

Formulation of *Phaseolus vulgaris* L. cream and its characterization

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Abstract: Red kidney beans have antioxidant effect and thereby can help in skin smoothening, moisturizing, whitening and have anti-wrinkles effect. The study was based on the formulation of a stable w/o emulsion possessing extract of *Phaseolus vulgaris* L. seeds, using paraffin oil with the aim to investigate its effect on various skin parameters. The extract, achieved by concentrating ethanolic extract of red kidney beans was embedded in the internal aqueous part of w/o emulsion. An active formulation possessing concentrated extract of red kidney beans and a placebo formulation having no active material in the aqueous phase were formulated and placed at various conditions for the duration of 28 days, to observe the stability of cream. The placebo and formulation were stable at different storage conditions in terms of phase separation and colour changes. Minute liquefaction was observed from 21st day up to 28th day in formulations which were kept at 40°C +75% RH (relative humidity). With the passage of time significant changes were observed in formulation pH while insignificant changes were observed at basic pH. Different effects of creams i.e placebo and formulations were observed on the human skin by applying them on the volunteer's cheeks for about 8 weeks. A stable w/o emulsion can be formulated by using red kidney beans' extract without any phase separation, liquefaction and colour change over 28 days storage.

Keywords: *Phaseolus vulgaris* L. seeds, red kidney beans extract, skin sebum, melanin, skin moisture, TEWL.

INTRODUCTION

Two immiscible liquids on proper mixing may constitute a two-phase system known as emulsion, which can be stabilized by the use of proper surfactants. Depending on the dispersed and dispersion phase, emulsions are broadly categorized as being water in oil (w/o) or oil in water (o/w) emulsions (Remington *et al.*, 2006). This system has been successfully employed to improve drug bioavailability and to avoid first pass effect when used topically (Marti-Mestres and Nielloud 2002). The emulsion formulation improves the therapeutic as well as distribution properties of the drug contents (Waqas *et al.*, 2010). Emulsion formulations can also be used as solvents to deliver drug substances to the body. These formulations can also be loaded with plant extracts to impart cosmetic properties (Waqas *et al.*, 2010). Numerous formulations with potential antioxidant and cosmetic properties have already been prepared. The w/o emulsions are used more extensively for dehydrated skin (Akhtar *et al.*, 2011).

Phaseolus vulgaris L. commonly known as red kidney beans (RKB), is an important legume crop that is used world-wide as food (Kereena and Vishnuvardhan 2012) (Wani *et al.*, 2017) for its taste and enriched protein,

vitamins and mineral contents like Fe (70 mg/kg) and Zn (33 mg/kg) and energy (32%) (Beebe *et al.*, 2000, Medina Velo *et al.*, 2017, Apodaca *et al.*, 2018). The RKB are also reported to contain unsaturated fatty acids (Kereena and Vishnuvardhan 2012), and a variety of glycosides (Choung *et al.*, 2003). Aqueous extracts of RKB hulls have strong anti-oxidant and anti-inflammatory activities when tested on COX-1 & COX-2 owing to their phenolic contents (Oomah *et al.*, 2010). The methanolic extract of RKB has exhibited multiple activities e.g. anti-mutagenic effect, free radical scavenging activity (Cardador-Martínez *et al.*, 2002), reduction in the risk of heart diseases and colon cancer (Kereena and Vishnuvardhan 2012) and improvement in metabolism (Grant *et al.*, 1995). Anti-oxidant effects of extracts containing phenolic compounds have already been established (Espinosa *et al.*, 2015). Phenolic compounds have applications as anti-wrinkle, whitening and sunscreen agents (Grant *et al.*, 1995, Hayat *et al.*, 2014).

The present study aimed at the development of w/o cream from RKB extract and to evaluate its physicochemical and physiological properties. The evaluation parameters comprise of colour, pH, liquefaction, electrical conductivity, phase separation, centrifugation, TEWL (Trans epidermal water loss) and dermatological tests.

