www.ijpbs.net

INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.47*

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Int J Pharm Bio Sci 2013 July; 4(3): (P) 838 - 853

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

FORMULATION AND EVALUATION OF HERBAL EXFOLIATE FILM CONTAINING *CYPHOMANRDA BETACEAE&PHASEOLUS MUNGO*

PRIYA ABRAHAM*

Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod, Kerala, India

ABSTRACT

Exfoliation involves the removal of the oldest dead skin cells on the skins outermost surface to help maintain healthy skin. The most serious form of skin cancer is caused by excessive exposure to the sun (ultraviolet radiation). Skin cells that are damaged are at greater risk of becoming abnormal and cancerous.Herbal skin exfoliates enriched with flavanoids penetrate deep into the skin, nourishes it, and prevents photo damage such as discolouration and hyper pigmentation. Since herbal exfoliate shows a good antimicrobial and antioxidant activity, it shields the skin from environmental toxins, pollution and micro organism. Herbal exfoliation may also prevent skin cancer.Advantages of facial peel which Improves the texture of the skin, makes the skin healthy, makes the skin look more radiant, reduces appearance of wrinkles and fine lines, removes the dirt clogged in the pores, prevents acne and aging of the skin .Disadvantages mainly High price of some of the products and methods used to achieve it, exfoliation will lead to some initial redness to the skin, At the end of chemical peels, the skin will frost, with colors varying from a bright white to grey on the skin surface, plastic beads or polymer often used as exfoliates are not biodegradable where they remain in the soil or river for a very long time polluting the environment. Considering the above disadvantages, effort had been taken to formulate an herbal facial peel which is cheap, ecofriendly and effective on human skin without causing irritation.Cyphomandra&phaseolus genus belonging to the family Solanaceae whichare used for this studies.

PRIYA ABRAHAM
Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod, Kerala, India

Corresponding author

INTRODUCTION

Plants have been used for health and medicinal purposes since several thousands of years. A majority of world population in developing countries still relies on herbal medicine to meet its health needs. Herbal medicines are often used to provide first line and basic health service, both to people living in remote areas where it is the only available health service and to the people living in poor areas where it offers the only affordable remedy. Even the areas where modern medicine is available, the interest on the herbal medicines and their utilization have been increasing rapidly in recent years. The following herbs are used for the study

Cyphomandrabetaceae&Phaseolusmungo.

Cyphomandrabetaceae is a red edible fruit is widely used as skin colorants by the native tribes.The rich flavanoid and pectin content of this fruit may be attributing to the antioxidant and cleansing activity on the skin, which enhances glossy appearance of the skin. The crude extract had also shown skin irritation at higher concentrationb.*Phaseolusmungo*is a traditionally known exfoliate; it helps to remove the dead skin cells.Herbal facial peel it reveals soft, supple, re-energized skin and prevents from premature skin aging. Herbal facial peel can also be used on dry skin after removing the top layers of dry, dead skin cells that inhibit moisture absorption in the deeper layers. Herbal facial peel is a cleansing formula and treatment mask that detoxifies the skin and stimulates its metabolism. It enhances the absorption and retention of moisturizing agents and restores the skins own natural moisture factor. The skin is continuously exposed to harmful rays of the sun and under attack of free-radicals. These daily aggressions may intensify melanin formation and damage cells, accelerating the process of skin-darkening and aging.

MATERIALS &METHODS

Collection and authentication of plant materials

Fruits of tree tomato and seeds of green gram were collected from the herbal garden in Connoor. Authenticated by Dr.Rajan, Field Botanist ,Nilgiris

PLANT PROFILE[1]
Fruits of tree tomato

Common name :Tamarillo, Tree tomato
Plant source : Cymphomandrabetaceae
Family : Solanaceae
Parts used : Fruit
Vernacular names : (i) English : Tree tomato (ii)Tamil : Marathakkali (iii) Newzeland: Tamarillo (iv) Brazil : Tomatae de arvore (v) coloumbia : Pepino de arbol (vi)indonesia: dutch egg plant
Constituents: flavanoids, pectin, gums and mucilage
Uses : Highest sources of vitamins, minerals, antioxidants etc, anti hypertyensive, hypolipidemic, obesity

2. Seeds of green gram[2]

Phaseolusmungo(MUNG BEAN)

Botanical name:Phaseolusmungo Family :Papilionaceae
Vernacular names: (i) English : Green gram (ii) Tamil : Pachhai-payaru
(iii) Malayalam : Cherupayar (iv)S anskrit: mudga
Constituents : starch, albuminoids, oil, fibre tc
Uses: To relieve thirst in fever, aperient, in ayurvedic treatment used for bath, nutrient, oedema, anxiety, diuretic,diabetes,folk remedy for arsenic poisoning ,bleeding disorders etc

1. EXTRACTION AND ISOLATION

A) Isolation of starch fromPhaseolusmungoseeds: 100g of powdered*Phaseolusmungo*seeds mixed with 200 ml of distilled water and this mixture was heated at a temperature of 68˚C till gelatinization occurred. This gelatinized mixture was filtered through muslin cloth. Filtrate was collected and it was dried by using the following techniques,

A) Sun drying
B) Freeze drying[3]
C) Microwave oven[14] methods.

