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EVALUATION OF SUNSCREENING AND ANTIBACTERIAL ACTIVITY OF THE CREAM CONTAINING POMEGRANATE PEEL EXTRACT

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ABSTRACT

Cosmetic products are formulated for application on the body for the purpose of cleansing, beautifying, or altering appearance and enhancing the beauty. The ozone layer in the stratosphere practically prevents the UV photons from reaching the earth's surface of concern for human skin damage, therefore, only UV-B and UV-A radiation are relevant. In the present study an attempt has been made to formulate a cream containing alcoholic extract of pomegranate peel extract. The quantity control parameters of *Punica granatum* were performed as per WHO guidelines. The cream was formulated using pomegranate peel extract in two concentrations (10% and 25%). The UV analysis of different concentrations of prepared creams are done along with standard cream which has TiO_2 as the active ingredient. The extraction of peel was carried out by continuous hot percolation using Soxhlet apparatus and the phytochemical screening revealed the presence of flavanoids, tannins, anthocyanins and polyphenolic compounds. The evaluation of antibacterial activity is done by cup and plate technique using ethanolic extract of pomegranate peel against *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The results showed that the ethanolic extract of pomegranate peel has very good sensitivity against both gram positive and gram negative organism.

KEYWORDS

UV analysis, Cup and plate method, Ethanolic extract of pomegranate peel, WHO guidelines, TiO_2

INTRODUCTION

The recent rapid growth of the sunscreen containing cosmetic market indicates that people are quite conscious of the possible dangers of premature skin aging and skin cancer that occur as a result of excessive exposure to UV radiation. A UV index (UVI) developed and forecasted by the united states Environmental protection Assay (EPA) and the National Wealth Service (NWS), with various other radiation indexes provides the general public with a daily an index and guidelines for the use of sunscreen products where outdoor activities are involved.[1]The majority of research has focused as pulp and juice of the fruit.[2,3] But new scientists from the institute of Hygiene and environmental medicine have reported that the peel offers high yields of phenolics, flavonoids and proanthocyanidins than the pulp. Owing to the antioxidant property of polyphenols particularly the flavonoids and tannins present in pomegranate peel, [5] we carried out sun screening activity of cream containing pomegranate peel extract. To evaluate the preliminary phytochemical study of the alcoholic extract of pomegranate peels, to determine the quality control of cream containing pomegranate peel extract and screening the antibacterial activity of pomegranate peel extract against *Staphylococcus aureus*, *Bacillus subtillis, Escherichia coli,* and *Pseudomonas aeruginosa.*[18]

MATERIALS AND METHODS

The *Punica granatum* fruits were collected from local market in Bangalore and authentified by ABIPER at Yelahanka. The collected plant material was free from contamination of other plant materials. Chemicals of analytical grade and glasswares, apparatus of borosil were used. The microorganisms were purchased on order from Biocon, Bangalore. UV-160A spectroscopy, Shimadzu.

Extraction procedure[8]

The air dried powdered peel was extracted in soxhlet assembly successively by using petroleum ether, benzene, chloroform, acetone, methanol, etc as solvent. Aqueous extract was obtained by simple maceration process. The order of solvent was from non polar to polar solvent by increasing the dielectric constant of the solvent system.

Ethanolic extract

70gm of the powdered material was packed in the column of soxhlet assembly and about 400 ml of ethanol was taken in a round bottomed flask. The apparatus was set up in the mantle and the extraction was carried out for 8 hrs at 46-70^{o}C.

Aqueous extract

100gms of powdered material was macerated with distilled water (350ml) for 48 hrs to obtain aqueous extract. Each extract concentrated by distilling off the solvent and then evaporate to dryness on the water bath. They are stored in desiccators until further use.

