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Research Article

Exploring the potential use of pomegranate (*Punica granatum* L.) and prickly pear (*Opuntia ficus indica* L.) peels as sources of cosmaceutical sunscreen agent for their antioxidant and photoprotective properties

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ABSTRACT

Nowadays, consumer's demand for natural sources-derived sunscreen products, to get photoprotection from harmful ultraviolet radiations is forcing cosmetic industry to develop such breakthrough formulations by incorporating antioxidants as active phytochemical ingredients. Plant extracts are currently being focused for the purpose, but bio-wastes including fruit peels remain yet unexplored and consequently, underutilized. The present study is aimed to evaluate the photoprotective and antioxidant potential of pomegranate (*Punica granatum* L.) and prickly pear (*Opuntia ficus indica* L.) fruit peels extracts and to valorize their potential use as sources in cosmeceutical sunscreen agents. The selected extracts were estimated for antioxidant activity by DPPH, ABTS, FRAP, CUPRAC and GOR methods and their antioxidant properties were correlated to reference standards (BHT and BHA). The sun protection factor (SPF) was used to assess their photoprotective effect (SPF) *in vitro*. It was found that hydroalcoholic peel fruit pomegranate (*Punica granatum* L.) extract possessed highest antioxidant activities significantly (p<0.0001) in all the methods compared to peel fruit prickly pear (*Opuntia ficus indica* L.) extract, possessing rich source of antioxidants. Hydroalcoholic peel fruit pomegranate (*Punica granatum* L.) extract, also presented the highest phenolic content. It was found that antioxidant activity was due to phenolic compounds containing flavonoids and confer photoprotection. The highest SPF was also related to hydroalcoholic peel fruit pomegranate (*Punica granatum* L.) extract with highest levels of protection against ultraviolet light with sun protection values ranging at 44.402±0.438. It is rational to apply these active compounds from waste to sunscreens, in order to increase UV protection.

Keywords:
Punica granatum L., *Opuntia ficus indica* L., Peel fruit extract, Antioxidant activity, Sun Protection Factor (SPF)

1. INTRODUCTION

The main growing segment in the world agriculture production is the fruits and vegetables, in which various products like which juices, jams, and dehydrated products are commercialized. The useful bioactive compounds (phenols, peptides, anthocyanin, fatty acids and carotenoids) fibers, and enzymes are of great interest and key components from the by-products of the agri-food industry (peels, seeds, shells, pomace, and leaves). They are source of functional foods and drugs against acute and chronic diseases. They add the values in food, pharmaceutical industries and cosmetic as well as contribute in economy[1-2].

The use of fruits and vegetables by-products as cosmetic ingredients is an excellent opportunity for the community to develop natural ingredients potential. Cosmetic preparation preferred by the public, especially women, is cream. Cream preparation is used as a protector for the

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outer skin layer from sun exposure that can easily damage skin tissues[3]. Inhabitants of countries like Middle East and Maghreb countries especially Algeria, need skin protection because they are exposed to sun while outdoor workings; so the skin health needs to be maintained; not be damaged by exposure to solar radiation and must have protection from impact of UVB and UVA radiations, because Ultraviolet (UV) rays of the sun can accelerate the aging process of the skin[4-5]. The long term (over the years) exposure to ultraviolet rays can cause structural skin disorders, especially skin cancer and premature skin aging[4-5]. These damages may be observed clinically, histopathologically, and functionally[6].

Prickly pear (*Opuntia ficus indica* L.) and Pomegranate (*Punica granatum* L.) are considered as highest source of micronutrients and antioxidants compared to other types of fruits; belonging to the most commonly consumed fruits in Algeria[7-9]. Unfortunately, the massive amount of by-products i.e. peels are produced during the large scale consumption of theses fruits.

According to the report of United Nations Food and Agriculture Organization (FAO), the fruits and vegetables by products account for 60% compared to other food products. Pomegranates and Prickly pear peels generally account for roughly 54% and 55% of the fruits, respectively[10-11]. There is approximately 1.62 million wastes of pomegranate from total of three-million tons waste from the fruits[12].

