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Development of Photoprotective Creams with Antioxidant Polyphenolic Herbal Extracts

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ABSTRACT

Herbs having polyphenolic constituents are antioxidant in nature and are used for combating the deleterious effects of ultraviolet radiations thus producing photoprotective effects. The herbs selected were turmeric (dried rhizomes) and pomegranate (dried seeds) which could produce photo protective effect. The alcoholic extracts were produced by continuous hot extraction method taking 90% v/v ethyl alcohol and preliminary phytochemical identification tests were performed for the detection of constituents. Total polyphenol content of the extracts was measured using the Folin-Ciocalteu colorimetric method and antioxidant effect by reducing power estimation method in which the antioxidant activity of extract was compared with that of standard (ascorbic acid). Creams were developed taking 0.5, 1 and 2% extracts individually and evaluated for physicochemical parameters and long term skin hydration studies. Present studies showed that the selected herbal extracts have photoprotective properties in which the total phenolic content and antioxidant activity of *Punica granatum* extract was obtained higher than *Curcuma longa* extract. The skin hydration studies of *P. granatum* extract loaded creams show significantly improved hydration as compared to *Curcuma longa* extract loaded creams.

Key words: Polyphenols, antioxidant, free radicals, photoprotectives, herbal creams

INTRODUCTION

Ultraviolet radiations cause excitation of electrons and thereby generate photochemical reactions leading to adverse biological effects like photoaging and photocarcinogenesis. Photoaging causes dermal damage with marked elastotic degenerative change, loss of collagen, reduction in number and size of fibroblasts, an increase in proteoglycans and a moderate mononuclear inflammatory cell infiltrate. Photoprotection could be achieved by the use of sunscreens, moisturizers, keratolytics and antioxidants (Chanchal and Swarnlata, 2009). Since, multiple pathways are involved in photocarcinogenesis so mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens could also be an effective approach for reducing ultraviolet generated reactive oxygen species mediated photodamage, immunosuppression and skin cancer in humans (Saraf and Kaur, 2010).

Natural phenolics act as antioxidants by preventing UV induced oxygen free radical generation and lipid peroxidation. Three important groups of phenolics include phenolic acids, flavonoids and high molecular weight polyphenols (Svobova *et al*., 2003). Various plant extracts which constitutes vitamins like ascorbic acid, vitamin E; phenolic compounds and enzymes possess the ability to reduce the oxidative damage (Ashawat *et al*., 2007a; Atrooz, 2009). Pomegranate

(*Punica granatum* family punicaceae) is a rich source of two type of polyphenolic compounds namely anthocyanidins (delphinidin, cyanidin and pelargonidin) and hydrolysable tannins (punicalin, peducalagin, punicalagin, gallic and ellagic acid esters. Pomegranate fruit extract acts as photochemopreventive by inhibiting the UV generated phosphorylation reactions (Afaq and Mukhtar, 2006). Turmeric (*Curcuma longa*, family Zingiberaceae) has established anti-inflammatory, hepatoprotective, anti-microbial, wound healing, anti-cancer, anti-tumor and anti-viral properties. The important active constituents include curcumin, demethoxycurcumin and bis-demethoxycurumin (Saraf and Kaur, 2010). These herbal extracts could also be incorporated into the cream formulations which could be used as the preventives from photoaging as used by Ashawat *et al.* (2007b) with few other herbal antioxidant extracts. The application of novel approaches can also improve its efficacy regarding continuous action of herbs on the human body. The penetrating power and hydrant properties of the components of the novel systems could also be utilised to synergise the photoprotective effect of these herbal extracts (Chanchal and Swarnlata, 2008). Recent advances in nanotechnology show their promise as potential cosmetics for poorly soluble, poorly absorbed and labile herbal extracts and phytochemicals. The aim of this study was to produce extracts of both the herbs; perform preliminary phytochemical evaluation, antioxidant and phenolic content determination and compare their photochemoprotective ability by preparing creams and compare their long term change in skin hydration.

MATERIALS AND METHODS

The dried rhizomes of *Curcuma longa* and dried seeds of *Punica granatum* were purchased from local authentic herbal distributor of Raipur, Chhattisgarh and were authentified with the help of herbarium of the Pharmacognosy department of University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, India. Double distilled water was used for all the studies and the reagents used were of analytical grade. Microcentrifuge (RM-12 C DX, Remi) and UV spectrophotometer (1700-Pharmaspec, Shimadzu, Japan) were used for the present study. Figure 1 shows the morphological appearance of the collected samples and the structure of the important phenolic component present in the selected herbs. Table 1 depicts the scientific classification, plant part used and the chemical constituents present in both the herbs.

