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Formulation & Evaluation of Antiwrinkle Ethosomal Gel using Allantoin

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Abstract

In present project exhaustive literature survey on ethosomal formulation and their topical application has been done. Selected drug allantoin was procured from SSSUTMS, and identified by solubility and spectral characters. Ethosomal preparation has been done on the variation of soya lecithin and ethanol concentration reported. The best ethosomal preparation (F7) has been observed on the basis of vesicle size and drug entrapment efficiency which has been reported. The best formulation (F7) has been further proceed for the gel formulation in which carbopol is used as gelling agent which has been reported In further process the gel has been evaluated on various parameters like physical characteristic, pH, washability, extrudability study, assay, spreadability, viscosity. On the basis of evaluation parameters (EG-2) has been found the best formulation for the anti-wrinkle activities.

Keywords: Ethosomes, Liposomes, Topical Drug Delivery

Introduction

The skin is well known to be the first element influencing human socio-cultural relationship. How we exteriorly look is very important for self-accepting and for the social life. The skin is where emotions take place and the expression of health and wellness status. The color, the opacity and the hydration levels give a signal of the psychophysical status.

Wrinkle

The wrinkle is a furrow on the skin surface. It is due to a progressive collagen loss, causing a low elasticity of the tissue and to a lower cellular reproduction (Draelos and Pugliese, 2011).

It appears during the natural life course as a phenomenon known as ageing. Skin ageing is due to intrinsic and extrinsic processes. The formers are due to the individual genetic background, are inevitable and not subject to the influence of the human behavior. The latters are due to external factors introduced into the human body, such as smoking, sun exposure, poor nutrition and excessive alcohol consumption (Baumann, 2007).

As stated above the skin is the organ that plays a fundamental role in social life, and a not natural ageing, the presence of wrinkles, scars and imperfections lead to the constant research of tools to slow down the ageing process and to maintain a good tone of the skin.

In present project exhaustive literature survey on ethosomal formulation and their topical application has been done on the variation of soya lecithin and ethanol concentration

Material methods & Results

Characterization of drug

Physiochemical properties of Allantoin

Organoleptic evaluation

It has been done by evaluation of physical characters like appearance, odor etc.

Table No 1: Organoleptic property of Allantoin (EP, 2017)

Color	White or almost white, crystalline powder
Odor	Odorless

Results: It has been found white or almost white powder, odorless powder.

Solubility (at room temperature)

The solubility of a substance is the quantity of that solute that will dissolve in a given quantity of solvent.

Procedure:

10mg of drug was weighed accurately and transferred to 5 different (10 ml) volumetric flasks. Different solvents (water, 0.1 N HCl, Ethanol, Methanol and Chloroform) were added to the flask respectively and the solubility was determined.

Table No 2: I.P. Ranges for Solubility (Indian Pharmacopoeia, 2007)

Descriptive term	Parts of solvent required for
Very soluble	Less than 1
Freely soluble	From 1to 10
Soluble	From 10 to 30
slightly soluble	From 100 to1000
Very slightly soluble	From 1000 to 10000
Practically	Insoluble 10000 or more

Table No 3: Result for Solubility studies of Allantoin in different solvent

S. No.	Solvent Used	Solubility
1	Water	Slightly Soluble
2	Methanol	Freely soluble
3	0.1N HCL	Slightly soluble
4	Ethanol	Slightly Soluble
5	Chloroform	Soluble
6.	0.1N NaOH	soluble
7.	7.2 phosphate buffer	Soluble

Results: It has been found that Allantoin was soluble in methanol, 0.1N NaOH, Chloroform and 7.2 phosphate buffers, slightly soluble in 0.1N HCL, water and Ethanol.

Identification Test by IR (Pavia *et al.,* 2001)

The drug sample was scanned on IR spectrophotometer between 400-4000 cm^{-1} using KBr disc. The obtained IR spectrum was interpretated with the structure of Allantoin. The assignments for the characteristic bands in the infrared spectrum are listed in Table 4 and spectrum shown in Figure 2.