To our knowledge, there is limited literature on the study of the cosmetic benefits of by-products of pomegranate and prickly pear. In the light of this, the purpose of our research focusing on hydroalcoholic peel fruit (by-products) extracts of pomegranate (*P. granatum* L.) and prickly pear (*O. ficus indica* L.) was conducted for the investigation of their antioxidant activities as well as potential photoprotective effects against the sun exposure effects on skin to evaluate the primary active ingredient in the formulation of cosmeceutical sunscreen products.

The estimation for *in vitro* sun-protection factor (SPF) and the photostability of crude extract was measured using a diffuse reflectance spectrophotometry. Additionally, we determined the total phenol, expressed in gallic acid equivalents (GAE); the flavonoid and flavonols contents by reactions with aluminum trichloride; expressed as quercetin equivalents (QE); and the antioxidant activity, using several tests including DPPH, ABTS, FRAP, CUPRAC and GOR methods, expressed as half maximal inhibitory concentration (IC_{50}) or half maximal effective concentration (EC_{50}).

2. MATERIALS AND METHODS

2.1. Chemicals

The solvents utilized were obtained from PROLAB, MERK EUROLAB. Chemicals i.e.1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), radical (2,6-Di-tert-butyl-a-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadiene-1-ylidene)-p-tolyloxy), Gallic acid, Quercetin, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), Folin-Ciocalteu 's phenol reagent, sodium carbonate (Na_2CO_3), Aluminum trichloride ($FeCl_3$), Sodium acetate (CH_3CO_2Na), potassium persulfate ($K_2S_2O_8$), chloride ($FeCl_3$) potassium ferricyanide [$K_3Fe(CN)_6$], Neocuproine ($C_{14}H_{12}N_2$) and Cupric chloride ($CuCl_2$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals and solvents used were of analytical grade.

2.2. Phytochemical characterization

2.2.1. Preparation of extract

Punica granatum L. and *Opuntia ficus indica* L. fruits peels were obtained in autumn 2020, from Skikda-Algeria. Samples were authenticated by botanists and their voucher specimens were deposited (No. F184/ 2020). The plant materials were allowed to dry at room temperature under dark conditions for 2 weeks. *Punica granatum* L. and *Opuntia ficus indica* L. dried fruits peels were grounded separately in mixer grinder. The grounded biomass was subjected to thorough maceration in methanol/water (70/30; *v/v*). The extractions were carried out at intervals of 24 h by an automatic shaker, assisted the extraction at room temperature for 30 min by ultrasound. The extraction was performed twice under the identical conditions of remaining material. The extracts were combined and filtered; which were further concentrated under vacuum at low temperature (<40°C) in rotary evaporator to afford crude extracts. Crude extracts were subjected to lyophilization immediately. Samples were stored under low temperature (-25°C) until use for further experimentation[13].

2.2.2. Determination of total phenolic content (TPC)

Folin Ciocalteu assay was performed to determine the total phenolic contents (TPC) of the plant samples as described by Zeghad et al.[9]. For this purpose 1 mL of extract dissolved in distilled water was mixed in 5 mL of Folin-Ciocalteu reagent (10%) with addition of 4 mL sodium carbonate (0.7 M). Sample was incubated at room temperature for 2 hrs and then the absorbance was recorded by spectrophotometer (Spectroscan 60 DV UV/Vis, Biotech Engineering Management Co. Ltd.) at 760 nm against water as blank. Standard; gallic acid (GA) solution in water (0-0.1 mg/mL) was used to construct calibration curve. All tests are performed in triplicate (n=3). TPC was expressed as mg GAE/g dry fruit extract with mean±standards deviation[9].

2.2.3. Determination of total flavonoid content (TFC)

TFC of the all the plant samples was estimated according to a colorimetric method[9]. In this process, extract (2 mL) was dissolved in ethanol then added 2 mL of $AlCl_3$ (2%) reagent in ethanol. After incubation period of 30 min at room temperature the absorbance of samples was recorded at 420 nm by spectrophotometer (Spectroscan 60 DV UV/Vis, Biotech Engineering Management Co. Ltd.) while using an ethanol as a blank. Calibration curve primed with standard quercetin in ethanol (0-0.1 mg/mL) was constructed. All tests are repeated in triplicate (n=3). TFC was expressed as mg QE g^{-1} dry fruit extract mean±standards deviation[9].