B) Extraction of aqueous extract from Cyphomandrabetacea.:Fully ripped *Cyphomandrabetacea* fruits were cleaned thoroughly.The fruits were cut in to small pieces and 100gms of the sliced fruit was boiled with 500ml of distilled water for 2hrs by maintaining the temperature at 60˚C. After 2hrs the whole mass was filtered through the muslin cloth to obtain a ruby-red coloured aqueous extract. 500ml of the obtained aqueous extract was concentrated for 1hr at 60˚C to obtain 100ml of aqueous extract.

2. STUDY OF FILM FORMING PROPERTY

A) Solution preparation for making formulation

1gmof the isolated starch sample added with 10 ml of distilled water subject for heating (68˚C), Gelatinization will takes placem. Filter it collect the filterate (2.5ml)+ 0.5 ml of tree tomato aqueous extract. Concentrate the mixture to (1ml)

B) Film casting: 1ml of the prepared solution was casted on a wax coated glass plate subject for drying

3. PHYSIOCHEMICAL EVALUATION

(i) VISCOCITY MEASUREMENT

The viscosity of the formulation solution was measured with the help of viscometer.[5]The results are tabulated in table no:1

Table No 1
Measurement of Viscosity of the solution

Sample	Rpm	Viscosity	Torque (%)
Formulation-1	50	1.6	0.2
Formulation-1	100	1.6	0.4

(ii) THICKNESS MEASREMENT

of the film was measured at different points using digimaticvernier caliper.[5]The average of 3 readings was noted. The results are tabulated in table no:2

Table No 2
Film thicknesses

Sample	Average thickness(mm)
Film -1	0.18mm
Film -2	0.16mm
Film -3	0.16mm
Film -4	0.18mm
Film -5	0.19mm

(iii) MOISTURE CONTENT

The moisture content of the films was determined using Sartorius moisture analyzer. The formulated film was exposed to moisture for 72 hrs and difference in initial and final weight was calculated.[27] The results are tabulated in table no:3

Table No 3
Moisture content of formulated herbal facial peel

Weight taken =0.658gm
Moisture, L =12.86%
Dry weight, R =87.14%
Ratio, LR =14.76%
Weight after drying =0.573gm
Residue, G / kg =871.42 g /kg.

L-Moisture R-Dry weight LR-Ratio G-Residue

(iv) PERCENTAGE MOISTURE LOSS

The film was fixed over the edge of glass vial using adhesive and vial was weighed and placed in desiccators containing 10gms of calcium chloride as desiccant. The vial is taken and weighed for 72 hrs speriodically.[5] The results are tabulated in

Table No 4
Percentage moisture loss

Sample	Percentage moisture loss in 24 hrs
Film -1	0.025%
Film -2	0.022%
Film -3	0.026%
Film -4	0.025%
Film -5	0.020%

(v) FOLDINGENDURANCE

The folding endurance was measured manually for the prepared films. A strip of film (3x3 cm) was cut evenly and repeatedly folded at the same place till it broke.The number of times the film could be folded at the same place with out breaking gave the exact value of folding endurance.[5]
The results are tabulated in Table No: 5

Folding endurance of film

Sl.No	Folding endurance
1	140
2	170
3	200

(vi) COMPATIBILITY STUDIES

(a)ACETONE EXTRACTION AND CHLOROFORM PARTITION OF ANTHOCYANINS: [6-9]

Procedure

1. 50 g aqueous extract was mixed with acetone 1:2 (w/v) using a general-purpose homogenizer.

(For most materials, a 1:1 ratio of sample to solvent should be used. For materials rich in pectic substances, a higher proportion of acetone may be required, e.g., 1:1.4 or 1:2. For materials with high sugar content, the sample needs to be dispersed in water before extracting with acetone.)

2. Separated the anthocyanin extract (filtrate) from insoluble material by filtering the slurry through a Whatman no. 1 filter paper by vacuum suction using a buchner funnel.

