (for variation between different time intervals) and LSD (least significant difference) test were used.

**Stability studies and characterization**

Stability tests were performed for active formulations and respective control formulations at different storage conditions i.e. $8^0C \pm 0.1^0C$, $25^0C \pm 0.1^0C$, $40^0C \pm 0.1^0C$, $40^0C \pm 0.1^0C$ with 75% relative humidity (RH) and $50^0C \pm 0.1^0C$. Physical characteristic of creams, such as any change in emulsion colour, creaming, phase separation and/or liquefaction were observed at various specific time intervals for a study period of 3 months. The pH values of emulsions and their conductivity data was taken with digital pH meter and digital electrical conductivity meter

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respectively. Centrifugation tests of emulsions were performed immediately after preparation and after 12, 24, 48 and 72 hours; then after 7, 14, 21, 28, 45, 60, 75 and 90 days of preparation.

**Patch Test**

Patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. 33 healthy human female volunteers (22-40 years) were included in this primary skin irritation test after their written informed consent were obtained. Volunteers were divided into 3 groups (11 in each) for the application of *Prunus-domestica*, *Prunus-persica* and *Prunus-avium* extract creams respectively. The emulsions were applied on forearms of each volunteer. 5cm X 4cm regions were marked on both the forearms. Basic values for erythema and melanin were measured with the help of Mexameter (Courage and Khazaka, Germany); before and after the application of creams. 1.0 g of base and formulation each were applied to the 5cm X 4cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. After 24 hr dressings were removed and the measurements of erythema (redness) and melanin were repeated on both forearms.

## Results and Discussion

DPPH radical scavenging assay (Table-II) showed that all the fruit extracts and emulsions loaded with these extracts have good percentage inhibition and a very less minimum inhibitory concentration ($IC_{50}$) is required for free radical scavenging which is a direct measure of antioxidant activity.

**Table 2:** *In-vitro* antioxidant activity of various fruit extracts alone and emulsions loaded with fruit extracts

| DPPH radical scavenging assay | | | | |
|---|---|---|---|---|
| **Fruit Extract** | **Activity (%) of Extracts** | **$IC_{50}$ µg/ml of Extracts** | **Activity (%) of Emulsions loaded with Extracts** | **$IC_{50}$ µg/ml of Emulsions loaded with Extracts** |
| ***Prunus domestica*** | 86.14±1.33 | 17.5±0.89 | 80.83±1.41 | 19.1±0.26 |
| ***Prunus persica*** | 78.19±1.10 | 18.2±0.73 | 72.69±1.23 | 21.2±0.52 |
| ***Prunus avium*** | 89.84±0.24 | 16.18±0.24 | 83.21±1.11 | 18.1±0.67 |

Data are expressed as means ± standard deviations ($n$ = 3); values in columns are not significantly different ($P$ > 0.05).

**Organoleptic evaluation**

The emulsions (control and active formulations) were kept at 8°C in refrigerator, at 25°C, 40°C, 40°C + 75% RH (Relative Humidity) and 50°C in thermal incubators for 90 days. They were organoleptically evaluated to note any change in odour, colour, liquefaction and phase separation for a period of 3 months in compliance with ICH guidelines at interval of 12, 24, 48 and 72 hours; then after 7, 14, 21, 28, 45, 60, 75 and 90 days of preparation. No differences were observed in the organoleptic properties among emulsions for the period of 3 months under different temperature conditions.

The freshly prepared control (without any extract) was white while the active formulations (loaded with fruit extracts) were light pink in colour. There was no change occurred in colour up to the observation period of 3 months. This showed that the emulsions were stable at different storage conditions i.e. 8°C, 25°C, 40°C, 40°C + 75% RH (relative humidity) except 50°C throughout 3 months study period. No change in the colour may be attributed to different factors contributing the emulsions stability.