2.2.4. Determination of flavonol content

Since the antioxidant activity was represented by main fraction of flavonol compounds. So, the flavonol content of all the plant samples was specifically determined[9]. For this process. 2 mL of the sample that was dissolved in ethanol; was mixed with 2 mL of $AlCl_3$ (2%), prepared in ethanol (96%) by the addition of 3 mL sodium acetate solution (50 g/l).The absorption was determined by spectrophotometer (Spectroscan 60 DV UV/Vis, Biotech Engineering Management Co. Ltd.) at 440 nm after incubation of 2.5 hrs at 20°C[9]. The construction of standard calibration curve primed with quercetin in ethanol (0-0.1 mg/mL) was performed. Tests were repeated in triplicate manner (n=3). Total flavonol content was expressed as mg QE g^{-1} dry fruit extract with mean±standards deviation.

2.3. Antioxidant activities

2.3.1. DPPH antioxidant assay

DPPH radical (1,1-diphenyl-2-picrylhydrazyl) assay was used for determination of free radical scavenging activity spectrophotometrically of the samples. For this process 2 mL of each plant sample (0.1 to 1 mg/mL) dissolved in methanol was added in equal amount of a methanolic solution of DPPH (0.004%)[9]. All samples were incubated for 30 min at room temperature and then absorbance was measured at 517 nm using the spectrophotometer (Spectroscan 60 DV UV/Vis Biotech Engineering Management Co. Ltd.). BHT and BHA were taken as positive controls. Calculation of scavenging activity (%) was performed from absorbance values using the equation below:

$$\text{Percentage Scavenging activity (\%)} = [(A-B)/A]\times 100$$

A: Absorbance value of control (DPPH solution without sample)
B: Absorbance value of sample

All tests are repeated three times (n=3). The antioxidant activity of samples and control was estimated by determining the IC_{50} (µg/mL) values, i.e. the concentration of samples required to scavenge 50% DPPH radicals, expressed as mean±standards deviation.

2.3.2. ABTS radical scavenging assay

ABTS° radical (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) produced by oxidation of $ABTS^{2-}$ through potassium persulfate was used for this assay. Radical scavenging assay by utilizing ABTS was performed following the method of Re et al.[14]. During this process, 7 mM ABTS solution (5 mM NaH_2PO_4, 5 mM Na_2HPO_4, 154 mM NaCl) at pH 7.4 was added in 2.5 mM potassium persulfate (v/v) to produce ABTS° radical, followed by storage for 16 hrs in dark before its use. To adjust absorption at 0.70±0.02 units at 734 nm by spectrophotometer (Shimadzu UV-1800, Shimadzu corporation Kyoto-Japan), the mixture was diluted with ethanol. During this process, 100 µl of various concentrations (0.1-1 mg/mL) of each sample were dissolved in tampon buffer (5 mM NaH2PO4. 5 mM Na2HPO4, 154 mM NaCl), and allowed to react with freshly prepared ABTS solution (900 µl) at room temperature[9]. The absorbance of each sample was measured at 734 nm after six min of mixing. BHT and BHA concentrations (0.01-0.1 mg/mL) were used to prepare the standard curve. All tests were performed in triplicate way (n=3) and then was also calculated the IC_{50} (mg/L) value, the concentration of sample that is required to scavenge 50% of ABTS radicals, expressed as mean±standards deviation. The same (above-mentioned) equation was used for ABTS assay.

2.3.3. Ferric reducing antioxidant power (FRAP)

The reducing power of *Punica granatum* L., and *Opuntia ficus indica* L. fruits peel extracts was determined by the method of Hseu et al.[15]. 1 mL of each sample (0.1-1 mg/mL) was mixed with potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1%) and phosphate buffer (1 mL, 0.2 M, pH 6.6). 20 min incubation was followed at 50°C. An aliquot of trichloroacetic aid (TCA) (1 mL, 10%) was poured in the solution, centrifuged at 3,000 rpm following for 10 min. Afterwards,$FeCl_3$ (150 µl, 0.1%) and distilled water (1.5 mL) were mixed in the supernatant, and then measured the absorbance at 700 nm by spectrophotometer (Spectroscan 60 DV UV/Vis, Biotech Engineering Management Co. Ltd.). BHT and BHA were used as a reference materials[9]. All tests were performed in triplicate manner (n=3) for all the samples. EC_{50} (µg/mL) signifying the effective concentration of an extract with which the absorbance is equal to 0.5 deduced from the curve, reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) in the presence of *Punica granatum* L. or *Opuntia ficus indica* L. was expressed as mean±standards deviation.