Phytochemical screening [10, 12]

The extracts are subjected to preliminary phytochemical screening for detection of various chemical groups present in them. A systematic study of a crude drug enhances through combination of both primary and secondary metabolites derived as a result of plant metabolism. The dried powder of peels of pomegranate was subjected to phytochemical screening for the detection of various plant constituents. The aqueous extract was prepared by cold

maceration. Extract was taken for performing the following phytochemical tests alkaloids carbohydrates, flavanoids, steroids, volatile oils, tannins, phenolic compounds, proteins and amino acids, saponins, aromatic acids triterpenoids, gum and mucilage.tab no 3.

STANDARDIZATION OF PLANT MATERIAL [6]

Herbal drug preparations are supposed to be produce with high "quality" Quality encompasses all the properties of the final product which makes its optimal suitable for its intended use. Reproducible quality is a goal which is among others achieved by the process of standardization.

Physical evaluation

Evaluation of the drugs on the basis of important physical properties or physical characteristics of the active constituents is known as physical evaluation. Many a times, a crude drug consists of cellular material which limits its direct use for the study of physical properties. For the easy of study, these methods can be divided into various categories like foreign material, ash values, extractive values, chromatographic methods, physical constants, and spectroscopic methods.

Crude fiber content[8]

Place about 2gm of powder accurately weighed in a 250 ml round bottom flask. Add 100ml of 0.128M sulphuric acid and reflux for one hour. Filter and wash the residue with water until it becomes neutral. To the residue add 0.313M sodium hydroxide and it reflux for one hour. Filter the ash less filter paper and wash the residue with water till the filtrate becomes neutral. Weigh the residue (A) and ignite to ash. Weigh the ash (B).The weight difference A-B represents the crude fibre on the percent dry weight basis. Percentage (%) crude fibre = $\frac{A-B}{2} \times 100$

All the above physicochemical parameters are evaluated and results are tabulated (tab No 4)

FORMULATIONS OF CREAMS CONTAINING POMEGRANATE PEEL EXTRACT, [5,7]

Tab. No.1

Formula of a cream containing 10% Pomegranate peel extract

Ingredients	Quantity
Liquid paraffin	6 gm
White Beeswax	2 gm
Borax	0.1 gm
Distilled water	2.66 ml
Perfume	2 drops
Glycerin	2 drops

Procedure:

About 1 gm of the crude extract was weighed and transferred in to a china dish. Then 6 ml of the liquid paraffin and 2 gm of beeswax were measured and transferred into china dish respectively. 2 drops of glycerin also added and kept for heating on water bath (oil phase).In another 100 ml beaker 0.1 gm of borax and 2.6 ml of distilled water are taken and kept for heating on a water bath (aqueous phase). Both the phases are simultaneously heated till they attain 70^{O}C temperature. When both the contents in beaker (Liquid phase) attain a temperature of 70^{O}C are mixed together with continuous stirring. As a result of which a homogenous cream was produce with shiny texture in the china dish. Finally two or three drops of perfume was added to cream and packed in a suitable container.

Tab.No.2

Formula of cream containing 25 % pomegranate peel extract

Ingredients	Quantity
Liquid paraffin	7.5 gm
White Beeswax	3 gm
Borax	0.2 gm
Distilled water	2.6 ml
Perfume	2 drops
Glycerin	2 drops

Procedure:

Same procedure is followed as mentioned in the formulation of cream containing 10% of peel extract. The obtained formulation is also packed in a suitable container.

Quality control tests for creams containing pomegranate peel extract [15,17]

Test of Non – Irritancy

The bases used in the formulation of ointments may cause irritation or allergic reactions. Non-irritancy of the preparation is evaluated by patch test. In this test 24 human volunteers are selected. Definite quantity of ointment is applied under occlusion daily on the back or volar forearm (intact skin) for 21 days. Daily the type of pharmacological action observed.