**Centrifugation Test**

Centrifugation test was performed using 5g of sample in disposable stopper tubes. This test was performed at 25°C (room temperature) and 5000rpm speed for the period of 10 minutes. No phase separation was observed in any of sample of control and active formulations (peach, plum and cherry) kept at different temperature conditions for the period of 3 months except at 50°C temperature where little phase separation was observed. It showed the proper homogenization speed during emulsion formulation prevented them from breakage during stress testing as described by Nour and Yunus (2006). Centrifugation is an extremely helpful means for assessing and predicting the shelf life of emulsions (Herbert et al., 1988).

**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 in pH values as a function of time and storage temperatures indicated that the examined emulsions were stable. The pH values of the emulsions kept at 8°C, 25°C, 40°C, 40°C + 75% RH (relative humidity) and 50°C for 3 months have been determined. Comparative variations in pH of *Prunus domestica*, *Prunus persica* and *Prunus avium* at different temperature conditions as a function of time (average values from zero hour to 90 days study period) has been given in Figure 1.

In present study, the pH of freshly prepared control was 6.68 whereas the pH of peach, plum and cherry fruit-derived extract based o/w emulsions was 6.65, 6.65 and 6.72 respectively, which is within the range of skin pH. The pH values of control and *Prunus domestica*, *Prunus persica*, *Prunus avium* formulation kept at different storage conditions i.e. 8°C, 25°C, 40°C , 40°C + 75% RH and 50°C was found to be increasing gradually in the 1st week and then it started to decline continuously till the 90th day of study period. At the end of study, pH of control at 8°C, 25°C, 40°C, 40°C + 75% RH and 50°C was 6.5, 6.4, 6.2, 6.2, and 6.01 respectively. Whereas pH of peach, plum and cherry emulsions, kept at 8°C, 2°C, 40°C, 40°C + 75% RH and 50°C showed gradual reduction with slight variations with time. There was greater decrease in pH at

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50°C temperature. The pH values of peach emulsions were 6.5,6.4, 6.2, 6.2, and 6.0 and pH value of plum emulsions were 6.5,6.5, 6.4, 6.4, and 6.1 whereas the pH values of cherry emulsions were 6.5,6.5, 6.3, 6.3, and 6.1 at 90th day respectively.

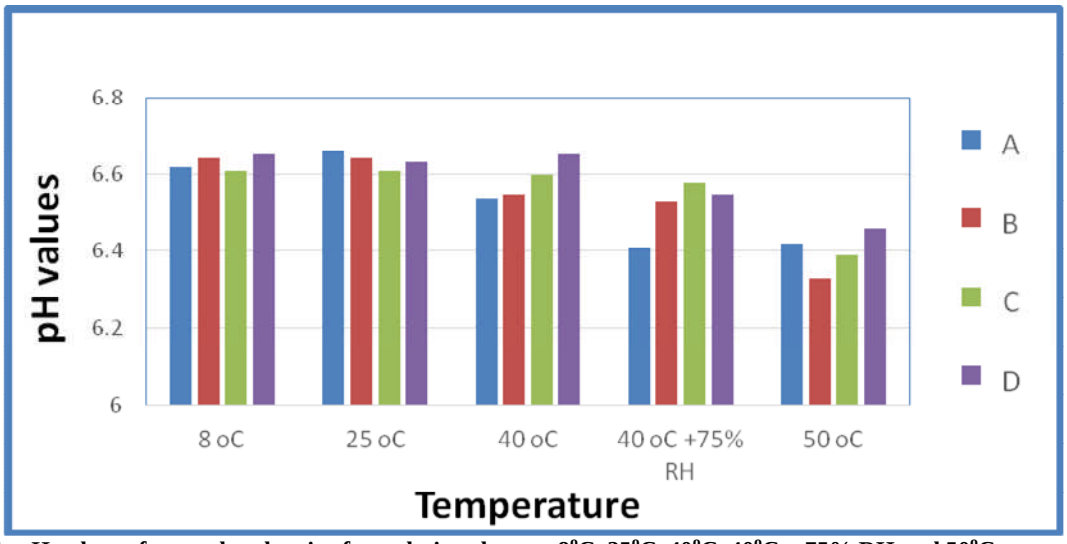


**Figure 1: pH values of control and active formulations kept at 8°C, 25°C, 40°C, 40°C + 75% RH and 50°C**
Where,
**A** = Control (without any extract)
**B** = Active Formulation loaded with PLUM extract
**C**= Active Formulation loaded with PEACH extract
**D** = Active Formulation loaded with CHERRY extract