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MATERIALS AND METHODS

Materials and apparatus

Red kidney beans (RKB), Distilled water, Abil-EM 90 (Franken Chemical, Germany), Lemon oil (Chemoflor Manufacturing Corp. Pakistan) and paraffin oil (Merck Germany). Digital pH-meter (WTW PH-17i, Germany), Conductivity meter (WTW COND-197i, Germany), Incubators (Sanyo, Japan), Water bath (HH-S 21-4, China), Digital humidity meter (TES Electronic Corp, UK), Centrifuge and Electrical balance (Precisa, Switzerland and Hettich, Germany), Mechanical mixer (IKA, Germany). TEWA meter and Corneometer MPA5 (Courgae + kazaka, Germany).

Preparation of plant extract

The weighed quantities of *Phaseolus vulgaris* L. seeds were pulverized and macerated in 80% ethanol solution for 3 days with occasional shaking (twice a day for ten minutes). The macerated pulverized material was filtered through 16-folds of muslin cloth (coarse filtration) and later through Whatman # 01 filter paper (fine filtration) to obtain the extract. Finally, the extract was concentrated on rotary evaporator (Rasul and Akhtar 2014) to obtain concentrated extract of red kidney beans.

Preparation of placebo cream

The w/o emulsion was prepared by mixing and agitating the aqueous and oil phases. For the preparation of placebo cream, an oily phase composed of a 3% surfactant Abil EM 90 and 14% paraffin oil 14% was heated up to 75°C ±1°C. Aqueous phase was also heated at the same temperature and mixed drop by drop with the oily phase (Waqas *et al.*, 2010) at 2000 RPM to achieve complete mixing of oil and aqueous phases. During mixing few drops of lemon oil were added to give pleasant fragrance to the emulsion. The cream was further mixed at relatively lower speeds, first at 1000 RPM and then at 500 RPM for 5 minutes each to complete the homogenization and allowed to cool down at room temperature (Akhtar *et al.*, 2010).

Preparation of formulation cream

Both aqueous and oil phases were prepared as explained earlier and heated separately until temperature reached to 75°C +1°C. Red kidney bean extract (3%) was added in the aqueous phase and then the aqueous phase was mixed drop by drop to the oily phase (Ali *et al.*, 2013). After the mixing of aqueous and oil phases same procedure was followed that was used in the placebo preparation (Akhtar *et al.*, 2010, Rasul and Akhtar 2014).

Cream characterization

The distinguished features of placebo and formulation creams were assessed by the determination of pH, centrifugation test, electrical conductivity test and physical analysis. Stability tests were also conducted under different storage conditions to observe the effects of

different temperature ranges on the emulsions (Akhtar *et al.*, 2011).

Evaluation of Creams on skin

Eleven healthy male volunteers between the age of 25 and 35 years (RCT codes = AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK, AL) were selected for the evaluation of cream on their skin with the selection criteria as; Only healthy volunteer, Not using any other cream during or at least two weeks before the study, Not allergic to any of the contents of cream and who gave written consent. The study was approved by the institutional ethics committee of Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan. Written detailed information was provided and informed consents were obtained from all the volunteers. Moreover, the dermatological examination of all the volunteers were also performed before the start of actual study. After assuring volunteers health, both placebo and formulation creams were applied to each volunteer's face (Ali *et al.*, 2013). Creams were marked 'left' or 'right' showing use of that cream on left and right cheeks. Volunteers applied the both creams for eight weeks as per instructions. Every volunteer was weekly examined by a Dermatologist to avoid any unwanted effects (Akhtar *et al.*, 2011).

Dermatological tests

In dermatological test melanin/erythema, moisture, sebum contents and also epidermal water loss (TEWL) were determined after 1, 2, 3, 4, 6 and 8 weeks from the start of cream application (Rasul and Akhtar 2014). Briefly, melanin and erythema contents were measured using mexameter (Courga+khazaka electronic GmbH). Measurements were taken on pre-defined time intervals after the application of either placebo or formulation creams. The sensitivity of placebo and formulation creams was checked by patch test prior to the actual experiment (Rasul and Akhtar 2014).

