Formulation Development and Evaluation of Sea Buckthorn (*Hippophae Rhamnoides*) Extracts Loaded Oral Herbal Gel

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Abstract

Objective: To formulate and evaluate Sea buckthorn (Hippophae Rhamnoides) extract loaded oral herbal gels.

Materials and Methods: Oral herbal gels were formulated incorporating the extracts of *Hippophae rhamnoides* using carbopol 934 and carbopol 940 as the gelling agent. Eight batches were formulated by varying the concentration of the extract and gelling agent (F1 to F8). The prepared formulations were evaluated for various parameters like physical appearance, pH, spreadability, viscosity, homogeneity, in-vitro release profile and antimicrobial activity against fungi and bacteria. The antimicrobial activity was also compared with a marketed gel formulation.

Results: The oral herbal gel containing extracts of *Hippophae rhamnoides* were prepared by simple dispersion method. Eight batches were prepared with two variables: varied drug concentration and polymer concentration. All the prepared formulation using different concentration of extract showed the percentage drug content ranging from 17.92% to 31.97%, pH values in between 6.0 to 7.2 and spreadability values ranged between the 4.0 to 6.0 cm. Formulation F2 and F4 showed highest percentage drug release among all the prepared formulations i.e., 78.12% and 76.56%. It also shows good zone of inhibition as compared to extract alone i.e., 31 mm with gel extract containing gel formulation and 40 mm zone of inhibition for extract alone. Out of all the formulations, formulation F4 containing herbal extracts showed a good spreadability and very promising antimicrobial activity comparable with a marketed gel.

Keywords: Herbal gel, *Hippophae rhamnoides*, sea buckthorn, viscosity, anti-microbial study.

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Introduction: The thorny nitrogen-fixing deciduous shrub known as Sea Buckthorn (SB) (*Hippophae rhamnoides* L., Elaeagnaceae) is grown across the world for its therapeutic and dietary benefits. The highly abundant vitamins A, B1, B12, C, E, K, and P, as well as flavonoids, lycopene, carotenoids, and phytosterols, make sea buckthorn fruits, seeds, and other portions highly prized. Due to its abundance in strong antioxidants, it is therapeutically significant. These substances are fascinating from a chemical perspective, but they're also interesting because many of them have biological and therapeutic properties, such as antioxidant, cardiovascular, cancer therapy, healing, anti-inflammation, anti-radiation effect, treatment of gastrointestinal ulcers, liver protective agent, antioxidant, platelet aggregation, and immunomodulator properties. The last two decades have seen a significant increase in research on SB, and modern phylogenetic methods have proved the existence of multiple subspecies. The eight subspecies of H. rhamnoides L., as established by Bartish et al. (2002), appear to be the most prevalent and widely accepted taxonomy at the moment (Sun et al. 2002; Ma et al. 2016). Increasingly research is being done on SB as it becomes more significant, particularly regarding the berry's makeup and health benefits (Zieliska and Nowak 2017; Kaur et al. 2017). (Olas 2018). Similar to this, there are several other study publications exploring the use of SB components in contemporary food products.

Although mechanical plaque control methods have the potential to maintain adequate levels of oral hygiene, clinical experience and population-based studies have shown that such methods are not being employed as accurately as they should be by a large number of people. Therefore, several chemotherapeutic agents have been developed to control bacterial plaque, aiming at improving the efficacy of daily hygiene control measures. The interest in plants with antibacterial and anti-inflammatory activities has increased to overcome the consequence of current problems associated with the wide-scale misuse of chemotherapeutic agents that induce microbial drug resistance [1].

Plant-based drugs have played an important role in the modern drug industry. Since ancient times, SB has been used to cure several ailments. SB has emerged as an important plant, which has been investigated for numerous pharmacological properties and shown to be beneficial in a number of therapeutic areas. Several clinical trials have demonstrated the therapeutic potential of SB for the treatment of many diseases including cardiovascular, inflammation, diabetes, platelet inhibition, etc. There is huge potential for developing standardized herbal products from different parts of SB. There are various advantages of using SB as medicinal agent. It is very beneficial for dental complications such as mouth ulcers, bleeding gums, gingivitis, tender gums, periodontitis, and even prevent foul odor in your mouth. It also helps in chronic problems like OSMF (Oral Submucosal Fibrosis) by decreasing the inflammation and burning sensation in mouth ultimately helps in mouth opening. In a recent study it is observed that SNEC oral gel consisting of curcumin is used as natural pain killer but now it can also minimize oral pain. It also consists of ayurvedic properties like golden herb turmeric/curcumin is antimicrobial and antifungal, it may help heal the reddening and bleeding of gums. In a study conducted by Ganju et al., 2005, he observed that immunomodulatory activity of SB leaf extract and suggested that the SBT leaf extract has a significant anti-inflammatory activity. Similarly, Balakrishna et al., 2019 certified that Sea buckthorn oil can be used as an anti-inflammatory nutraceutical and Mehani et al., 2021 prepared novel poly herbal mucoadhesive formulation for treatment of oral aphthous ulcer.