It was established that the production of metabolites or decomposition of any ingredient at accelerated conditions causes the decrease in pH at different storage conditions (Akhtar, 2001). Using two-way analysis of variance (ANOVA) technique at 5% level of confidence, it was found that changes in pH of different samples of control and active formulations were insignificant with respect to time and temperatures. When LSD (least significant difference) test was applied to check the individual average effects of the pH of the samples of control at different temperatures with passage of time by taking average pH values of zero hours as standard, it demonstrates insignificant changes for both, control and active formulations.

**Electrical conductivity**

The conductivity (µS/cm) of the prepared emulsions was measured immediately after preparation. Then all the samples were stored at various temperature conditions such as at 8°C (refrigerator), 25°C (incubator), 40°C (incubator) and 40 °C + 75% relative humidity (incubator) and 50°C (incubator). Electrical conductivity was then measured after specific time intervals i.e. 12 hours, 2412 hours, 4812 hours and 72 hours; then after 7 days, 14 days, 21 days, 28 days, 45 days, 60 days, 75 days, and 90 days of study period. For each sample conductivity was measured in triplicate.
The comparative conductivity difference of control and active formulations (peach, plum and cherry) at different temperature conditions as a function of time (average values from zero hour to 90 days study period) has been represented in Figure 2.

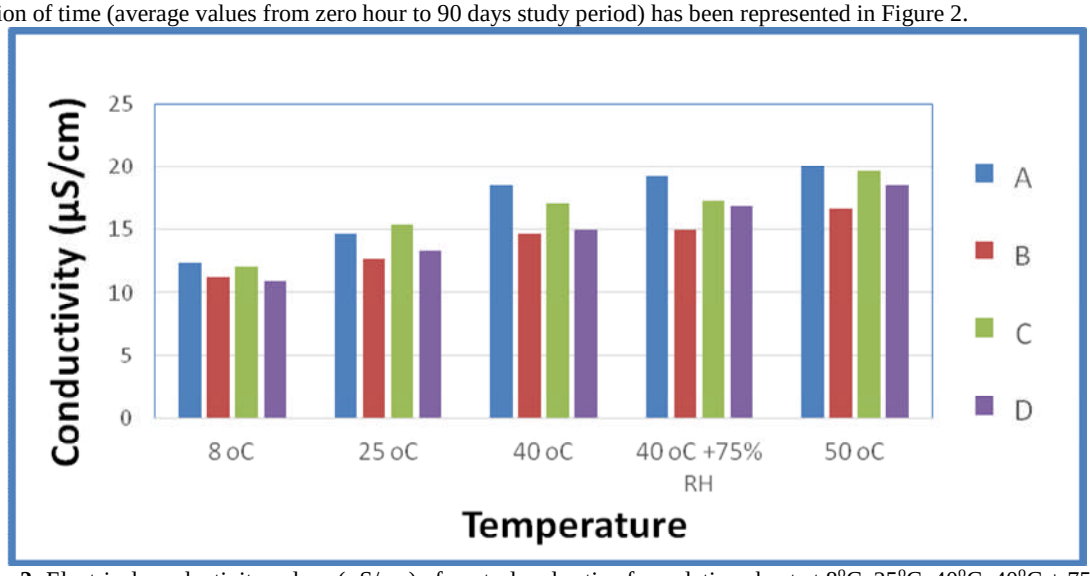


**Figure 2:** Electrical conductivity values (µS/cm) of control and active formulations kept at 8°C, 25°C, 40°C, 40°C + 75% RH and 50°C
Where,
**A** = Control (without any extract)
**B** = Active Formulation loaded with PLUM extract
**C**= Active Formulation loaded with PEACH extract
**D** = Active Formulation loaded with CHERRY extract
Emulsion type and stability was confirmed by performing the electrical conductivity test. Variation in electrical conductivity is an indication of phase separation and creaming process in an emulsion (James et al. 2000). In the current work, electrical conductivity of all the emulsion samples