IR Spectra of Allantoin

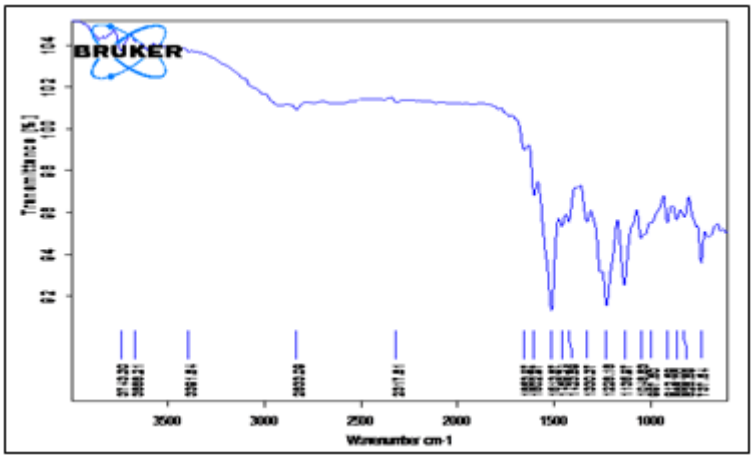

Figure 1: IR Spectra of Allantoin

Table No 4: Interpretation of Allantoin

S. No.	Peak Position	Interpretation
1.	C-H stretching	2833.03
2.	NH stretch	3743.30
3.	C=O stretch	1672.49
4.	C-H bending	1330.31

Loss on drying (Zeljka Marjanovic-Balaban, 2013)

Loss on drying was directly measured by IR moisture balance. Firstly the instrument was calibrated by rotating knob. 1gram powdered drug was weighed accurately. The temperature was fixed at 100°C to 105°C for 5 minutes and constant readings was taken by setting the knob and % moisture was determined.

Result: The percentage of loss on drying of Allantoin has been found 0.25% w/w.

Table No 5: Loss on Drying

Drug	% of LOD
Allantoin	0.25%

Melting point (The Pharmaceutical Codex, 1994)

It is one of the parameters for the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drugs contain the mixed chemicals, they are described with certain range of

melting point.

Procedure for determine melting point

A small quantity of drug was placed into a capillary tube, and then it was placed in the digital melting point apparatus containing liquid paraffin (Chemline). The temperature of the liquid paraffin was gradual increased automatically and reading was taken at which sample started to melt till all sample gets melted.

Table No 6: Melting point range of Allantoin

S. No.	Melting Point		Result
	Onset	Complete	
1	230°C	231°C	230-231°C
2	231°C	232°C	
3	230°C	231°C	

Result: Melting point determined by Melting point apparatus and has been found 230-231°C.

Determination of λmax

The absorption maxima of Allantoin were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Igile *et al.,* 2014).

Procedure for the Determination of λ max

Accurately weighed 10 mg of Allantoin separately and dissolved in 10 ml of 7.2 pH Buffer in 10 ml of volumetric flask and prepared suitable dilution to make it to a concentration of 10 µg/ml make adequate of sample with concentration range of 5- 25µg/ml Allantoin and calculate the spectrum of this solution was run in 200-400 nm range in U.V spectrophotometer. (Labindia U.V. 3000 +)