2.3.4. Cupric reducing capacity (CUPRAC)

For the determination of cupric ions (Cu^{2+}) reducing ability of the extracts, the method of Apak et al.[16] with little modification was used. In this procedure, 50 µl (0.01 M) $CuCl_2$ solution, 50 µl (7.5×10^{-3} M) ethanolic neocuproine solution, and 60 µl (1 M, pH=7) ammonium acetate ($C_2H_7NO_2$) buffers were mixed in test tube, followed by addition of 40 µl of extract having various concentrations (0.1-1 mg/mL). After the incubation of mixture sample at room temperature for 60 min, the absorbance was measured at 450 nm (EnSpire Multimode Plate Reader, PerkinElmer, France) using methanolic solution as blank. The reference standards used for antioxidant were BHT and BHA. All tests were performed in triplicate way (n=3). Expressed the EC_{50} value (µg/mL) signifying the effective concentration of an extract, with which the absorbance is equal to 0.5 deduced from the curve reduction of Cu^{2+} in the presence of *Punica granatum* L. or *Opuntia ficus indica* L. as mean±standards deviation.

2.3.5. Glavinoxyl (GOR) radical scavenging assay

The galvinoxyl radical (2,6-Di-tert-butyl-a-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadiene-1-ylidene)-p-tolyloxy) was found to be more reactive against phenolic compounds and this assay is recommended for compounds that are donor of electron and hydrogen[17]. Method of Shi et al.[17] was used to evaluate Galvinoxy free radical scavenging activity. During this process, in 40 µL of extracts (samples), 160 µL of Galvinoxyl radical 0.1 mM (4 mg in 100 mL MeOH) was added. The absorbance was measured at 428 nm after the incubation of mixture solutions for 120 min. Galvinoxyl solution in methanol was taken as a blank while the BHT and BHA solutions were used as antioxidant standards. All the tests were performed in triplicate (n=3). The antioxidant activity of samples and control was estimated by determining the values of IC_{50} (µg/mL), i.e. the concentration of samples needed to scavenge 50% GOR radicals, expressed as mean±standards deviation.

2.4. Determination of sun protection factor (SPF)

The values of *Punica granatum* L. and *Opuntia ficus indica* L. fruits peels for the Sun Protective Factor (SPF) were conducted *in vitro*, by method of Mansur et al.[18]. The stock solution (1 mg/ml) of dried extract, was prepared and then different concentrations were made using distilled water. Spectrophotometric (Shimadzu UV-1700, Japan) readings were taken for these solutions with the wavelength ranging 290 to 320 nm, using quart cell (1 cm) whereas water was used as a blank. The absorption reading was noted for every 5 min. At each point quadruplicate reading was taken and then SPF value of formulations was determined by the Mansur equation below.

$$SPF=CF\times\sum_{290}^{320} EE(\lambda)\times I(\lambda)\times Abs(\lambda)$$

Where, CF=10 (correction factor), EE (λ)=erythemogenic effect of radiation at wavelength λ, I (λ)= intensity of solar light at wavelength λ, and Abs (λ)= absorbance of wavelength by a solution. The absorbance values obtained were multiplied by the EE (λ) values; The correlation factor 10 was multiplied by summation taken[19-20].

2.5. Statistical analysis

All the results were expressed as mean±SD. The Tukey (HSD) test was used to determine the level of significance. The $p<0.05$ value was considered as significant. The obtained results were compared to standard control. The XL Stat version was used for Statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