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stored at various temperatures was measured for a time period of 3 months at specific time intervals. Results revealed that there was an insignificant difference in electrical conductivity of emulsion samples which were placed at 8°C temperature, 25°C temperature, 40°C temperature and 40°C temperature + 75% relative humidity; from its preparation (o hour) to three months study duration indicating the stability of emulsions. The electrical conductivity of emulsion samples kept at 50°C showed decline which indicated instability according to Martin (1970).

**Patch test**

Adverse skin reactions i.e., redness, inflammation, itching, irritation or any change in skin morphology were checked for a period of 24 h (Akhtar et al., 2008). No skin irritation, redness or inflammation was observed; rather all the emulsions loaded with 6% natural fruit extracts produced cooling effects. The melanin and erythema contents were significantly decreased after application of active formulations of plum, peach and cherry extract (Figure-III).

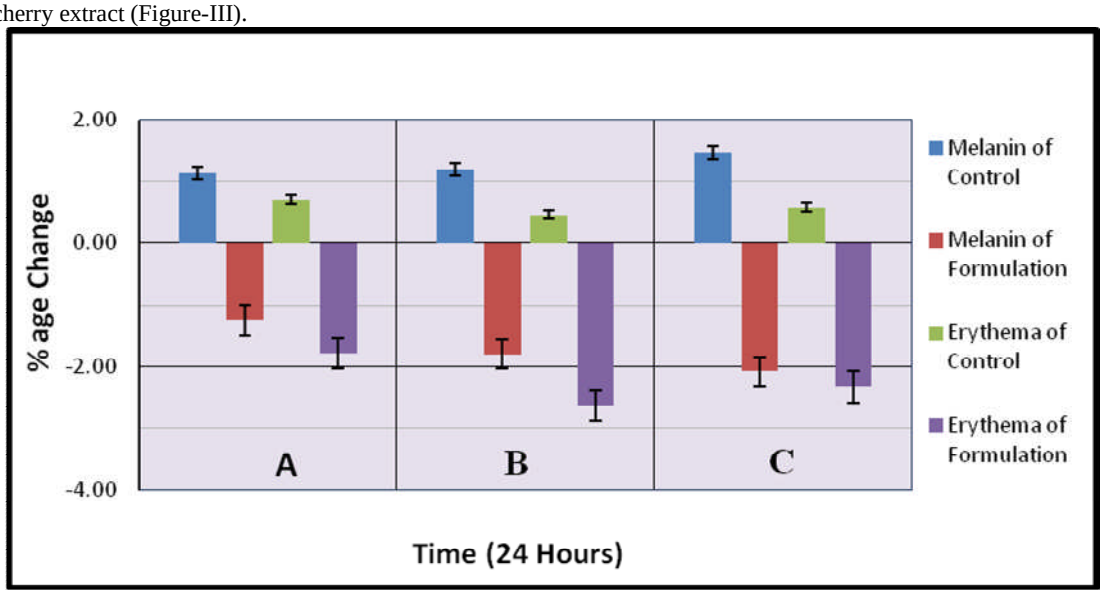


**Figure 3:** Patch test values (percentage changes in melanin and erythema contents) of control and active formulations
Where,
**A** = Percentage changes in Melanin and Erythema contents of Active Formulation loaded with *Prunus domestica* extract and its control
**B**= Percentage changes in Melanin and Erythema contents of Active Formulation loaded with *Prunus persica* extract and its control
**C** = Percentage changes in Melanin and Erythema contents of Active Formulation loaded with *Prunus avium* extract and its control

**Conclusion**

It was concluded that the *Prunus domestica*, *Prunus persica* and *Prunus avium* extracts and novel oil-in-water emulsions loaded with natural extracts have good and correlated radical scavenging activity and desired stability. These *Prunus* fruit extracts of same family *Rosaceae* could be considered for preparing natural topical formulations for use in skin care.

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