3. Reextracted with 70% (v/v) aqueous acetone until a faintly colored solution is obtained and pooled the filtrates. (The use of acidified acetone ensures that the aqueous fraction will be at a low pH where the anthocyanins are more stable and that the chloroform/acetone solvents will be in an acidic environment).

4. Transfered filtrate to a separating funnel, added 2 volumes of chloroform, and gently mix by turning funnel upside down. Store sample overnight at 4°C or until a clear partition between the two phases is obtained.

5. Transferred the aqueous phase (upper portion) to a 500-ml boiling flask. Removed residual acetone/chloroform in a rotary evaporator at 40°C under vacuum. The presence of anthocyanin pigments in the aqueous phase is evidenced by the pink to red color of the solution. Prolonged evaporation time should was avoided to minimize pigment degradation. Evaporation was completed in 10 min.

6. Made up remaining aqueous extract to a known volume (100 ml) with acidified deionized distilled water. Sample was stored at 4°C. Avoided repeated freezing and thawing.

(b) HPTLC STUDIES

HPTLC studies were performed to quantify the main phytoconstituent namely quercetin present in the formulation and to confirm its compatibility with other phytoconstituents present in the formulation using CAMAG Linomat IV instrument. The data obtained suggested that there were no interaction between the phyconstituents and also confirms that the quantity of the constituent remain the same before and after the formulation.

The results are tabulated in table no:6

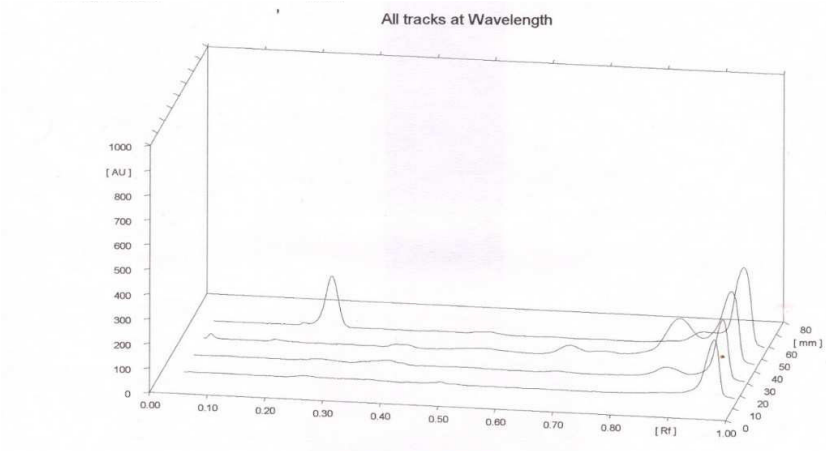
COMPATIBILITY STUDIES

Table No 6
HPTLC Analysis

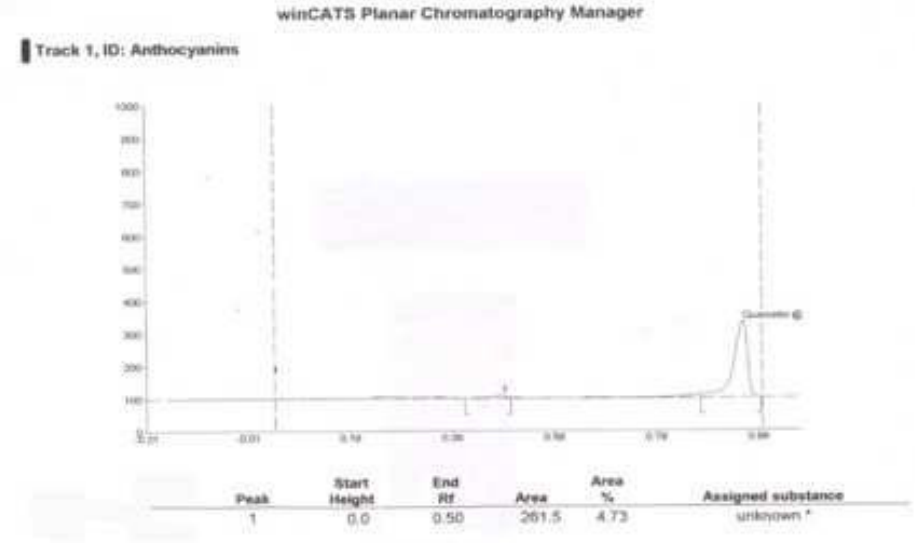
Sample	R_f	Amount of quercetin present in µg/ 5µl	Amount of quercetin present in %	Solvent system
1. Herbal facial peel formulation	0.96	1.318	2.64	Ethyl acetate: Glacial acetic acid: Formic acid: Water. (100:11:11:26)
2. Aq .extract of *C.betacea*	0.96	1.349	2.70	
3. Chloroform extract of *C.betacea*	0.95	2.175	4.35	
4. Standard Quercetin	0.96	20.00	100	

The details of the study are shown in table -11 and graph 1-7.