Both pomegranate and prickly pear fruits contain many different kinds of antioxidants[9,21-22]. Polyphenolic compounds, flavonoids and flavonols are the most effective antioxidative constituents in fruits peels[23]. Thus, it is important to quantify their contents and to assess their contribution to antioxidant activity. Phenolic content, flavonoids and flavonols contents are expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively. Our results have indicated that hydroalcoholic fruits peels extract of pomegranate (*Punica granatum* L.) has significantly ($p<0.0001$) a higher TPC, TF and TFL values than fruits peels extract of *Opuntia ficus indica* L. The highest TPC, TF and TFL were obtained for hydroalcoholic fruits peels extract of pomegranate (*Punica granatum* L.) (590.07±13.58 mg/g GAE g^{1-}, 227.85± 46.48 mg/g QE g^{1-}, 91.57±5.79 mg/g QE g^{1-}, respectively) (Table 1). The current results show that main compounds are flavonoids and flavonol derivatives in the extracts of pomegranate fruits peels, which is in agreement with findings of studies performed previously[24-26]. It is further reported that level of phenolic compounds is changed with fruits parts and their cultivars as discussed by Sushil Belkacem et al.[22]; Linlin et al.[23] and Kumar Middha et al.[27].The peel extract contained the highest level of total phenolic content that were obtained in all cultivars.

To evaluate the antioxidant activity of plant extracts, there are two types of typical assays. The potential of plant extract to cause reduction of ions or oxidants (by acting as reducing agents) like ferric ion, cupric ion measures the first category. FRAP (measures the reduction potential of ferric to ferrous ion), and CUPRAC

Table 1. Total phenolic contents (TPC), total flavonoid contents (TFC) and total flavonol contents (TFLC) of *Punica granatum* L. and *Opuntia ficus indica* L. wastes and their antioxidant activities.

Samples	TPC (mg/g) GAE g-1	TFC (mg/g) QE g-1	TFCL(mg/g) QE g-1	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)	FRAP EC50 (µg/ml)	CUPRAC EC50 (µg/ml)	GOR IC50 (µg/ml)
Punica granatum L.	590.07 ± 13.58*	227.85 ± 46.48**	91.57 ± 5.79*	75.04 ± 0.00	26.91 ± 0.0004	350.83 ± 0.18	66.36 ± 0.004	2.55 ± 0.00
Opuntia ficus indica L.	110.51 ± 30.81	74.40 ± 0.91	25.24 ± 1.76	2600.82 ± 0.00$^{\#}$	841.06 ± 0.03$^{\#}$	4744.62 ± 0.41$^{\#}$	701.86 ± 0.04$^{\#}$	733.64 ± 0.04$^{\#}$
BHT	/	/	/	22.32 ± 1.00	1.29 ± 0.30	6.77 ± 1.15	8.97 ± 0.94$^{\#\#}$	13.90 ± 2.54
BHA	/	/	/	5.73 ± 0.00	1.81 ± 0.10	34.30 ± 2.38	5.35 ± 0.71$^{\#\#}$	2.98 ± 0.38

GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent. Values are expressed as Mean±SD (Tukey HSD-test, n=3); *p<0.0001, **:p<0.01 vs *Opuntia ficus indica L.* $^{\#}$:p<0.0001, $^{\#\#}$:p<0.05 vs *Punica granatum*.

Table 2. Values of sun protection factor of *Punica granatum* L., *Opuntia ficus indica* L. peel fruits extracts and commercial sunscreens (Avène® 30+ and Avène® 50+).

λ (nm)	*Punica granatum* L.		*Opuntia ficus indica* L.		Commercial sunscreen (Avène® 30+)		Commercial sunscreen (Avène® 50+)	
	ABS	EE(λ)xI(λ)x Ab (λ)1	ABS	EE(λ)xI(λ)x Ab (λ)1	ABS	EE(λ)xI(λ)x Ab (λ)1	ABS	EE(λ)xI(λ)x Ab (λ)1
290	4.445 ± 0.301	0.667 ± 0.045	2.810 ± 0.048	0.422 ± 0.007	4.457 ± 0.026	0.669 ± 0.004	5.141 ± 0.048	0.771 ± 0.007
295	4.195 ± 0.494	3.427 ± 0.404	2.269 ± 0.019	1.854 ± 0.015	4.465 ± 0.024	3.648 ± 0.019	5.492 ± 0.019	4.487 ± 0.015
300	4.746 ± 0.000	13.640 ± 0.000	1.977 ± 0.020	5.682 ± 0.057	4.409 ± 0.037	12.672 ± 0.105	5.010 ± 0.020	14.398 ± 0.057
305	4.445 ± 0.301	14.571 ± 0.987	1.814 ± 0.024	5.947 ± 0.080	4.416 ± 0.037	14.476 ± 0.120	4.934 ± 0.024	16.173 ± 0.080
310	4.212 ± 0.404	7.851 ± 0.752	1.718 ± 0.024	3.202 ± 0.044	4.428 ± 0.046	8.254 ± 0.085	5.021 ± 0.024	9.359 ± 0.044
315	4.244 ± 0.174	3.552 ± 0.145	1.654 ± 0.018	1.384 ± 0.015	4.415 ± 0.024	3.705 ± 0.020	4.827 ± 0.018	4.040 ± 0.015
320	3.853 ± 0.317	0.694 ± 0.057	1.602 ± 0.015	0.288 ± 0.003	4.490 ± 0.050	0.808 ± 0.009	4.880 ± 0.015	0.879 ± 0.003
(SPF)		44.40 ± 0.44		18.78 ± 0.21*		44.23 ± 0.35^{NS}		50.11 ± 0.53*