Preparation of herbal extracts: Plant materials were cleaned and were ground to a coarse powder separately and each herb were extracted with ethyl alcohol (90% v/v) at 60-70°C for 24 h

Table 1: Details of the selected photoprotective herbs

Properties	Turmeric	Pomegranate
Scientific classification	Kingdom: Plantae	Kingdom: Plantae
	Order: Zingiberales	Division: Magnoliophyta
	Family: Zingiberaceae	Class: Magnoliopsida
	Genus: Curcuma	Subclass: Rosidae
	Species: *C. longa*	Order: Myrtales
		Family: Lythraceae
		Genus: *Punica*
		Species: *P. granatum*
Plant part used	Dried rhizomes	Dried seeds
Active constituents present	Curcuminoids, which include mainly curcumin (diferuloyl methane), emethoxycurcumin, and bisdemethoxycurcumin	Anthocyanins,Ellagitannins, gallotannins, ellagic acid,

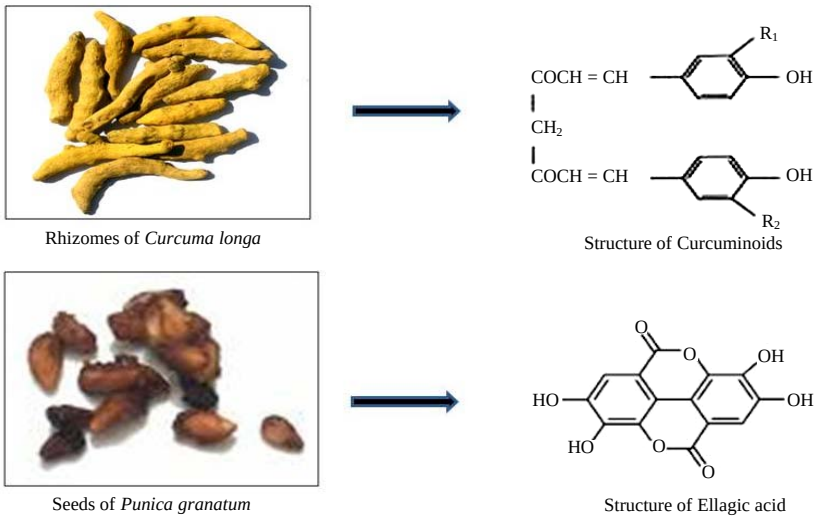

Fig. 1: Photoprotective herbs with their important phytoconstituent

by a continual hot extraction method, until complete exhaustion of the drug using a soxhlet apparatus. The obtained extracts were evaporated under reduced pressure (AU 5 psi) at 50±5°C for 5-15 min and concentrated extracts were dried to obtain actual yields (Kaur and Saraf, 2011).

Phytochemical screening: Phytochemical screening was performed for the extracts for detection of various chemical groups present in them (Harborne, 1973). Extracts were taken for performing the phytochemical tests of triterpenoids, phenolic compounds, xanthoprotein, saponin, flavonoids, tannins, aromatic acid, reducing sugar, alkaloids, steroids and cardiac glycosides (Table 2).

Analysis of phenolic content of each extract: Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method (Gao *et al*., 2000; Zongo *et al*., 2010). Herbal extracts (100 μL) were mixed with Folin-Ciocalteu reagent (0.2 mL) and H_2O (2 mL) and incubated at room temperature for 3 min. Following the addition of 20% sodium carbonate (1 mL) to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a UV-VIS spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. The results were expressed as Gallic Acid Equivalents (GAE), milligrams per g of dry weight. All determinations were performed in triplicate (n = 3).

Antioxidant activity determination: The relative reducing activity in terms of antioxidant activity of both the extracts was determined by reducing power estimation method using 5 mg of extract as well as its combination with equal amount of ascorbic acid. The absorbance was measured at 700 nm by UV-visible spectrophotometer (1700-Pharmaspec, Shimadzu, Japan) by taking as blank the mixture of all the reagents in the same quantity as added while preparing samples (Kaur and Saraf, 2011). Increased absorbance of the reaction mixture indicated increased antioxidant activity via reducing power with reference to equal amount of standard ascorbic acid

(Rajeshwar *et al.*, 2005; Ashawat *et al.*, 2007c). Similar procedure was repeated to know combination antioxidant power of each extract with ascorbic acid.