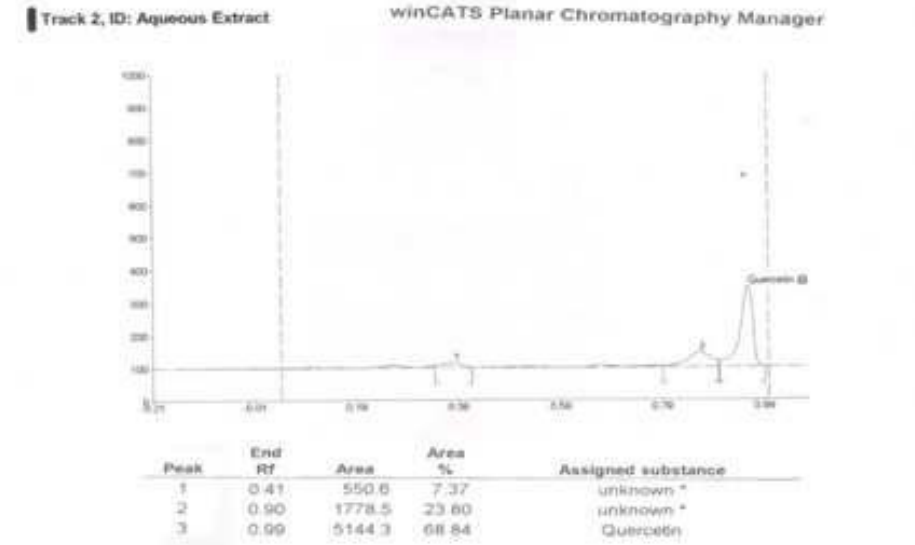
HPTLC STUDIES



GRAPH-1



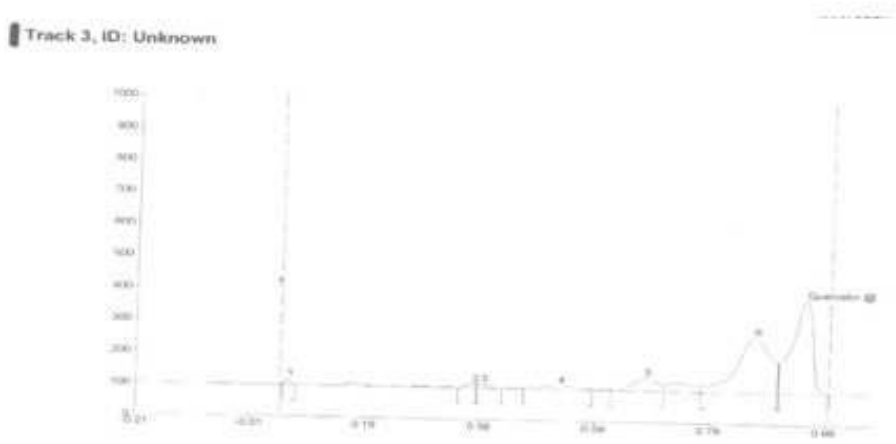
Peak	Start Height	End Rf	Area	Area %	Assigned substance
1	0.0	0.50	261.5	4.73	unknown *



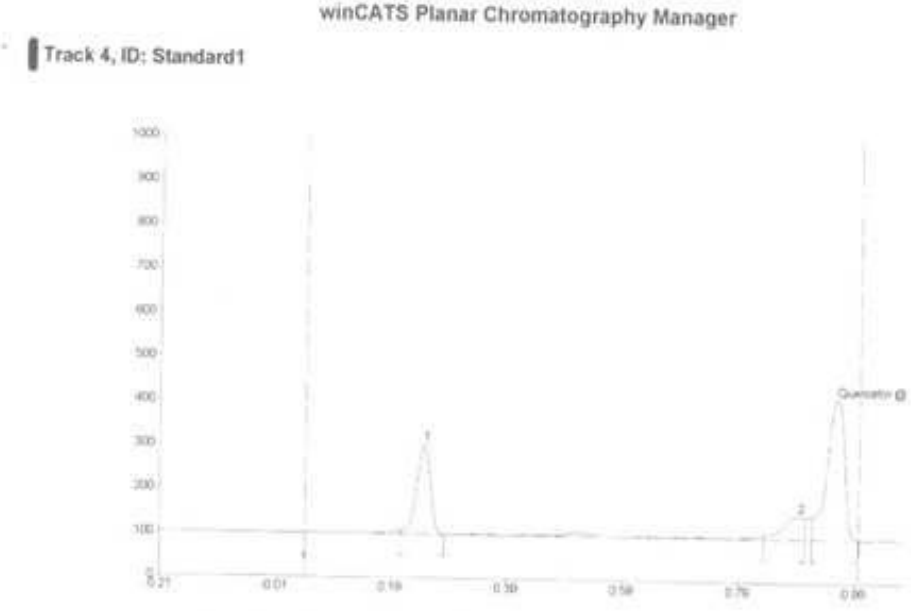
Peak	End Rf	Area	Area %	Assigned substance
1	0.41	550.6	7.37	unknown *
2	0.90	1778.5	23.80	unknown *
3	0.99	5144.3	68.84	Quercetin