Test of rate of Penetration [14]

The rate of penetration of a semisolid dosage form is crucial in the onset and duration of action of the drug. Weighed quantity of the preparation should be applied over selected area of the skin for a definite period of time. Then the preparation left over is collected and weighed. The difference between the initial and final weights of the preparation gives the amount of preparation penetrated through the skin and this when divided by the area and time period of application gives the rate of penetration of the preparation. The test should be repeated twice or thrice. This procedure is tedious and not followed anymore. Using flow-through diffusion cell or micro dialysis method, the rate of penetration of the preparation can be estimated. Animal or human skin of definite area should be collected and tied to the holder present in diffusion cell. The diffusion cell is placed in a fluid bath. Measured

quantity of the preparation is applied over the skin and the amount of drug passed into the fluid is measured at regular intervals by analyzing the aliquots of fluid using a spectrophotometer.

Test of Rate of drug release

There are three methods by which the rate of drug release from the ointment base can be evaluated.

Method – I

A clean test tube is taken and the internal surface is coated with the preparation as a thin layer. Saline or serum is poured into the tube. After a certain period of time, the saline is analyzed for the quantity of the drug. The amount of drug when divided by the time period gives the rate of drug release.

Method –II

Empty ointment jar is taken and filled with the preparation and the mouth is closed with cellophane. The jar is place in a water bath in inverted position for a definite period of time. The water is analyzed for the drug content and the rate of drug release is determined.

Method – III

This method is suitable for drugs having bactericidal action. Five nutrient agar broth tubes are taken and the medium is melted. At 40 – 45^{O}C *Staphylococcus aureus* organism is inoculated into the tubes. Under aseptic conditions the inoculated medium is transferred into sterile petriplates. In each plate at the central portion the medium is streaked into a small cup-like surface. Over the cup a small quantity of the preparation is placed under aseptic conditions. All the plates are incubated and after that the diameter of zone of inhibition of microbial growth is measured in all the plates. The average diameter of zone of inhibition is calculated and interpreted as the rate of drug release results tabulated in table no. 7.

Tab. No. 3

Preliminary phytochemical screening of pomegranate peel extract

Sl.no	Plant constituents	Aqueous extract	Ethanolic extract
1.	Alkaloids	+	-
2.	Carbohydrates	+	-
3.	Glycosides	-	+
4.	Saponins	-	-
5.	Proteins & aminoacids	-	-
6.	Phenolic compound & Tannins	-	+
7.	Gums & mucilage	-	+
8.	Flavonoids	-	+
9.	Volatile oils	-	-
10.	Triterpinoids	-	+
11.	Steroids	-	+
12.	Aromatic acids.	-	-

\+ = Present

\- = Absent

***Determination of* pH**

pH was determined by preparing 1:20 gel to the distilled water mixture was subjected to pH determination by a digital pH meter.

***Test of microbial content*[18]**

Micro-organisms like *Pseudomonas aeruginosa* and *Staphylococcus aureus* may contaminate the preparation and finally infect the skin. So semisolid dosage forms should be tested for the absence of such micro-organisms.

Test for Pseudomonas aeruginosa

Pesudomonas aeruginosa is a gram-negative organism and it contains abundant amounts of cytochrome oxidase enzyme. The presence of the enzyme in the preparation is interpreted as the presence of the organism. Solutions of different samples of the semisolid dosage form are prepared. Each sample is inoculated into nutrient broth medium present in the test tubes. All the test tubes are incubated at 37^{O}C for 18-24 hours. After incubation 0.2 ml of α - naphthol and 0.3 ml of p-aminodimethylaniline oxalate are added to each tube. Immediately the test tubes are shaken vigorously for thorough oxygenation of the contents.

Test for Staphylococcus aureus

This test is also called coagulate test. Solutions of different samples of the preparation are made. Each sample is inoculated into separate volume of 0.5ml of rabbit's plasma under aseptic conditions and incubated at 37 ^{O}C for 1 – 4 hours results tabulated in table no. 7.

Stability study

The formulation was kept at different temperatures (10^{o}C, 30^{o}C and 45^{o}C). The samples were observed for pH, viscosity and their appearance for 8 weeks.