Preparation of cream: Cream formulations were prepared by using a phase inversion technique (Forster and Tesmann, 1991). The oily constituents include cetyl alcohol (3.56% w/w), stearic acid (4.80% w/w), olive oil (5.78% w/w), jojoba oil (0.50% w/w), tea tree oil (0.51% w/w), lemon grass oil (3.53% w/w) and lavender oil (0.25% w/w); emulsifying agents used were polysorbitan monooleate (Span 60) (1.78% w/w), polysorbitan monostearate (Tween 80) (0.75% w/w) and aqueous phase comprised of propylene glycol (4.02% w/w), glycerin and double distilled water. Herbal extracts were added individually in quantity 0.5%w/w, 1% w/w and 2% w/w to produce PC1, PC2 and PC3 with *P. granatum* extract and CC1, CC2 and CC3 with *C. longa* extract. Base Cream (BC) was prepared similarly excluding the herbal extracts (Ashawat *et al.*, 2008).

Skin hydration determination: Hydration of the epidermis (stratum corneum) was determined with a non-invasive, skin capacitance meter (Corneometer ® CM 820, Courage Khazaka, Ko¨ln, Germany). Corneometry is an established method for the determination of skin hydration (Barel, 1995). The acceptance is due to high reproducibility, easy handling, short measuring time and economy (Leydenn, 1995). The device determines the water content of the superficial epidermal layers down to a depth of about 0.1 mm and expresses the values in arbitrary units. The average values of four measurements were used in subsequent calculations. 5±1 mg per cm^2 to a 2 cm^2 area of the formulations were applied twice daily, in the morning and in the evening continuously for 6 weeks at volar forearm and the measurements were taken after 1, 2 and 6 weeks.

Statistical analysis: Statistical analysis was carried out using STAT software14, the measurements were taken thrice and the values were expressed in Mean±Standard Deviation. All parameters were statistically analyzed at 95% confidence level. One-way ANOVA analysis, with Tukey correction, was used to determine significant differences for the comparison. Differences were considered statistically highly significant if $p<0.001$ and non significant if $p>0.05$.

RESULTS AND DISCUSSION

After complete extraction the extracts were dried and practical yield were calculated. The practical yield obtained was 16.86% w/w for *P. granatum* extract and 15.35% for *C. longa* extract (Table 3). Preliminary phytochemical evaluation of both the herbs was done to depict the functional groups present in the herbal extracts (Table 2). The studies show the presence of triterpenoids, phenolic compounds, saponin, flavonoids, aromatic acid, tannins, reducing sugar, alkaloids and steroids and absence of xanthoprotein, in *C. longa* extract. While *P. granatum* extract shows the presence of triterpenoids, phenolic compounds, saponin, xanthoprotein, flavonoids, aromatic acid, tannins and reducing sugar and absence of alkaloids and steroids.

The results of the total phenolic content determination of the examined plant extracts, using Folin-Ciocalteu method, are presented in Table 3. The content of total phenols in extracts, expressed as Gallic Acid Equivalents (GA) per gram of dry extract, was 18.23±1.50 mg GAE/g and 78.94±6.40 mg GAE/g for *C. longa* and *P. granatum* extract. Higher is the phenolic content better will be the photoprotective effect. The higher total phenolic content of *P. granatum* extract shows better photoprotective ability as compared to I extract, but it could be further justified by the development of formulation and their activity analysis.

Table 2: Phytochemical evaluations

Evaluation parameters	Turmeric	Pomegranate
Triterpenoids	Present	Present
Phenolic compounds	Present	Present
Saponin	Present	Present
Xanthoprotein	Absent	Present
Flavonoids	Present	Present
Aromatic acid	Present	Present
Tannins	Present	Present
Reducing sugar	Present	Present
Alkaloids	Present	Absent
Steroids	Present	Absent

Table 3: Photoprotective analytical data of the antioxidant herbs

Properties	Turmeric	Pomegranate
Practical yield obtained	15.35% w/w	16.86% w/w
Phenolic content obtained	18.23±1.50 mg GAE/g	78.94±6.40 mg GAE/g
Antioxidant activity as compared to ascorbic acid	12.57±2.26%	29.26±1.88%
Synergistic antioxidant activity of extract with ascorbic acid	128.33±1.87%	142.58±2.82%