Graph:2 ,3

GRAPH-4



Peak	End Rf	Area	Area %	Assigned substance
1	0.07	135.2	0.69	unknown *
2	0.38	173.3	0.88	unknown *
3	0.42	202.0	1.03	unknown *
4	0.58	761.2	3.86	unknown *
5	0.71	1460.2	7.41	unknown *
6	0.91	8480.3	43.05	unknown *
7	0.99	8487.3	43.08	Quercetin

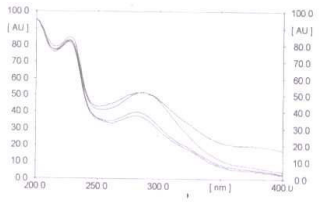


Peak	End Rf	Area	Area %	Assigned substance
1	0.28	3879.6	29.29	unknown *
2	0.90	1560.3	11.78	unknown *
3	0.99	7805.3	58.93	Quercetin

GRAPH-5

GRAPH – 6

Quercetin on all Tracks



T	Rf	Substance	Max. @
1	0.96 Rf	Quercetin	200 nm
2	0.96 Rf	Quercetin	200 nm
3	0.95 Rf	Quercetin	200 nm
4	0.96 Rf	Quercetin	200 nm

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sample	1	Anthocyanins
2	Sample	2	Aqueous Extract
3	Sample	3	Unknown
4	Standard1	4	

Table of substances

Substance	Position MD mm	Tracks 1	2	3	4
Quercetin	82.2	A	A	A	A

Results per track

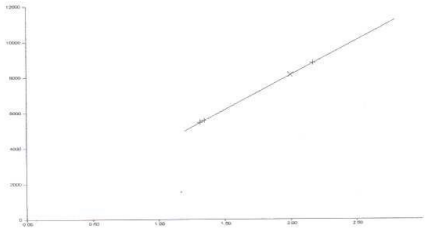
Substance: Quercetin @ 254 nm

Regression via area: Single level Y = 0 + 3903 * X r = 0.00000 sdv = 0.00

Track	Vial	Rf	Amount	Height	X(Calc)	Area	X(Calc)	SampleID/Remark
1	1	0.96				5263.22	1.349 µg	Anthocyanins
2	2	0.96				5144.28	1.318 µg	Aqueous Extract
3	3	0.95				8487.27	2.175 µg	Unknown
4	4	0.96	2.000 µg			7805.26		

GRAPH – 7

winCATS Planar Chromatography Manager



winCATS summary report

Calibration results per Analysis

Sample from vial 1: Anthocyanins
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Quercetin	0.96	1.349 µg	0.000	1	

Sample from vial 2: Aqueous Extract
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Quercetin	0.96	1.318 µg	0.000	1	

Sample from vial 3: Unknown
Result via area

Substance	Rf	X(average)	CV [%]	n	Remark
Quercetin	0.95	2.175 µg	0.000	1	

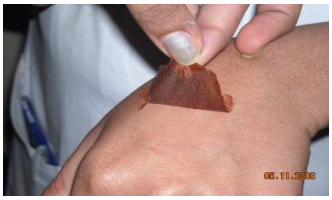
4.STUDY ON THE PEEL OFF PROPERTY OF THE FILM

(a) Human skin

The prepared formulation when applied on human skin dried with in 20 minutes and revealed a good peeling property. (Fig No: 1)

FIGURE NO 1

Formulated film on human skin (Peeling property



(b) White pigskin

After the application of formulation on pigskin the film dried with in 30 minutes and revealed a good peeling property. (Fig No:2)

FIGURE NO 2

Formulated film on animal skin (Peeling property)

(c) Glass slide

The prepared formulation was applied on glass slide, dried with in 15 minutes and showed a good peeling property. Fig(No:3)

FIGURE NO 3

Formulated film on glass slide (Peeling property).