***Determination of total fat substances*[9]**

Weigh accurately about 2g cream into a clean beaker and add 25 ml of water, 25 ml of dilute hydrochloric acid, & a few drops of methyl orange indicator. The solution in beaker should have a red colour. Warm the contents till the fatty matter forms a clean layer on the top. Cool to room temperature and transfer to a separating funnel. Rinse the beaker three times with 25 ml of ethyl ether and transfer the rinsing to the separating funnel. Shake vigorously. Set the funnel aside for the two layers to separate out the aqueous and other phases and extract the aqueous phase twice more with 25 ml each of ethyl ether. Combine all the ether extracts and wash well with water until free of acid. Filter the ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried and weighed. Wash the sodium sulphate on the filter paper with ether and combine the washings and filtrate. Distil off the ether and dry the material remaining in the flask at a temperature of 60 ± 2^{O}C to constant mass.

***Evaluation of sunscreen activity of cream containing pomegranate peels extract*[2, 15]**

A sunscreen sunburn preventive agent is an active ingredient that absorbs 95% or more of the light in the U.V. range of 290-320nm. A sunscreen sun tanning agent is an active ingredient that absorbs up to 85% of light in the UV range of 290-320 nm but transmits light at wavelength longer than 320 nm. A sunscreen opaque sun block agent is an opaque agent that reflects or scatters light is the UV and visible at wavelength of 290-777 nm.[16] The sun emits energy in a continuous band throughout the electromagnetic spectrum. The shorter wavelengths are absorbed in the upper atmosphere so that at sea level the radiation extends from a cut off near 290 nm through the near UV to the conventional end of the UV range, which is near 400 nm. The intensity of the radiation varies nonlinearly through out this range. The production of erythema and the subsequent production of melanin pigment are both maximum with 296.7 nm radiation. As the wavelength increases both responses fall rapidly, so that 10 $\mu W/cm^2$ of 307 nm

radiation, 100 $\mu W/cm^2$ of 314 nm, 1000 $\mu W/cm^2$ of 330 nm and 10,000 $\mu W/cm^2$ of 340 nm radiations are required to equal the effect 1 $\mu W/cm^2$ of 296.7 nm radiation in the production of erythema. A unit of erythemal flux, the E-viton is equivalent to the erythema induced by 10 $\mu W/cm^2$ of 296.7 nm radiation. The response of the skin to an E-Viton (viton) is constant: irradiation by 10 vitons for one hour produces the same erythemal responses as 5 vitons for two hours. About 20 min exposure to mid summer sunlight (or 40 viton min) are needed to produce a minimum perceptible erythema (MPE) on normal Caucasian skin. With the realization that prolonged exposure to sunlight produces most of the aging effects on skin and the clear implies of exposure in at least three types of skin cancers; use of products that protect the skin from excessive exposure has becomes increasingly widespread.

Antibacterial activity of pomegranate peel extract

The cultures are taken and inoculate in the nutrient broth and allow it for 24hrs for providing of good growth that cultures from that, the cultures are inoculated in to the nutrient agar medium into a petridish by using of sterile cotton swabs. The cultures are spreaded uniformly in all over area of medium in petridish. Make a cavity (bore) in the Petridish by the use of sterile bore. The cup plate depends on the diffusion of antibiotic from vertical cylinder through its solidified. In the cavity pour the solution of extract (*Punica granatum* in DMSO in three concentrations) and allow it for diffusion of drug to media. The agar plates are inverted and incubated for 37^OC for 24 hrs. *Invitro* evaluation of antibacterial activity for different concentration of *Punica granatum* ethanolic extract was carried by antibiotic cup plate method using amoxicillin as standard. The antibacterial activity of different concentration was measured in terms of zone of inhibition. The maximum activities were measured with two different concentrations against the *S.aureus* (gram +ve) and *P*seudomonas *aeruginosa* (gram –ve). For comparison purpose we have used antibiotic amoxicillin 5µgm/ml as positive antibacterial control. The antimicrobial activity was evaluated in terms of MIC by plotting a graph of zone of inhibition (mm) Vs concentration of extract used and is compared with antibacterial activity of standard amoxicillin and ethanolic extract of *Punica granatum.*[18, 19]