Seabuckthorn (SB) has received worldwide attention for therapeutic, nutraceutical and cosmetic purposes. It is used for the treatment of a number of diseases. Hundreds of commercial products containing SB are available in the market. SB has a rich history of use in treating numerous medical conditions. It has been called a "wonder" plant in many Asian countries, including China, India, and Pakistan. In Mongolia, extracts from the leaves and branches of the plant are used medicinally to treat colitis and enterocolitis in humans and animals. In Middle Asia, the leaves are used to treat GI and skin disorders, and are topically applied to treat rheumatoid arthritis. Sea buckthorn contains carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, and tannins. The aim of present study was to prepare an oral herbal gel consisting of *Hippophae Rhamnoides* for maintaining oral hygiene by avoiding misuse of chemotherapeutic agents that generally produce microbial drug resistance. So by preparing an oral herbal gel will be beneficial in controlling mechanical plaque to maintain appropriate levels of oral hygiene.

Material and methods

Material

Standardized 75% dried ethanolic extract of SB leave was obtained as a gift sample from Aushadhi Herbals New Delhi, India. Gallic acid was purchased from Sigma. All other solvents and reagents used were of analytical grade.

Method

Procedure for calculating gallic acid equivalent (GAE):

10 mg of 75% dried ethanolic extract of SB leaves was dissolved in 10 ml of water i.e.; 1mg/ml solution was prepared. Then to the aliquot (50 microliter) taken from stock solution(1mg/ml) of the 75% dried ethanolic extract of SB leaves, 3.5ml of distilled water and 250 microliter of Folio's Ciocalteau Reagent was added, then the mixture was kept at room temperature for 1-8 min. Then 750 microliter of saturated sodium carbonate solution was added and mixture was kept at room temperature for 2 hours and absorbance of color developed was recorded at 765nm with the help of UV-Visible spectrophotometer against blank. Total phenols content(mg/g) in the SBT leaf extract was expressed as Gallic Acid Equivalent using standard curve (R^2=0.986) prepared from Gallic acid (0.1mg/ml) solution. Then, sodium carbonate solution was dissolved in 200 gm anhydrous sodium bicarbonate in 800 ml of water if bring to boil, after cooling a few crystals of sodium bicarbonate was added and wait for 24 hours at room temperature, then filtered through what man filter paper and then 200 ml water was added and stored at room temperature.

Calculate Gallic acid equivalent formula:

$$\mathbf{C = C_1 \times V/M}$$

Where,

C = Total content of phenolic compounds in mg/g, in GAE (Gallic acid equivalent).

C_1= The concentration of Gallic acid established from the calibration curve in mg/ml

V = The volume of extract in ml
M = The weight of plant extract in gm

Measurement of gallic acid equivalent to extract in phosphate buffer (7.4):

10 mg gallic acid was dissolved in 10 ml of water and 50 microliters was taken from stock solution(1mg/ml) of the 75% dried ethanolic extract of SB leaves, 3.5ml of distilled water and 250 microliter of Folin's Ciocalteau Reagent was added, then mixture was kept at room temperature for 1-8 min and 750 microliter of saturated sodium carbonate solution was added. Mixture was kept at room temperature for 2 hours and absorbance of color developed was recorded at 765nm with the help of UV-Visible spectrophotometer against blank.