5. MICROSCOPICAL EVALUATION OF THE FILM

Scanning electron microscope: The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical

conductivity.[10]Magnification in a SEM can be controlled over a range of up to 6 orders of magnitude from about x25 to x 250,000 and exceptionally to 2 million times in the Hitachi S-5500 in-lens Field Emission SEM, imaging a specimen area about 60nm wide with resolution up to 0.4 nm. sometimes in combination with formaldehyde and other fixatives, and optionallyfollowed by post fixation with osmium tetroxide. The fixed tissue is then dehydrated. Because air-drying causes collapse and shrinkage The results are tabulated in table no:7

Table No 7
Skin pore size measurement before and after the application of herbal exfoliate formulation

S.NO	PORE SIZE (µm)Before	PORE SIZE(µm) After(5 minutes)	PORE SIZE After treatment (1hr)
1	4.03	4.84	2.08
2	3.69	4.31	1.61

SCANNING ELECRON MICROSCOPY

FIGURE NO 4
(a)Skin before treatment

Presence of dead skin on skin surface

FIG NO 4(b) *Skin after treatment*
Removal of dead skin on skin surface

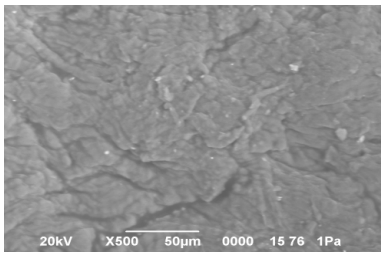
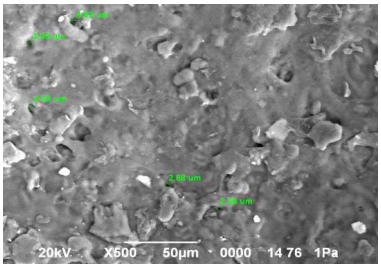


FIGURE NO 4(c)
Skin before treatment (Normal Pore size)



(2) System microscope - Motic image

The film was analyzed microscopically using system microscope (motic image).

FIGURE NO 4(d)
System microscope image of formulation on pigskin

Enlarged pores after skin treatment

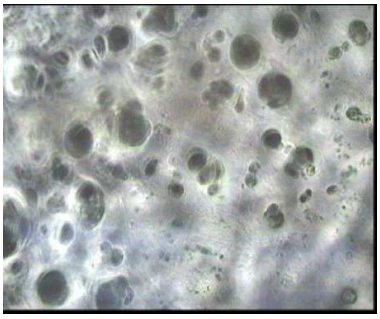
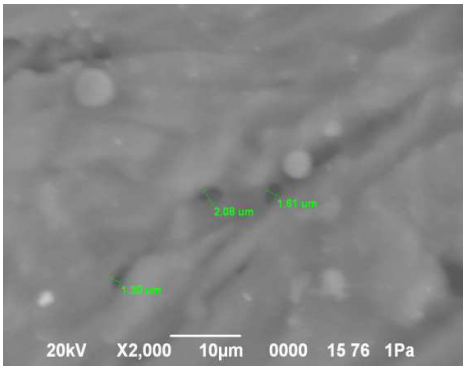


FIGURE NO 4(e)
Skin after treatment (Pore size)



6.STABILITY TESTING OF THE FORMULATION

Guidelines: ICH guidelines.[11 (a) (b)]
Duration: 6 months
Parameters:
(i) Effect of temperature on physical appearance of the formulation.
(ii) Microbial stability of the formulation.

The purpose of the ICH is to "make recommendations on ways to achieve greater harmonization on the interpretation and application of technical guidelines and requirements for product registration in order to reduce or obviate the need to duplicate the testing carried out during the research and development of new medicines".

ICH topics are divided in to quality, safety (non clinical safety studies), clinical efficacy and multidisciplinary areas.

Quality includes issues relating to chemical and pharmaceutical quality assurance.

Safety includes issues relating *in vitro* and *in vivo* non clinical studies.

Efficacy includes matters relating to clinical studies in humans.

The results are tabulated in table no: 13(a) &13(b).