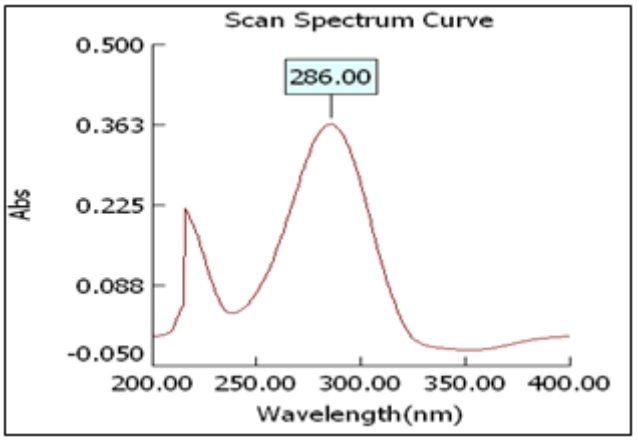

Figure 2: Determination of λ max of Drug

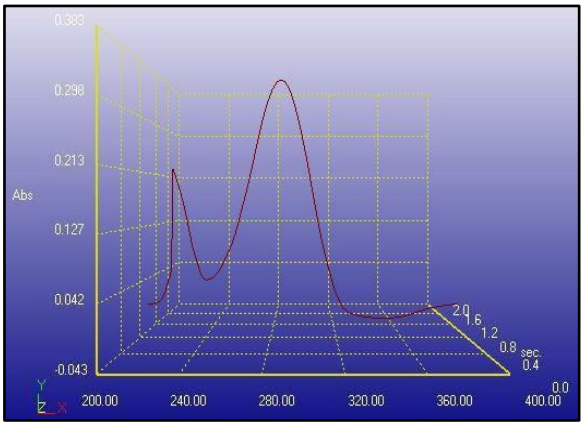

Figure 3: 3D spectra of determination of λ max of Drug

Calibration curve of Allantoin (Igile *et al.,* 2014).

Preparation of Standard Stock Solution

10mg of Allantoin was weighed accurately and transferred to 10 ml volumetric flask, and the volume was adjusted to the mark with the 7.2 pH Buffer to give a stock solution of 1000 ppm or µg/ml.

Preparation of Working Standard Solution

From stock solutions of Allantoin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with 7.2 pH Buffer, gives standard drug solution of 5, 10, 15, 20, 25 µg/ml concentration.

Table No 7: Readings for calibration curve of Allantoin

S. No.	Concentration (µg/ml)	Mean Absorbance*
1.	5	0.180±0.002
2.	10	0.365±0.001
3.	15	0.541±0.001
4.	20	0.725±0.002
5.	25	0.915±0.003

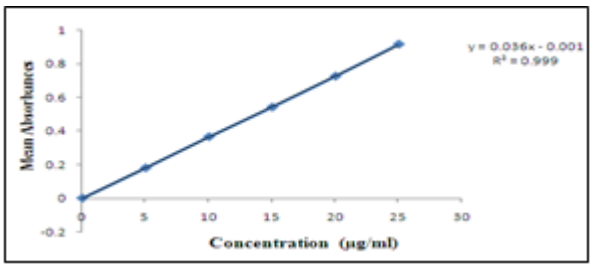

Figure 4: Calibration curve of Allantoin

Table No 8: Statically Data for Linearity

S. No.	Parameter	Remark
1	Linearity Range	5-25 µg/ml
2	Regression Equation	0.036x-0.001
3	Correlation Coefficient	0.999

FORMULATION AND CHARACTERIZATION

Preparation of Ethosomes

Preparation of Allantoin Ethosomes

Ethosomal formulations were prepared by cold method (Supraja *et al.,* 2017). In brief the lecithin (1-4%w/v) and PEG was taken in small round bottom flask and solubilized with ethanol (10-50%) containing drug under mixing with a magnetic stirrer. The round bottom flask was covered to avoid ethanol evaporation. Distilled water was added slowly with continuous stirring to obtain the ethosomal colloidal suspensions. The final suspension of ethosomes was kept at room temperature for 30 min. under continuous stirring. Formulation were stored in the refrigerator and evaluated for vesicle size, vesicular shape, surface morphology, entrapment efficiency and *in vitro* permeation study.

Table No 9 : Different Composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid	Ethanol (%	PEG	Water
F1	50	0.25	10	20	100
F2	50	0.25	20	20	100
F3	50	0.25	30	20	100
F4	50	0.5	10	20	100
F5	50	0.5	20	20	100
F6	50	0.5	30	20	100
F7	50	0.75	10	20	100
F8	50	0.75	20	20	100
F9	50	0.75	30	20	100

Evaluation of Allantoin loaded Ethosomes (Maghraby *et al.,* 2000)

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba scientific, nanoparticle analyzer instrument the results shown in table 8.2.