Values as expressed as Mean±SD (Tukey HSD-test, n=3); *p<0.0001, NS: Not Significant p>0.05.
EE(λ): erythemal effect spectrum; I(λ): solar intensity spectrum; SPF: sun protection factor.

(measures the reduction of cupric to cuprous ion) are two main assays. The free radical (stable free radicals like DPPH, ABTS and GOR) scavenging ability of plant extract is exhibited by second type of assay[28].

In the present study, hydroalcoholic fruits peels extract of pomegranate (*Punica granatum* L.) exhibited significantly (p<0.0001) the greater antioxidant activities than those from hydroalcoholic peel fruit extract of prickly pear (*Opuntia ficus indica* L.) against DPPH ABTS and GOR radicals (as shown in Table 1). The experimental results revealed that the pomegranate peel extract was efficacious in scavenging of different radicals.

The results of reducing power to FRAP (reduction Fe^{3+} to Fe^{2+}) and CUPRAC (reduction Cu^{3+} to Cu^{2+}) indicate that pomegranate fruits peels extract was significantly (p<0.0001) stronger than prickly pear fruit peels extract (Table 1), with strong ability to reduce Fe^{3+} to Fe^{2+} and Cu^{3+} to Cu^{2+} as reported by Qabaha et al. and Benslimane et al.[28-29] using the FRAP and CUPRAC assays.

The antioxidant activity of plants is exhibited by the presence of phenylpropanoid derivatives i.e. polyphenols besides the secondary metabolites. The fruits peels extract have the antioxidant activities due to presence of the polyphenol content. Flavonoids and phenolic acids are potential radical terminators and main components of phenolic compounds in the natural products, playing key role for antioxidant activities i.e., the electrons to radicals are donated by them[27].

Correlation results show that the phenolic contents, flavonoids and flavonols contents of both extracts were correlated positively with DPPH (r^2=0.997, 0.894 and 0.988, respectively) ABTS (r^2=0.992, 0.889 and 0.987, respectively) and GOR (r^2=0.988, 0.888 and 0.987, respectively), in agreement with previous reports. Total phenolic, flavonoids and flavonols contents of both tested extracts were also correlated positively to FRAP (r^2=0.976, 0.887 and 0.97, respectively) and CUPRAC (r^2=0.979, 0.884 and 0.985, respectively). These correlations indicate that the antioxidant activity of fruits peels extracts significantly depend on their polyphenolic content including flavonoids, flavonols, condensed and hydrolysable tannins, that account 92% antioxidant activities by them[22]. Our results are in agreement with prior studies as reported by Middha et al.[27].

The difference in the composition of phenolic compounds of the peel extract of pomegranate and pear prickly ascribed the differences in antioxidant activities. There is more need of studies for the isolation and characterization of individual phenolic compounds for the elucidation of different antioxidant mechanisms possibly existing among the compounds and their synergism.

3.2. *In vitro* SPF assay

The SPF (Sun Protection Factor) is a measurement for the effectiveness of sunscreen formulation quantitatively. It provides an idea that how much long an individual can stay without getting burn from sunrays while staying in the sunshine. Following standard procedures reported by Mansur et al.[18], SPF was assessed. The absorbance and SPF values for *Punica granatum* L and *Opuntia ficus indica* L. peels extracts and commercial sunscreen calculated by UV-Spectrophotometrically are indicated in Table 2.