Table No 8
Stability testing of the formulation

S.No	Temperature	Duration (days)	Microbial contamination	Physical Appearance
1	15˚C - 35˚C	20	NIL	Good
2	15˚C - 35˚C	40	NIL	Good
3	15˚C - 35˚C	60	NIL	Good
4	15˚C - 35˚C	80	NIL	Good
5	15˚C - 35˚C	100	NIL	Good
6	15˚C - 35˚C	120	NIL	Good
7	15˚C - 35˚C	140	NIL	More viscous
8	15˚C - 35˚C	160	NIL	More viscous
9	15˚C - 35˚C	180	NIL	More viscous

Table No 9
Stability testing of the formulation

S.No	Temperature	Physical appearance	pH
1	10˚C	Good	6.8
2	20˚C	Good	6.8
3	30˚C	Good	6.8
4	40˚C	Good	6.8
5	50˚C	More viscous	6.3
6	60˚C	Solidified	6.0

The results are tabulated in table no:8& 9

7.SKIN IRRITATION STUDY

The formulated herbal facial peel l should not produce any skin irritation or skin sensitization, after its application on the skin or else it will be unsuitable for application on to the skin. Hence the herbal exfoliate formulation was subjected to skin irritation study using Draize modified scoring technique. [12]

1) Test compound: Herbal facial peel formulation containing *Phaseolusmungo*and*Cyphomandrabetacea.*

2) Standard compound: Orange facial Peel (Marketed product).

Skin irritation studies were performed on healthy rabbits, average weight: 1.5 Kg. The institutional Animal Ethics Committee approved the experimental procedures (Approval number: J.S.S.C.P/ IAEC/ M.Pharm/ Ph.Cog/ 05/ 2008-09). The dorsal surfaces (50cm2) of the rabbits were cleaned and the hair was removed by shaving. The skin was cleaned with diluted dettol. The sample and standard formulations were applied over the skin individually. The animal was kept under observation for skin irritation after1 hour and 2 hours of application of the sample and standard formulation. After the removal of the film the animals were kept under observation for 24, 36, 48 and 72 hrs examined for dermal reactions. The resulting reaction was evaluated by using Primary dermal irritation index. The readings are tabulated in table no: 10,11

(Fig No:5,6,7,8).

SUMMARY OF SKIN IRRITATION SCORES

Table No 10
EDEMA FORMATIONS

Animal No:	Sex	Hours after treatment with herbal exfoliates(1hr)	24hrs	48hrs	72hrs
1	Male	0/0	0/0	0/0	0/0
2	Male	0/0	0/0	0/0	0/0
3	Male	0/0	0/0	0/0	0/0

Table No 11
ERYTHEMA FORMATIONS

Animal No	Sex	Hours after treatment with Herbal Exfoliate (1 hr)	24hrs	48hrs	72hrs
1	Male	0/0	0/0	0/0	0/0
2	Male	1/0	0/0	0/0	0/0
3	Male	1/0	0/0	0/0	0/0

TOTAL: 2/0 =2
MEAN: 2/4 =0.5
Primary Dermal Irritation Index (PDI) =PDI for 1, 24, 48 and 72 hrs
4
= 0.5 / 4
= 0.125
Classification Non- IrritatingCLASSIFICATION SYSTEM

PDI	Classification
Less than 0.5	Non-Irritating
0.5-2.0	Slightly Irritating
2.0-5.0	Moderately Irritating
More than 5.0	Severely Irritating

FIGURES

FIGURE NO 5
Plain rabbit skin

FIGURE NO 6
Rabbit skin with standard (Facial orange peel)

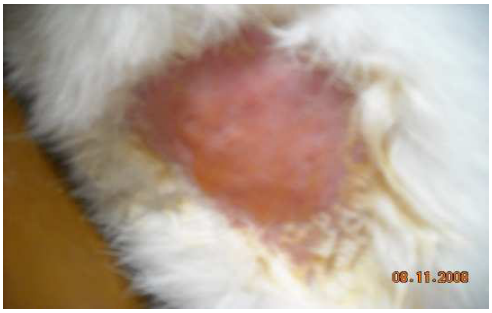


FIGURE NO 7
Rabbit skin with sample
(Herbal facial peel formulation)

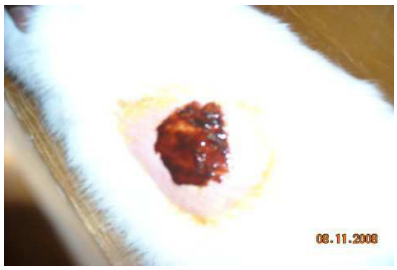


FIGURE NO 8
Rabbit skin after removing standard
(Facial orange peel)

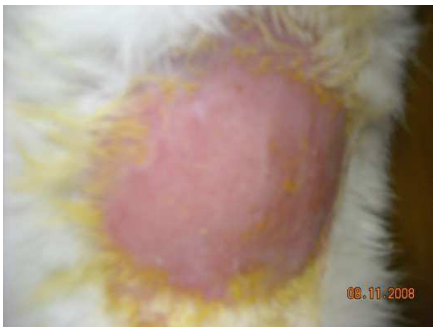


FIGURE NO 9
Rabbit skin after removing sample
(Herbal facial peel formulation)