Entrapment efficiency (Vijay *et al.,* 2010)

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 10,000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without Allantoin was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 286nm.

Table No 10: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size (nm)	Entrapment
F1	250.26±1.21	79.98±0.12
F2	245.56±3.45	82.25±0.45
F3	236.65±2.14	84.45±0.65
F4	252.23±4.56	79.98±0.25
F5	265.47±4.12	75.65±0.36
F6	240.45±3.12	82.12±0.78
F7	**224.56±4.56**	**86.65±0.65**
F8	235.65±3.12	76.65±0.41
F9	241.15±3.45	75.45±0.21

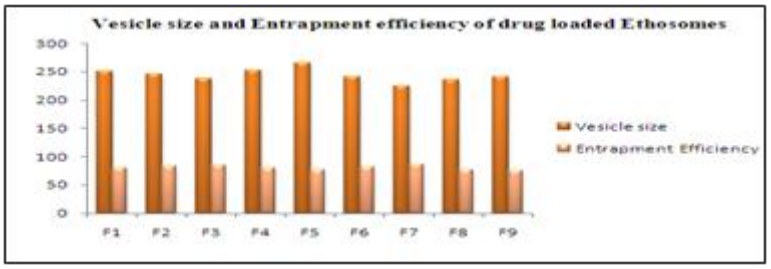

Figure 5: Graphical representation of vesicle size and entrapment efficiency

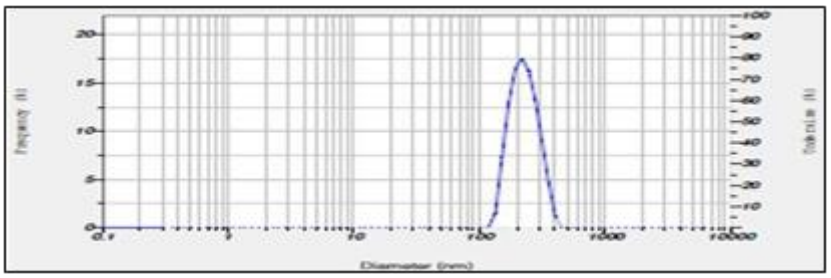

Figure 6: Vesicle size of optimized formulation

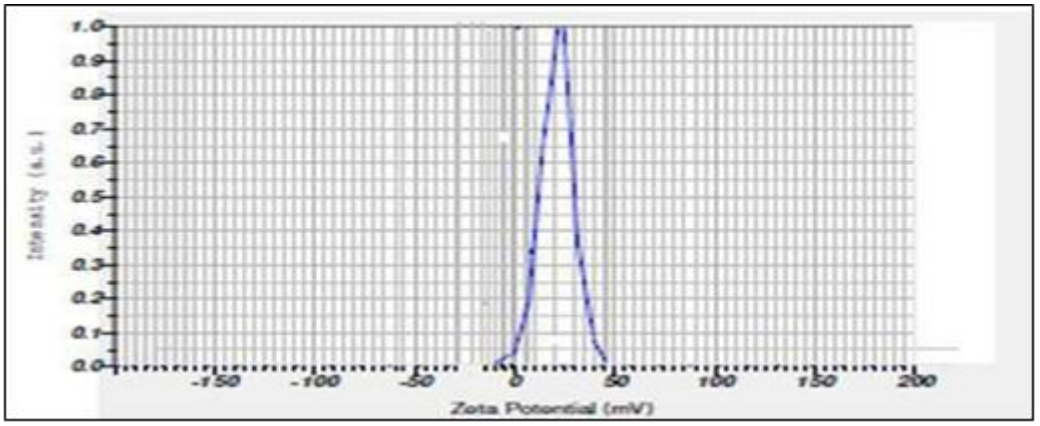

Figure 7: Zeta potential of Optimized formulation

Table No 11: Vesicle size and entrapment efficiency of optimized ethosomes

Formulation	Vesicle size (nm)	Entrapment	Zeta potential
F7	**224.56±4.56**	**86.65±0.65**	-35.56mv