Mathematical and statistical analysis

To analyse the differences in different dermatological parameters among the individuals, the assays were performed in triplicates and results were expressed as mean values + S.D. (standard deviation). The calculated data was subjected to the one-way analysis of variance (ANOVA), using the statistical package (SPSS 26.0). Differences between placebo and formulation creams were determined by paired t-test, while variations among different time intervals were evaluated by Tukey's test at p<0.05. Asterisks indicate significant differences.

RESULTS

Stability testing

Four separate samples of placebo and formulation were prepared and placed at various temperature conditions i.e., 8+0.1°C in refrigerator, 25+0.1°C, 40+0.1°C in incubator

and $40 \pm 0.1^\circ\text{C}$ in incubator having 75% relative humidity (Rasul and Akhtar 2014). Samples were observed for the time intervals till 28 days under various storage conditions. The properties like colour change, liquefaction and phase separation were observed (Badiu et al., 2009).

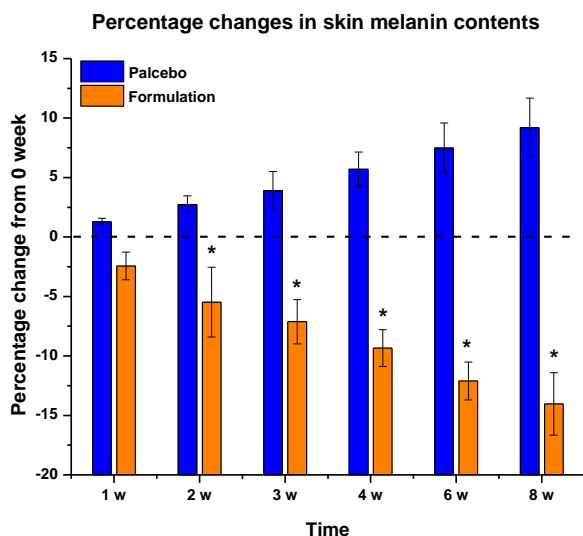


Fig. 1: Percentage change in skin melanin content after application of placebo and formulation creams

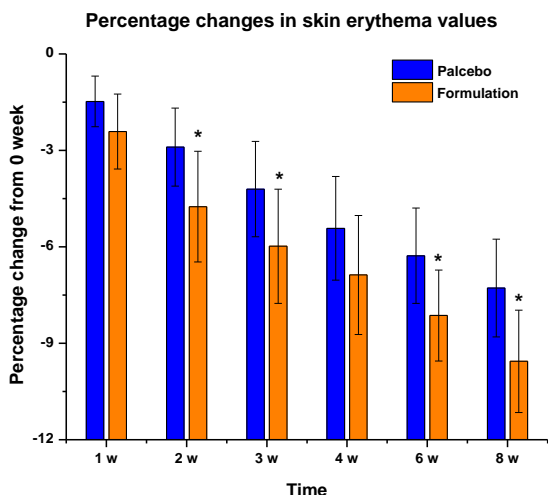


Fig. 2: Percentage change in skin erythema content after application of placebo and formulation

No change in colour was observed. All the samples of placebo and formulation placed at various conditions of temperature and humidity were observed for liquefaction. The slight liquefaction was observed in both placebo and formulation samples kept at accelerated temperature (40°C) without humidity and with humidity on 28th day. Considering phase separation as a stability parameter, the formulations were comparatively more consistent than the placebo stored at the elevated temperatures (Badiu et al., 2009).

In centrifugation test, separation of phases was not observed in the preparations after keeping them under various storage conditions till 28th day of study period (Waqas et al., 2010). The electrical conductivity tests of all the placebo samples as well as formulations were performed by using conductivity meter after storage under several different temperature conditions for four weeks at specific time intervals e.g. for 24 hours, 7 days, 14 days, 21 days and 28 days (Akhtar et al., 2011). No electrical conductivity was observed in placebo samples as well as formulations up to 28 days.