CONCLUSION

Herbal facial peel are cleansing formula and treatment mask that detoxifies the skin and stimulates its metabolism. It enhances absorption and retention of moisturizing agents and restores the skin's own natural moisture factor. Herbal exfoliates can also be used on dry skin after removing the top layers of the dead skin cells that inhibit moisture absorption in the deeper layers.Some of the major disadvantages of the commercially available herbal facial peel formulation are higher price of the products, inconvenience in application and irritation or reddening of the skin. Near the end of facial peels, the skin will frost, with colors varying from a bright white to gray on the skin surface Polymers often used as exfoliates are not biodegradable and pollutes the environment. Considering the

above disadvantages effort had been taken to formulate an herbal herbal facial peel which is cheaper, ecofriendly and effective on human skin without causing skin irritation. The aim of the present study was to formulate and to evaluate herbal facial peel containing traditionally used herbs namely *Phaseolusmungo* and *Cymphomandrabetaceae.* The formulation showed a good peel off property on human and animal skin without causing skin irritation or edema.The study also revealed that the formulation is capable of enlarging the pores and enhancing the cleansing of the skin by removing dead skin on the surface. The skin pores were also observed to be retaining their normal size with in an hour of treatment; thus retaining the moisture and nutrients with in the skin. The formulation also showed a good microbial stability without adding any antimicrobial agents. The variation in the climatic condition did not affect the physical property of the formulation. Skin treatment with herbal facial peel formulation proves to be more convenient and effective when compared to facial exfoliate scrub. The freeze dried starch of *Phaseolusmungo*was selected for the study due to smaller particle size, absence of lumpy formation in aqueous solution and lower gelatinization at lower temperature. The formulation showed a good peel off property on human and animal skin without causing skin irritation or edema.The study revealed that the formulation is capable of enlarging the pores and enhancing the cleansing of the skin by removing dead skin on the surface. The skin pores were also observed to be retaining their normal size within an hour of treatment; thus retaining the moisture and nutrients with in the skin. The formulation showed a good microbial stability without adding any antimicrobial agents. The variation in the climatic condition did not affect the physical property of the formulation The method of formulation is simple and economically feasible. Hence it can be promoted under small scale industry.

REFERENCES

1. Friedhelm Mark, Research report, University of Bonn, Institute of Nutrition and Food Sciences, Germany, October 9-11, 2007.
2. Donns ., Film and pharmaceutical hard capsule formation properties of Mung bean and water chest nut starches., Food chemistry,106, 2006, 56.
3. H.Panda, The complete Technology book on herbal perfumes and cosmetics, Page no: 403-404.
4. Herrmann, K., Constituents and uses of important exotic fruit varieties. VI. Solanaceae - naranjilla, lulo, topiro, pepino, tamarillo, Judas cherry and tomatillo, IndustrielleObst- und Gemueseverwertung, 79 [6], (1994).
5. Prashant.M.Satturwar, Education of polymerized rosin for the formulation and development of transdermal drug delivery system: A technical note, *AAPS Pharmscitech*, 6(2005), 49-51.
6. Baublis, A., Spomer, A., and Berber-Jimenez, M.D. 1994.Anthocyaninpigments:Comparison of extract stability. J. Food Sci 59:1219-1221, 1233.
7. Fuleki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanins. Extraction and determination of total anthocyanin in cranberry juice. J. Food Sci. 33:72-78.
8. Markakis, P. 1974. Anthocyanins and their stability in foods. CRC Crit. Rev.Food Technol. 4:437.
9. Strack, D. and Wray, V. 1989. Anthocyanins. In Methods in Plant Biochemistry, Vol. 1: Plant Phenolics (P.M.DeyandJ.B.Harborne,eds.), pp. 325-359. Academic Press, San Diego.
10. Russell, S.D.; Daghilian, C.P. (1985). “Scanning electron microscopic observations on deembedded biological tissue sections: Comparison of different fixatives and embedding materials”. Journal of Electron Microscopy Technique 2 (5): 489-495.
11. (a) ICH M3 [International Conference on Harmonization of Technical

Requirements, Volume 43, Supplement 2002.

(b) Non-climical safety studies for the conduct of human clinical trials for pharmaceuticals. Recommended for Adoption at step 4 of the ICH process on 16 July 1997 by the ICH Steering Committee and amended on 8th November 2000 by the ICH Steering Committee.

12. Draize, J.H., MWoodard , G.&Calvery ,H.O.(1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous memberane. J.Pharmacol and Exp.Therapeutics, 82, 377-390.