Values are Mean±Standard Deviation (SD) for n = 3; $p<0.001$

The antioxidant activity of the individual extracts was compared with the standard ascorbic acid (Table 3). The antioxidant activity of ascorbic acid was considered 100% and activity for the other extracts was determined with respect to it. The antioxidant activity was obtained 12.57±2.26% for *C. longa* extract alone while 128.33±1.87% with ascorbic acid in combination similarly 29.26±1.88% for *P. granatum* extract alone and 142.58±2.82% with ascorbic acid in combination. As reported by various scientists it was observed that antioxidant activity was produced due to the presence of phenolic compounds (Ashawat *et al*., 2007b; Adesegun *et al*., 2008; Akond *et al*., 2011). The higher antioxidant activity of *P. granatum* extract may be due to the presence of phenolic contents like punicalagins, ellagic acid etc and presence of curcuminoids support for the activity of *C. longa* extract.

Amit *et al*. (2007) reported that various functional cosmetics could be prepared for improving skin appearance which could act at structural and physiological level of skin. Similarly Ashawat *et al*. (2007b) prepared creams with combination of various herbal extracts for combating photo-aging. In present study creams were prepared taking extracts individually and comparison in improvement in skin hydration was assessed.

Creams were prepared and were optimized for their stability at room temperature for six months. The formulations showed no signs of bleeding at room temperature, which indicated that uniform mixing and the desired consistency remained in the control base cream formula. The different physicochemical parameters determined show that the on comparison with base cream the difference was highly significant ($p<0.001$) for pH, non volatile percent, fatty Concentration, layer thickness and viscosity; it was non significant ($p>0.05$) for spreadability, ash exam, microbial count and some of the acid values. Non significant values depict that incorporation of the extract produced no change in those physicochemical parameters.

The acid value is associated with the free fatty acid and volatile content. The saponification value of the formulations reflects the presence of free esters, which may influence the formula stability. To safeguard the formulation's damage during storage and handling, cosmetic formulation's thermal stability and viscosity are the prime parameters which should be regulated.

Table 4: Physicochemical evaluation parameters of creams

Formulation code	pH	Non volatile (%)	Saponification value	Acid value	Fatty concentration (% w/w)	Spreadability (%)	Layer thickness (μm)	Ash exam	Viscosity (Cps)	Microbial count (CFU g^{-1})	Erythemal score
BC	5.83±0.01	14.91±0.5	24.0±0.5	6.73±0.3	15.8±0.9	90±2	6.73±0.3	0.06±1	5967±60	30±2	0
PC_1	5.53±0.02***	22.24±0.1***	24.20±0.2ns	6.95±0.5ns	13.23±0.4***	93±2ns	4.24±0.4***	0.02±2ns	5900±10ns	33±2ns	0
PC_2	5.32±0.02***	20.26±0.4***	24.34±0.2ns	7.21±0.4ns	12.26±0.2***	92±1ns	3.67±0.2***	0.03±2ns	5800±10***	34±1ns	0
PC3	5.20±0.02***	19.82±0.3***	28.10±0.4***	8.68±0.5***	12.20±0.3***	93±3ns	3.24±0.3***	0.02±1ns	5725± 15**	34±2ns	0
CC_1	5.46±0.03***	23.12±0.2***	20.30±0.3***	6.50±0.5ns	12.84±0.4***	92±1ns	4.57±0.3***	0.04±1ns	5870±10***	32±1ns	0
CC_2	5.40±0.01***	21.14±0.2***	21.12±0.1***	7.32±0.3ns	12.53±0.3***	93±2ns	4.35±0.4***	0.02±2ns	5760±10***	33±2ns	0
CC3	5.34±0.02***	19.98±0.4***	22.14±0.4***	8.54±0.2*	12.32±0.3***	94±2ns	4.14±0.2***	0.03±1ns	5620± 15***	34±1ns	0

All the values are represented as Mean±SD (n = 3), *** is highly significant ($p<0.001$), **Significant ($p<0.01$), *Significant ($p<0.05$), ns is non significant ($p>0.05$) in the column. The comparison is done with the base cream in the same column. BC= Base cream, PC_1 PC_2, PC_3= Cream with 0.5%, 1% , 2% *P. granatum* extract, CC_1 CC_2, CC_3= Cream with 0.5, 1 and 2% *C.longa* extract respectively.