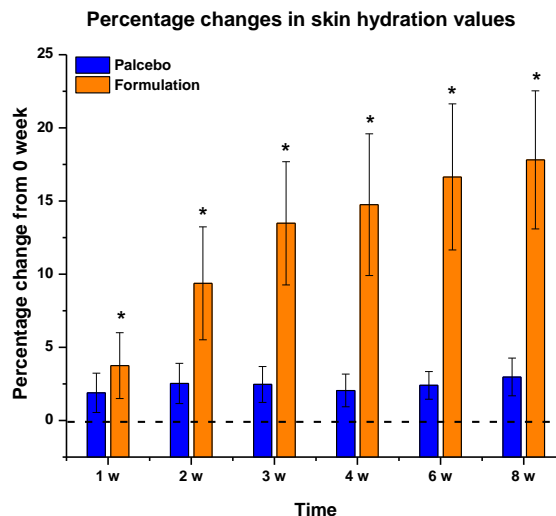


Fig. 3: Percentage change in skin hydration content after application of placebo and formulation creams

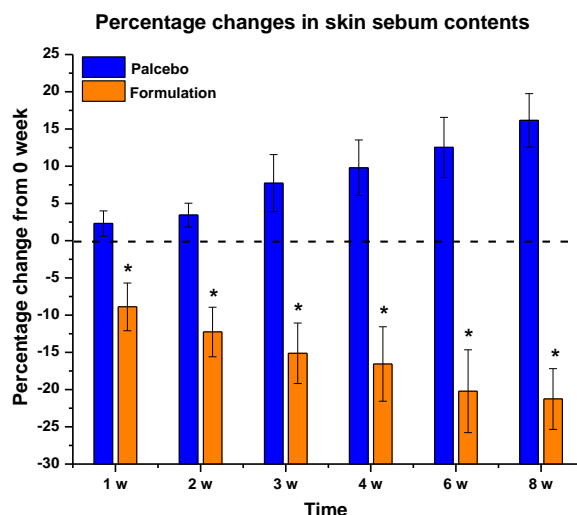


Fig. 4: Percentage change in skin sebum content after application of placebo and formulation creams

pH of both the placebo and formulation samples showed a timely decline from 24 h to 28 days as given in table 1 (Akhtar et al., 2011). However, significant variation in the pH of formulation samples was observed under

Table 1: The pH values of placebo and formulation kept at 8°C, 25°C, 40°C and 40°C + 75% RH (P = placebo, F= formulation, RH = relative humidity).

Time	8°C		25°C		40°C		40°C + 75% RH	
	P	F	P	F	P	F	P	F
Fresh	5.92	5.67	5.92	5.67	5.92	5.67	5.92	5.67
12hours	5.85	5.62	5.78	5.64	5.78	5.59	5.71	5.56
24hours	5.72	5.53	5.61	5.59	5.64	5.53	5.43	5.49
36hours	5.68	5.48	5.43	5.54	5.45	5.29	5.25	5.43
48hours	5.43	5.39	5.28	5.48	5.35	5.11	4.98	5.38
72hours	5.31	5.28	5.12	5.33	5.21	4.95	4.92	5.24
7days	5.14	5.16	4.94	5.21	5.09	4.87	4.8	5.09
14days	4.98	5.03	4.81	4.98	4.93	4.84	4.71	4.92
21days	4.72	4.92	4.68	4.72	4.82	4.72	4.67	4.81
28 days	4.59	4.78	4.49	4.67	4.71	4.68	4.24	4.73

various temperature conditions. In least significant difference (LSD) test the change in the pH of formulation samples were significant in similar storage condition with the time intervals. Aldehyde and organic acids production by paraffin oil oxidation may be the main cause of decrease in formulation pH at different storage conditions (Rowe *et al.*, 2009).

Dermatological tests

Melanin

During the evaluation of the effect of placebo and formulation on the formation of skin melanin by using mexameter (Courga + khazaka electronic GmbH), the skin melanin contents were increased over the time (insignificant change) by placebo cream while the formulation significantly decreased the skin melanin contents from week 0 (represented by dotted line) as shown in fig. 1.