% of Inhibition = [100-(C-A/C×100)]

Where: C: area of total media in petridish, A: area of inhibition

RESULTS

Tab. No. 3 Preliminary phytochemical screening of pomegranate peel extract
Tab No. 4 Standardization of plant material of *Punica granatum*
Tab No. 5 Quality control tests for cream containing pomegranate peel extract
Tab.no.6 Evaluation of sun screening activity of cream containing pomegranate peel extract
Tab. No.7 Anti-bacterial activity of pomegranate peel extract

Tab No. 4

Standardization of plant material of Punica granatum

TEST FOR EXTRANEOUS MATERIAL	
FOREIGN MATTER	*2%*
SAND AND SILICA	*Absent*
INSECT INFESTION	*Nil*
MOISTURE CONTENT	*2%*
CRUDE FIBRE	*91.1%*
PHYSICO- CHEMICAL ANALYSIS	
ASH VALUES:	
TOTAL ASH	*18%*
WATER SOLUBLE ASH	*11.7%*
ACID INSOLUBLE ASH	*0.92%*
EXTRACTIVE VALUES	
AQUEOUS EXTRACT	*89.1%*
ETHANOLIC EXTRACT	*12.4%*

Tab No.6

Evaluation of sun screening activity of cream containing pomegranate peel extract

Wavelength(nm)	**290**	**300**	**310**	**320**	**330**	**340**	**350**	**360**	**370**	**380**	**390**	**400**
Standard:												
% Absorbance	1.811	1.36	1.01	0.73	0.57	0.52	0.49	0.47	0.46	0.41	0.34	0.28
% Transmittance	1.98	5.13	10.62	19.26	27.18	32.04	33.66	33.75	33.84	37.8	43.2	49.95
10% cream:												
%Absorbance	1.4	1.14	0.857	0.633	0.493	0.426	0.4	0.391	0.38	0.329	0.28	0.235
% Transmittance	3.7	7.2	13.8	23.2	32.1	37.6	39.7	40.1	41.6	46.8	52.4	58.1
25% cream:												
% Absorbance	1.647	1.238	0.927	0.669	0.519	0.447	0.426	0.428	0.423	0.376	0.318	0.255
% Transmittance	2.2	5.7	11.8	21.4	30.2	35.6	37.4	37.3	37.6	42	48	55.5

Tab No. 5
Quality control tests for cream containing pomegranate peel extract

SL.NO	TEST	INFERENCE
1	Non-irritancy	No visible reaction occurred
2	Rate of penetration	Spectrophotometric analysis was done
3	Rate of drug release	Zone of inhibition has observed
4	pH	6.8
5	Microbial content	No Blue color – absence of *P.aeruginosa* No clot formed absence of *S.aureus*
6	Stability	*No significant change occurred*

Tab. No.7
Anti-bacterial activity of pomegranate peel extract

Organisms	Concentration of Ethanolic extract of *P. granatum*	Zone of inhibition (Diameter in cm)	% of inhibition 100-(C-A/C×100)
Staphylococcu aureus	50mcg/ml	1.0	11.25%
	100mcg/ml	1.4	13.62%
	200mcg/ml	3.0	17.25%
Pseudomona aeruginosa	50mcg/ml	0.6	10.36%
	100mcg/ml	0.8	10.96%
	200mcg/ml	1.2	12.23%