Standard curve of gallic acid in water:

100 mg of gallic acid was dissolved in 10 ml of water and first stock was prepared. 10ml from primary stock solution was taken out and make up the volume in 100 ml of volumetric flask was done and second stock was prepared. Again 1ml was pippet out in second stock and volume makeup was done in 10 ml of volumetric flask (third stock) and the aliquot (50 microliter) were taken from stock solution (1mg/ml) of the 75% dried ethanolic extract of SB leaves, 3.5ml of distilled water and 250 microliter of Folin's Ciocalteau Reagent was added, then mixture was kept at room temperature for 1-8 min and 750 microliter of saturated sodium carbonate solution was added. Mixture was kept at room temperature for 2 hours and absorbance of color developed was recorded at 765nm with the help of UV-Visible spectrophotometer against blank.

Preparation of gel

Sea-buckthorn gels were prepared by simple dispersion method. Eight batches were prepared using varying polymer & 75% dried ethanolic extract of SB leaves concentration. Carbopol 934 was soaked in distilled water overnight and dispersed using mechanical stirrer in 1200 rpm. Another solution containing varying concentrations of extract and the required quantity of methyl paraben and propyl paraben were added with continuous stirring. Propylene glycol was also added to the solution. This prepared solution was further mixed with Carbopol 934 solution thoroughly with continuous stirring, volume was made upto 25ml with water. pH was adjusted by drop wise addition of triethanolamine to get gel of required consistency.

Table 1: Results of Preformulation Studies of extract

1.	UV-Spectrophotometery	PBS 6.8	PBS 7.4	Distilled water
a.	**Absorption maxima**	750 nm	740 nm	765 nm
b.	**Beer's Law limit**	5-25µg/ml	10-50 µg/ml	10-50 µg/ml

c.	**Regression equation**	y= 0.9177x+0.9215	y= 0.0329x	y=0.1053x
d.	**R^2**	0.9834	0.9838	0.9867
2.	**Log P**	1.5		

Table 2: Formulation of sea-buckthorn gel with various polymer concentration

Batch No.	F1	F2	F3	F4	F5	F6	F7	F8
Extract (gm)	0.5	0.5	0.5	0.5	0.75	1	1.25	1.5
Carbopol 934 (gm)	0.5%	0.75%	1%	1.25%	1%	1%	1%	1%
Triethanolamine (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
DMSO (ml)	5	5	5	5	5	5	5	5
Ethanol (ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Methyl paraben (gm)	0.2	0.2	0.2	0.2	0.2	0.2		
Propyl paraben (gm)			0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (ml)	50	50	50	50	50	50	50	50

Evaluation of SB oral herbal gel

Visual appearance

The prepared gels were tested for color, clarity, texture, transparency and presence of any gritty particles.

pH determination: The value of pH of SB gel was measured using digital pH meter at the room temperature.

Spread ability

The spread ability of gel formulation was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after 1 min. The standard weight applied on the upper plate was 125 gm.

Texture analysis: The texture analysis was done using texture analyser.

Homogeneity

All developed gel formulations were tested for homogeneity by visual inspection after the gels have been set in to the container. They were tested for their presence and appearance of any aggregates.

Rheological properties: Viscosity of SBT gel formulation was measured using Brookfield viscometer with helipath, using spindle number 96 at 10 rpm.

Stability studies: Sea buckthorn gel was subjected to stability studies as per ICH guidelines. Formulations were studied at 25°C ± 2°C/ 60 ± 5% RH and 40°C ± 2°C/75 ± 5% RH

Anti-microbial study:

Anti-microbial activity was checked by agar well diffusion method. In this method two microbial cultures Candida albicans (fungi) and E Coli (bacteria) were used. Muller Hinton agar (anti-bacterial) and sabouraud dextrose agar (anti-fungal) media was prepared. The agar was then poured into petri plates after complete solidification liquefied medium, which had been inoculated with the test organisms, the well was made aseptically with flamed cork borer on the surface of the agar plate. The gel and leaf extract were delivered into each wells. These were incubated at 37°C for 24 h and were made, zone of inhibition for comparison with those of extract, from the inhibition of zones antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured**.(Table 3 & Figure 1)** Triplicates were carried out for each extract against each of the test organism.

In-vitro release study:

In-vitro release test was performed with Franz diffusion cell by dialysis membrane for the study. One gram of the gel was placed outside the membrane (donor compartment). The entire surfaces of the membrane were in contact with the receptor compartment containing 50 ml of phosphate buffer pH 6.8. The donor compartment was covered with parafilm to prevent the drying out of the gel. The receptor compartment was continuously stirred (50 RPM) using magnetic stirrer at 37°C. The study was carried out for 4 to 5 h. The sample was withdrawn at predetermined time intervals and the same volume was replaced with fresh buffer medium. The absorbance of the withdrawn sample was measured after suitable dilutions at 765 nm to estimate gallic acid using the same method as discussed earlier in this paper. The experiment was carried out in triplicate and average values were reported.