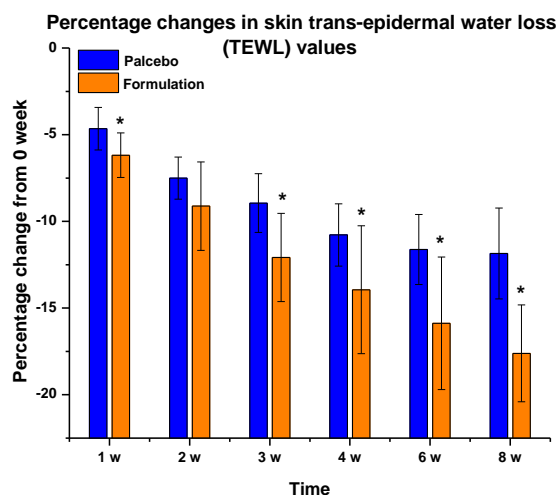


Fig. 5: Percentage change in trans-epidermal water loss after application of placebo and formulation creams

Erythema

The erythema contents were slightly decreased (insignificant change) after the application of placebo,

while in case of formulation a significant decrease in the skin erythema contents was observed from 1st to 8th week of observation as shown in fig. 2.

Skin moisture contents

The skin moisture contents were recorded by Corneometer (Courga + khazaka electronic GmbH) and the values are shown in figure 3. Intergroup differences at different time intervals were also analysed using one way ANOVA and post hoc Tukey’s test to observe the variations and it was observed that the placebo cream showed insignificant variations while formulation has significant difference in the study of 8th week.

Skin sebum contents

The effect of placebo as well as formulation creams on sebum contents of human skin was examined by using Sebometer (Courga+ khazaka electronic GmbH) and the values are shown in fig. 4. When LSD test was performed, it was observed that the variation in the skin sebum contents recorded at specific periods after applying placebo and formulations were significant.

Trans-epidermal water loss (TEWL)

A significant variation in TEWL was observed after the application of both placebo and formulation as shown in fig. 5.

DISCUSSION

The developed w/o cream from RKB extract was evaluated for its physicochemical and physiological properties like colour change, liquefaction and phase separation (Badiu *et al.*, 2009).

The stability in colour of placebo and formulation was most probably due to the presence of paraffin oil (Waqas *et al.*, 2010). The active ingredients i.e. red kidney beans extract contains polyphenols (Cardador-Martínez *et al.*, 2002) that kills most of the bacteria owing to their antimicrobial effect (Lopez-Hernandez *et al.*, 1993).

Thereby help to protect the active formulation contents from microbial growth and the colour change of the active formulation during storage. The slight liquefaction was observed in both placebo and formulation samples kept at accelerated temperature (40°C) without humidity and with humidity on 28th day, which might be due to the increased temperature. The consistency of the emulsion in term of phase stability at the elevated temperatures (Badiu *et al.*, 2009) was due to increase phase viscosity at low temperature (Waqas *et al.*, 2010). In centrifugation test, separation of phases was not observed in the preparations after keeping them under various storage conditions till 28th day of study period (Waqas *et al.*, 2010).

The main cause of decrease in formulation pH at different storage conditions of both the placebo samples as well as formulations were may be due to production of aldehyde and organic acids by paraffin oil oxidation (Rowe *et al.*, 2009).

The effect of placebo and formulation on the formation of skin melanin was also observed. The decrease in skin melanin contents by the use of formulation was due to its antioxidant and free radical scavenging activity, owing to the presence of polyphenols (Choung *et al.*, 2003, Waqas *et al.*, 2010, Marathe *et al.*, 2016, Roy *et al.*, 2019) and diglycosides of quercetin and kaemferoll (Cardador-Martínez *et al.*, 2002, Lin *et al.*, 2008). The slight decrease in erythema contents after the application of formulation cream was due to Carotenes present in the red kidney beans (Lopez-Hernandez *et al.*, 1993) which also consists of tocopherols and are effective in scavenging reactive oxygen species (Tsuda *et al.*, 1994). These ingredients are responsible for anti-erythema effects of formulations. The significant increase in moisture contents by formulation cream may be due to vitamins C in the red kidney beans extract. Vitamin C helps in the collagen synthesis (Colven and Pinnell 1996). Hydration level also increases when collagen level is increased (Sharma *et al.*, 2008). Placebo cream increased the sebum contents that may be due to thick viscous oily nature of paraffin oil, whereas, formulation cream contains flavanol glycosides, pro-anthocyanidins (Beninger and Hosfield 2003) and anthocyanin are also present in red kidney beans coat (Choung *et al.*, 2003), which reduce the sebum contents in the skin (Ali *et al.*, 2013). A significant variation in TEWL was observed after the application of both placebo and formulation creams, which may be due to the fatty acids available in red kidney beans extract and they help in preventing TEWL, retain skin hydration and keep it healthy for longer periods.