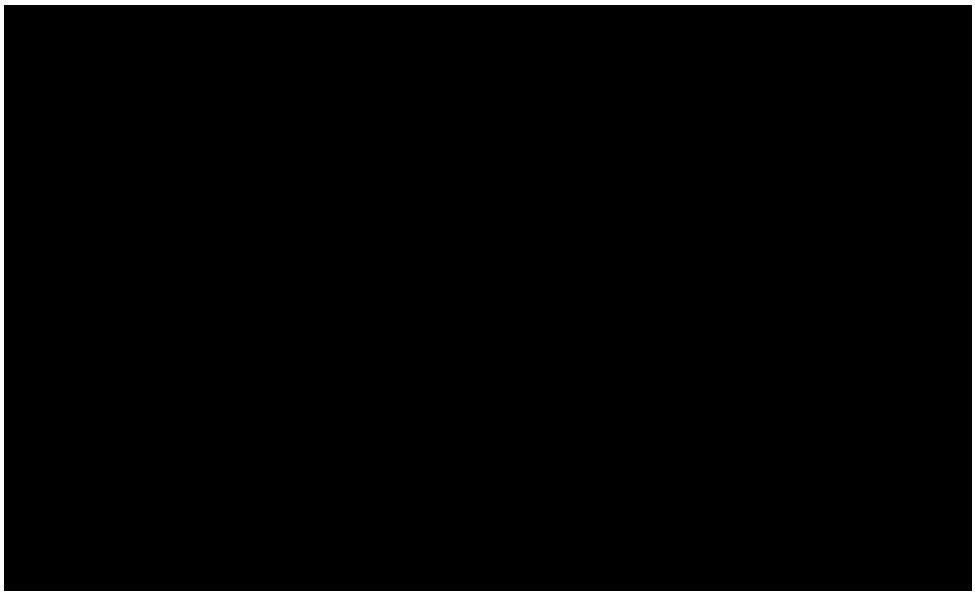
DISCUSSION

The recent rapid growth of the Sunscreen and Sunscreen containing cosmetic market indicates that people are quite conscious of the possible dangers of premature skin, aging and skin cancer that occurs as a result of excessive exposure to UV radiation. The ozone layer in the Stratosphere practically prevents the UV photons from reaching the earth's surface of concern for human skin damage, therefore, only UV-B and UV-A radiation are relevant. In the present study an attempt has been made to formulate a cream containing alcoholic extract of pomegranate peel extract. The quantity control parameters of *Punica granatum* were performed to determine the quality of crude drug as per WHO guidelines shown in tab.no.5. The extraction of peel was carried out by continuous hot percolation using Soxhlet assembly and phytochemical screening has been carried out which reveals the presence of flavanoids, tannins, anthocyanins and polyphenolic compounds shown in tab.no.3. The cream has been formulated using the pomegranate peel extract in two concentrations (10% and 25%). The UV analysis of different concentrations of prepared creams are done along with standard cream which has TiO_2 as the active ingredient. The result showed (tab.no.6 and fig.no.1 and 2) that the cream has significant sunscreening activity compared to the standard thus can be successfully used as sunscreening agent for protection against UV rays. The pomegranate peel extract also said to exhibit effective antibacterial activity. Thus antibacterial activity of the crude drug was evaluated by using ethanolic extract. The evaluation of antibacterial activity is carried out by cup and plate technique using the ethanolic extract of pomegranate peel against *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The efficacy of antibacterial activity was measured in terms of the zone of inhibition. From the obtained results (tab.no.7) it was clearly established that the ethanolic extract of pomegranate peel has very good sensitivity against both gram positive and gram negative organism. Thus the *Punica granatum* peel extract is confirmed to possess the significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Fig. No1 Evaluation of sun screening activity of cream containing pomegranate peel extract

Fig.No.2

Evaluation of sun screening activity of cream containing pomegranate peel extract



CONCLUSION

In the present study an attempt has been made to formulate a cream containing alcoholic extract of pomegranate peel extract in different concentrations of 10% and 25% cream. The formulated cream containing pomegranate peel extract is dose dependent and the percentage transmittance of the cream reduced in the UV region when compared to other regions. The preliminary phytochemical screening revealed the presence of polyphenolic compounds anthocyanins and flavanoids which are responsible for the antioxidant and sunscreening activity of respective cream. The significant zone of inhibition was seen when compared with that of standard and these results shows that the respected ethanolic extract effectively inhibited growth of *P.aeruginosa* and *S.aureus.*

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