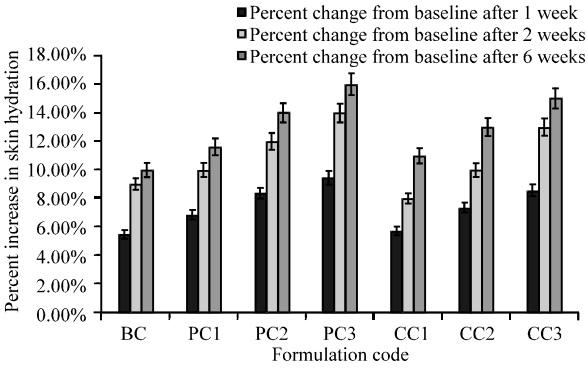

Fig. 2: Percent increase in Skin hydration after 1 week, 2 weeks and 6 weeks application. $p<0.05$ for the formulations with respect to base line (control). BC: Base cream, PC_1 PC_2, PC_3: Cream with 0.5, 1 and 2% *P. granatum* extract, CC_1 CC_2, CC_3: Cream with 0.5%, 1%, 2% *C. longa* extract, respectively

The viscosity of all formulations was between 5000 and 6000 cps (Table 4). With respect to safety and the irritant test evaluation, all the formulations showed erythema score 0 indicating no irritation (no redness) by visual observation according to COLIPA and BIS guidelines. This low erythema score is presumably because of the higher acid value and lower pH of the formulations.

Significant increases in the water content of stratum corneum readings ($p<0.05$) relative to baseline were observed 1, 2 and 6 weeks after the application of all formulations (Fig. 2). The improvement started after one week period and consistently improved till 6 weeks. After 1 week base cream produced 5.47±1.2% improvement in skin hydration while the improvement reached upto 10±2 percent increase with respect to control (baseline) after 6 weeks. Similarly for *P. granatum* extract loaded creams improvement was 6.84±3.0% after 1 week and 16±1% after 6 weeks; while *C. longa* extract incorporated creams produced 5.84±1.8% increase after 1 week and 14±1 percent increase after 6 weeks period. The effect was dose dependent, the improvement was more with 2% w/w extract loaded creams than with creams having 0.5% w/w extract. The enhancement of skin hydration by application of the prepared formulations show the significance of the active constituents present in the extract.

Similarly in the studies performed by Saraf *et al.* (2010) at marketed moisturizers it was reported that formulation containing wheat germ oil, aloe vera and turmeric extract in combination improved skin hydration than using formulations having these components individually showing that herbal constituents enhance the efficacy of the formulation system.

The present work compare the improvement in skin hydration by both the extract containing creams and showed that both the types of creams were effective but the results of *P. granatum* extract loaded creams were better than *C. longa* extract loaded creams. However, when these formulations were compared with each other, the water content of the stratum corneum values obtained with the formulation containing 2% of *P. granatum* extract was significantly higher.

Base cream also improved skin hydration showing that the components present in cream also improve skin properties, but on inclusion of herbal extracts the effect was enhanced significantly showing the photoporotective effect of these extracts. The different oils used in the preparation also produce photoprotective effect as shown by Kaur and Saraf (2010). The mechanical properties of the skins are influenced by the collagen matrix which is composed of mechanically coupled cytoskeletons of adjacent cells and proteins in extracellular regions and have a fixed composition of lipids, phosphates, ceramides and fatty acids. The imbalance in its composition affects mechanical

properties of skin. The herbal extracts maintain the composition and function of the cytoskeletons and also protect the skin from ultraviolet radiations by scavenging free radicals (Ashawat *et al.*, 2008). Herbal ingredients like ceramides, triglycerides, steroids, polyphenols and fatty acids act as an intercellular cement, essential for regulating the passage of water through the skin inhibiting the evaporation of water from the skin.

CONCLUSION

The studies performed showed the photoprotective nature of the selected herbs *Curcuma longa* and *Punica granatum* by the phytochemical evaluation, total phenolic content determination and antioxidant activity analysis. These studies also show comparison between both the herbs in which the total phenolic content and antioxidant activity of *Punica granatum* extract was obtained higher than *Curcuma longa* extract. The developed cream formulations were physicochemically stable and improved skin hydration significantly as compared to base line and base cream. The increase in skin hydration was in the order *P. granatum* extract loaded creams> *C. longa* extract loaded creams> Base cream.

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