CONCLUSION

The objective of this study was to develop a w/o cream from the extract of *Phaseolus vulgaris* L. seeds, using

paraffin oil and to investigate its effect on various skin parameters.

It can be concluded that a stable w/o emulsion can be formulated by using red kidney beans extract without any phase separation, liquefaction and colour change over 28 days storage. When tested on human volunteers, RKB formulation cream successfully whitened the skin, reduced skin melanin contents, skin erythematic values, TEWL, skin sebum levels and enhanced skin moisture contents in a significant manner.

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REFERENCES

- Akhtar N, Anwar M, Khan BA, Mahmood T and Shahiq-Uz-Zaman (2011). Formulation development and pharmaceutical evaluation of a w/o emulsion of Coleus extract. *Ind. J. Pharm. Edu. Res.*, **45**(3): 236-241.
- Akhtar N, Khan BA, Mahmood T, Parveen R, Qayum M, Anwar M and Farooq M (2010). Formulation and evaluation of antisebum secretion effects of sea buckthorn w/o emulsion. *J. Pharm. Bioall. Sci.*, **2**(1): 13.
- Akhtar N, Khan HMS, Iqbal A, Khan BA and Bashir S (2011). *Glycyrrhiza glabra* extract cream: Effects on skin pigment melanin. International Conference on Bioscience, Biochemistry and Bioinformatics IPCBEE. Iacsit Press, Singapore.
- Ali A, Akhtar N, Khan MS, Khan MT, Ullah A and Shah MI (2013). Effect of Moringa oleifera on undesirable skin sebum secretions of sebaceous glands observed during winter season in humans. *Biomedical Research*. **24**(1): 127-130.
- Apodaca SA, Medina-Velo IA, Lazarski AC, Flores-Margez JP, Peralta-Videa JR and Gardea-Torresdey JL (2018). Different forms of copper and kinetin impacted element accumulation and macromolecule contents in kidney bean (*Phaseolus vulgaris*) seeds. *Sci. Total Environ.*, **636**: 1534-1540.
- Badiu D, Roncea F and Roşoiu N (2009). Formulation and pharmaceutical evaluation of three W/O Emulsions with *Mytilus galloprovincialis* Imk. and *Rapana venosa* lipid extracts. *Farmacia*, **57**(2): 212-217.
- Beebe S, Gonzalez AV and Rengifo J (2000). Research on trace minerals in the common bean. *Food Nutr. Bull.*, **21**(4): 387-391.
- Beninger CW and Hosfield GL (2003). Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *J. Agr. Food Chem.*, **51**(27): 7879-7883.