Formulation of ethosomal gel (Touitou *et al.,* 2001)

Preparation of ethosomal gel: 0.5%, 1%, 1.5% w/w gel base was prepared by carbopol in distilled water containing 0.02% methyl paraben and 0.2% propyl paraben, using magnetic stirrer. Carbopol was used as gelling agent and methyl paraben and propyl paraben were used as preservatives. Equivalent to 1% of ethosomes were incorporated into gel base then evaluated and further for physical characteristic, pH, washability, extrudability study, assay, spreadability, viscosity and drug release.

Table No 12: Composition of different gel base

S. No.	Formulation	Carbopol (%)
1.	EG-1	0.5
2.	EG-2	1
3.	EG-3	1.5

Evaluation of gel

Physical Characteristic (Lopez-Pinto *et al.,* 2005)

The Physical Characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 8.5.

Determination of pH (Cevc *et al.,* 1995)

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was
noted. The measurements of pH of each formulation were replicated two times

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation.

Assay (Cevc *et al.,* 1995)

Weight equivalent to 10 mg of ethosomal gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance at 286nm and calculate using calibration curve of linearity.

Spreadability Principle:

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application area. The therapeutic efficacy of a formulation also depends on its spreading value.

A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip of from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 76 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±/1^0C) before the measurements.

Results of evaluation of gel formulation

Table No 13: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EG-1	+++	16.23±0.25	+++	**Good**
EG-2	+++	14.65±0.21	+++	**Good**
EG-3	++	13.25±0.45	+++	**Good**

+++ Good ++ Average

Table No 14: Results of pH, Viscosity and % Assay

Code	pH	Viscosity (cps)	% Assay
EG-1	7.05±0.21	3550±25	96.65±0.25
EG-2	6.98±0.15	3320±32	99.58±0.35
EG-3	7.05±0.045	3240±41	95.45±0.45