P. granatum L. peel fruit extract showed significantly highest (p<0.0001) SPF activity (SPF=44.402±0.438) when compared with *Opuntia ficus indica* L. fruits peels extract (SPF=18.780±0.214). The least activity was also significantly (p<0.0001) noticed in *Opuntia ficus indica* L. bark fruit extract when compared with the two commercial sunscreens Avène® 30+(SPF=44.230±0.347) and Avène® 50+(SPF=50.107±0.527).

The SPF values are key parameters according to which sunscreen products are categorized. The values from 2 to under 12 are classified as "minimal sun protection" and 12 to under 30 are "moderate sun protection" and the products that have SPF values 30 and above are defined as "high sun protection," respectively[11]. The good sunscreen products have higher efficacy of SPF values and it depends on the duration of exposure to sun before feeling the burn effect[11].

Irradiation of UV rays on the skin increases collagenase activity and contributes to the formation of wrinkles through degradation of collagen in the extracellular matrix of the skin. Human skin as the main organ of the human body, is constantly exposed to external factors, which can generate free radicals, so there is a continual need for testing the antioxidative properties of several plant extracts. It is commonly known that the topical use of antioxidants in cosmetics can better protect and possibly correct the damage of the skin by neutralizing free radicals and, in the final effect, act as an antiaging agent[29]. Phenolic content, especially flavonoids have an excellent antioxidant effect because they have a chromophore group that can absorb UV A and UV B rays thereby reducing its intensity on the skin[30]. This suggests that the capacity of *Punica granatum* L. peel extract as sunscreen and photo-protector against UVB radiation is related to its phenolic compounds, that act as filters absorbing UV A and UV B photons and by its antioxidant activity by preventing photooxidative damage[31-32], preventing UV-induced oxygen free radical generation, lipid peroxidation and DNA damage[33].

Although, our results showed that the peels extract of pomegranate show high protection against UV radiation, it can be assumed they have the potential to be applied in cosmetic formulations, particularly in sunscreen cosmetics. The skin health products can be integrated by the bioactive compounds of pomegranate rind (biowaste) especially flavonoids (good candidates for use in photoprotective products) and may be converted into value-added products. The bioactive compounds; Ellagic acid

and punicalagin promote skin health by inhibiting tyrosinase and initiating anti-inflammatory and anti-fungal effects are present in pomegranate rind[34-36]. Pomegranate seed oil has rich content of punicic acid, which gives anti-inflammatory and protective characteristics against UV-induced radiation[37]. In addition, aging-induced glycation, a process that negatively affects the elasticity of skin is also inhibited by the Pomegranate oils[38]. The results deduced from these studies show that pomegranate extract has promising pharmaceutical and cosmetic applications. The disorders e.g. UV-induced hyperpigmentation, decreased skin elasticity and skin wrinkling may be effectively treated by pomegranate fruits peels extracts[39-40]. In our future work, several cosmetic formulations using the above-mentioned extracts are planned for study.

4. CONCLUSION

The current study has been reported first time for the bioactivity potent of *Punica granatum* L. and *Opuntia ficus indica* L. fruits peels extracts against UV light and radiations. Especially, the pomegranate fruits peels have excellent antioxidant and photoprotective properties with high SPF value. Findings based on this research, *Punica granatum* L fruits peels extract is main component as substitute the synthetic sunscreen agents in cosmetic industry due to presence of naturally active ingredients. The consumption of bio-waste plant extract in cosmetic industry will minimize the skin disorder that are mostly caused by use of artificially synthesized ingredients in cosmetic products commercially. Not only that, in processing plant the load of waste will be reduced by using modern processing techniques or the in plant treatment and agricultural waste reuse. The development of nano-emulsion-based topical sunscreen formulation by incorporating *Punica granatum* L peel fruit extract as the main active ingredient and its *in vivo* photoprotection efficiency is further under work progress.

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NZ methodology, data analysis and interpretation of results. NZ and EA article structuring and writing. MZK and AB revision and supervision. All authors have read and approved the manuscript for publication.

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