- Cardador-Martínez A, Loarca-Piña G and Oomah BD (2002). Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *J. Agr. Food Chem.*, **50**(24): 6975-6980.
- Choung MG, Choi BR, An YN, Chu YH and Cho YS (2003). Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). *J. Agr. Food Chem.*, **51**(24): 7040-7043.
- Colven RM and Pinnell SR (1996). Topical vitamin C in aging. *Clin. Dermatology*, **14**(2): 227-234.
- Espinosa R, Inchingolo R, Alencar SM, Rodriguez-Estrada MT and Castro IA (2015). Antioxidant activity of phenolic compounds added to a functional emulsion containing omega-3 fatty acids and plant sterol esters. *Food Chemistry*, **182**: 95-104.
- Grant G, Dorward PM, Buchan WC, Armour JC and Pusztai A (1995). Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: Effect on body composition and organ weights. *Brit. J. Nutr.*, **73**(01): 17-29.
- Hayat I, Ahmad A, Ahmed A, Khalil S and Gulfranz M (2014). Exploring the potential of red kidney beans (*Phaseolus vulgaris* L.) to develop protein based product for food applications. *J. Anim. Plant Sci.*, **24**(3): 860-868.
- Kereena C and Vishnuvardhan Z (2012). Effect of soya and red kidney bean supplementation on nutrient intakes of head and neck cancer patients. *Ind. J. Fund. and Appl. Life Sci.*, **2**(1): 266-275.
- Lin LZ, Harnly JM, Pastor-Corrales MS and Luthria DL (2008). The polyphenolic profiles of common bean (*Phaseolus vulgaris* L.). *Food Chemistry*. **107**(1): 399-410.
- Lopez-Hernandez J, Vazquez-Oderiz L, Vazquez-Blanco E, Romero-Rodriguez A and Simal-Lozano J (1993). HPLC determination of major pigments in the bean *Phaseolus vulgaris*. *J. Agr. Food Chem.*, **41**(10): 1613-1615.
- Marathe SA, Deshpande R, Khamesra A, Ibrahim G and Jamdar SN (2016). Effect of radiation processing on nutritional, functional, sensory and antioxidant properties of red kidney beans. *Radiat. Phys. Chem.*, **125**: 1-8.
- Marti-Mestres G and Nielloud F (2002). Emulsions in health care applications — An overview. *J. Disper. Sci. Technol.*, **23**(1-3): 419-439.
- Medina Velo I, Barrios A, Zuverza-Mena N, Hernandez-Viezas J, Chang C, Ji Z, Zink J, Peralta-Videa J and Gardea-Torresdey J (2017). Comparison of the effects of commercial coated and uncoated ZnO nanomaterials and Zn compounds in kidney bean (*Phaseolus vulgaris*) plants. *J. Hazard Mater.*, **332**: 214-222.
- Oomah BD, Corbé A and Balasubramanian P (2010). Antioxidant and anti-inflammatory activities of bean (*Phaseolus vulgaris* L.) hulls. *J. Agr. Food Chem.*, **58**(14): 8225-8230.
- Rasul A and Akhtar N (2014). Anti-aging potential of a cream containing milk thistle extract: Formulation and *in vivo* evaluation. *Afr. J. Biotech.*, **11**(6): 1509-1515.
- Remington JP, Troy DB and Beringer P (2006). Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins.
- Rowe RC, Sheskey PJ and Quinn ME (2009). Handbook of Pharmaceutical Excipients, Pharmaceutical Press.
- Roy M, Sarker A, Azad MAK, Shaheb MR and Hoque MM (2019). Evaluation of antioxidant and antimicrobial properties of dark red kidney bean (*Phaseolus vulgaris*) protein hydrolysates. *J. Food Meas. Charact.*, DOI 10.1007/s11694-019-00292-4.
- Sharma S, Poddar R, Sen S and Andrews J (2008). Effect of vitamin C on collagen biosynthesis and degree of birefringence in polarization sensitive optical coherence tomography (PS-OCT). *Afr. J. Biotech.*, **7**(12): DOI: 10.5897/AJB07.486.
- Tsuda T, Osawa T, Ohshima K and Kawakishi S (1994). Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris* L. *J. Agr. Food Chem.*, **42**(2): 248-251.
- Wani IA, Sogi DS, Wani AA and Gill BS (2017). Physical and cooking characteristics of some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. *J. Saudi Soc. Agr. Sci.*, **16**(1): 7-15.
- Waqas MK, Akhtar N, Ahmad M, Murtaza G, Khan H, Iqbal M, Rasul A and Bhatti NS (2010). Formulation and characterization of a cream containing extract of fenugreek seeds. *Acta Pol. Pharm.*, **67**(2): 173-178.