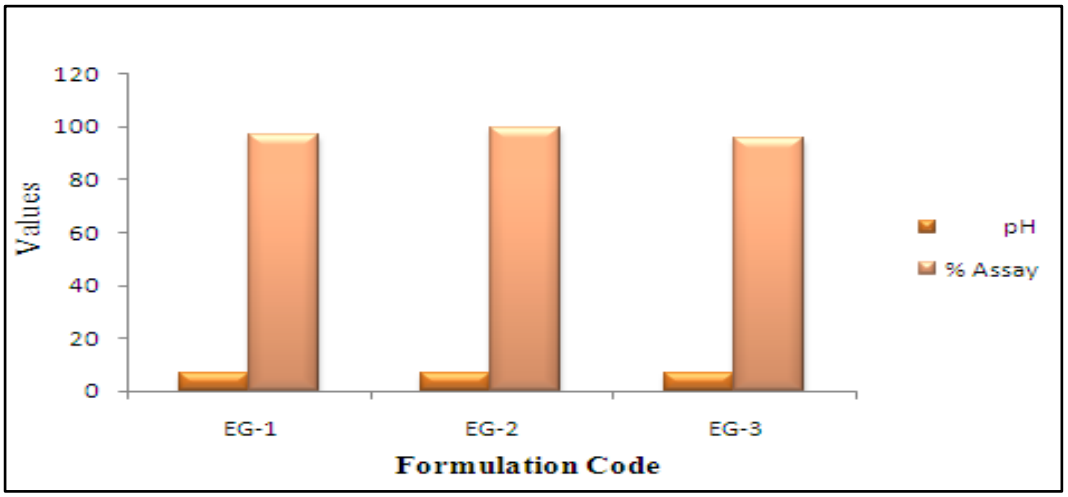

Figure 8: Graphical representation of pH and % Assay

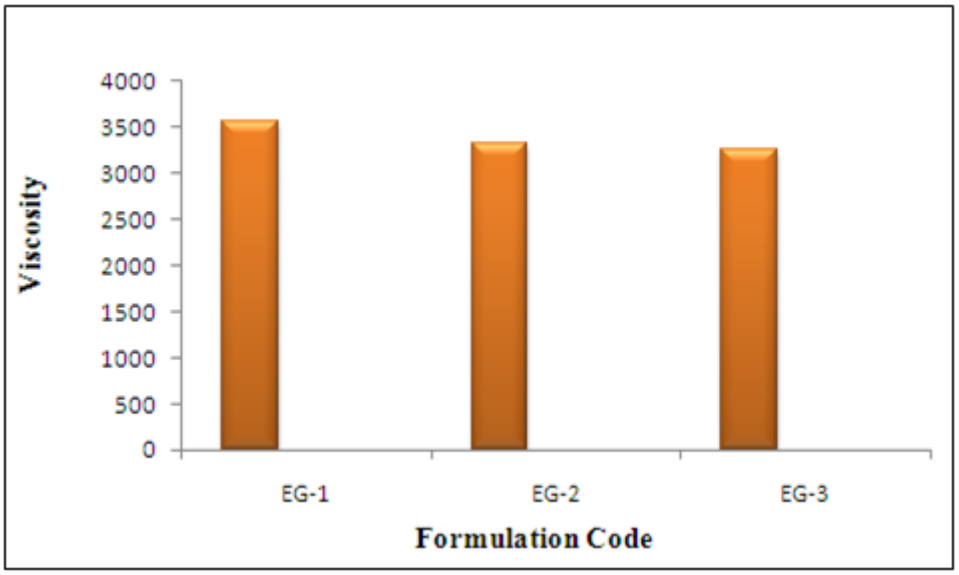

Figure 9: Graphical representation of Viscosity

Conclusion

In present project exhaustive literature survey on ethosomal formulation and their topical application has been done on the variation of soya lecithin and ethanol concentration. The best ethosomal preparation (F7) has been observed on the basis of vesicle size and drug entrapment efficiency. The best formulation (F7) has been further proceed for the gel formulation in which carbopol is used as gelling agent and has been evaluated on various parameters like physical characteristic, pH, washability, extrudability study, assay, spreadability, viscosity. This study disclosed that (EG-2) has been found the best formulation for the anti-wrinkle activities among all prepared formulations.

REFERENCES

1. Draelos, Z.D., Pugliese, P.T., 2011. Physiology of the Skin. Allured Publishing Corporation.
2. Baumann, L., 2007. Skin ageing and its treatment. J. Pathol. 211, 241–51. doi:10.1002/path.2098.

3. Kligman, A.M., Zheng, P., Lavker, R.M., 1985. The anatomy and pathogenesis of wrinkles. Br. J. Dermatol. 113, 37–42.
4. Kusin, S., Lippitz, J., 2009. Skin Fillers. Disease-a-Month 55, 236–256.
5. Fischer, J., Metzler, G., Schaller, M., 2007. Cosmetic permanent fillers for soft tissue augmentation: a new contraindication for interferon therapies. Arch. Dermatol. 143, 507–510.
6. Bhalla, M., Thami, G.P., 2006. Microdermabrasion: Reappraisal and brief review of literature. Dermatol. Surg. 32, 809–14.
7. Camargo Cristina, P., Costa Caroline, S., Gemperli, R., Tatini Maria, D.C., Bulsara Max, K., Riera, R., 2014. Botulinum toxin for facial wrinkles. Cochrane Database Syst. Rev.
8. Hruza, G.J., Geronemus, R.G., Dover, J.S., Arndt, K.A., 1993. Lasers in Dermatology
- 1993. Arch Dermatol 129, 1026– 35.
9. Binic, I., Lazarevic, V., Ljubenovic, M., Mojsa, J., Sokolovic, D., 2013. Skin ageing: Natural weapons and strategies. Evidence-based Complement. Altern. Med. 2013, 1– 10.
10. Bae, J.Y., Choi, J.S., Kang, S.W., Lee, Y.J., Park, J., Kang, Y.H., 2010. Dietary compound ellagic acid alleviates skin wrinkle and inflammation induced by UV-B irradiation. Exp. Dermatol. 19, 182–190.
11. Abhishek Chandel, Vishal Patil, Rohit Goyal, Hitesh Dhamija and Bharat Parashar, International journal of pharmaceutical and chemical sciences, 2012, Vol. 1 (2):pp 563-569.
12. Anju Dhiman, Deepika Singh, IJBR, 2013, Vol 4(10).
13. Ankit Acharya, Mohammed Gulzar Ahmed, Baba Datteshwara Rao, Vinay C H, Journal of Pharmaceutical Sciences, 2016, Vol. 2(1): pp13.
14. Bhalaria, M.K., Naik, S., Misra, A.N., Indian J. of Exp. Bio. 2009, 47, pp 368-375.
15. D. Prasanth, P. K. Lakshmi, International Current Pharmaceutical Journal 2012, 1(11): 370-375.
16. Gajendra Singh Rathore, Yuvraj Singh Tanwar, Anshu Sharma, Pharmaceutical and Chemical Journal, 2015, Vol. 2(1): pp41-50.
17. Jain S., Bhandra D., Jain S., and Jain N. K., 1st Edn, CBS Publishers and Distributors, New Delhi 1997, pp 426-451.
18. Maestrelli, F., Capasso, G., Maria, L., Rodríguez, G., Rabasco, A.M., Ghelardini, C. and Mura, P., J. Lipo. Resear. 2009, pp 1-8.
19. Manosroi, A., Jantrawut, P., Khositsuntiwong, N., Manosroi, W. and Manosroi, J., Chiang Mai. J. Sci. 2009, Vol. 36(2): pp168-178.
20. Marwa H. Abdallah, Int J Pharm Pharm Sci, 2013, Vol. 5(4): pp560-567.
21. Mohammed Irfan, Sushma Verma, Alpana Ram, Asian J Pharm Clin Res, 2012, Vol. 5(3): pp 162-165.
22. Neelam Indora, Dinesh Kaushik, International Journal of Engineering Science Invention Research & Development, 2015, Vol. I (VIII): pp280-306.
23. Pravin P. Aute, Meghana S. Kamble, Dr. Pravin D. Chaudhari, Dr. Ashok V. Bhosale, International Journal of Research 2012-13, Vol. 2(1), pp 218-224.
24. Preeti, Murugesan Senthil Kumar, Indian Journal of Pharmaceutical and Biological Research, 2014; Vol. 2(2): pp 7-13.
25. Rahul G.S. Maheshwari, Rakesh K. Tekade, Piyoosh A. Sharma, Gajanan Darwhekar, Abhishek Tyagi, Rakesh P. Patel, Dinesh K. Jain, Saudi Pharmaceutical Journal, 2012, Vol. 20(20): pp 161–170.

26. Roshan Dhruv Patel, Sudarshan Singh, Sheth Navin, Gendle R J. Pharm. Sci. & Res. 2009, Vol.1(4): pp 71-80.
27. Sheo Datta Maurya, Sunil Kumar Prajapati, Anish Kumar Gupta, Gyanendra Kumar Saxena and Ram Chand Dhakar, Indian J.Pharm. Educ. Res. 2010, Vol. 44(1): pp102- 108.
28. Subheet Jain, Ashok K. Tiwary, Bharti Sapra, and Narendra K. Jain, AAPS PharmSciTech, 2007, Vol. 8(4): pp249.
29. Sujitha B, Krishnamoorthy B, Muthukumaran M, Int J Adv Pharm Gen Res, 2(1), 2014; 34-45.
30. Touitou E., US Patent 1995, Vol. 38: pp 934.