Stability studies:

Selected formulations (F1, F2, F3, F5 and F6) were subjected to stability study. Formulations were stored at room temperature for two months. Stability was evaluated by pH measurements of the gel formulations.

Results

Pre-formulation studies: The result of pre-formulation studies of 75% dried ethanolic extract of SB leaves was observed in **Table 1.**

Formulation of herbal gel: Eight formulations of herbal gels were formulated by varying the concentration of polymer and herbal extract in each of the formulation as shown in **Table 2.**

Percentage drug release and pH measurement: The results for percentage drug release profile and pH values were observed in **Table 4.**

Stability studies: The result of stability studies of various formulations F1 to F8 can be observed in **Table 5.**

Drug release kinetics study: The result for drug release kinetic values of various formulations can be observed in **Table 6.**

Discussion

The present research work was undertaken to explore the potential of SB extract loaded gel in keeping oral cavity healthy. The phenolic components of the extract can inhibit the harmful bacteria and fungi and maintain oral hygiene. The prepared gel also has local anti-inflammatory, local analgesic, antiseptic and anti-oxidant effects. Balakrishna et al., 2019 proved that Sea buckthorn oil can be used as an anti-inflammatory nutraceutical. Mehani et al., 2021 prepared novel poly herbal muco-adhesive formulation for treatment of oral aphthous ulcer.

Results of prefomulation studies of SB extract are tabulated in table 1 .

All the prepared gel formulations were evaluated for parameters such as physical appearance, pH, homogeneity, spread ability, clarity, viscosity drug content and in-vitro drugrelease. The observation reveals that the gels were having smooth texture and were elegant in appearance. The pH of all prepared gels was found to be in range of 6.0-6.8. All the gels showed good spreadability. Also from the data it was observed that increase the concentration of plant extract increases the spreadability. All the prepared gels showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent. The viscosity of all the developed gels was found to be excellent and within the range 4650-8900 cps.

The antimicrobial activity was studied using the well diffusion method. Out of all the formulations, F6 gel containing highest concentration of extract showed the highest zone of inhibition that can be observed in **Table 3** against Candida albicans & E. coli. Ganju et al., 2005 conducted immunomodulatory activity of Seabuckthorn (SBT) leaf extract and suggested that the SB leaf extract has a significant anti-inflammatory activity. The result is shown in **Figure 1**. The percentage drug release graph of optimized formulation of SB extract is shown in **Figure 2.**

In-vitro release test was performed with Franz diffusion cell by dialysis membrane in buffer pH 6.8 for 6 hours for optimized formulation F6 **(Fig 5)**. Percent drug release was fitted in different kinetic equations and R square value is tabulated in **Table 6**.

Table 3: Zone of Inhibition

Micro-Organism	Preparation	Zone of Inhibition (mm)			
		0.5%	0.75%	1%	1.5%
	Gel	31	32.5	35	38

E coli **Candida albicans**	**formulation**				
	Gel Formulation (F6)	25 mm	32	33	35

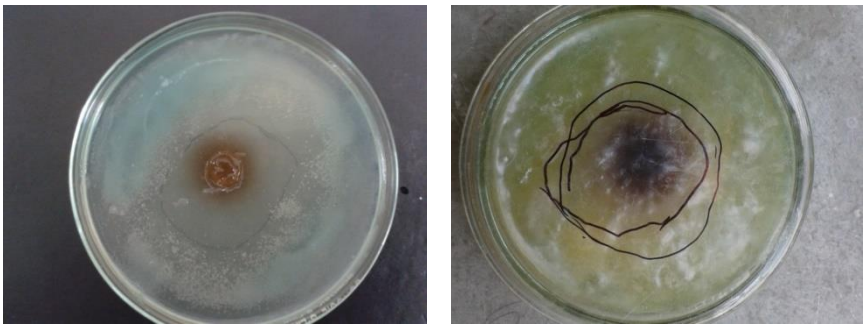

Figure 1: Prepared gel against *Candida albicans* and *E. coli*

Table 4: Results for evaluation parameters

Formulation Code							ug content (mg GAE/g) (%)
F1							17.92%
F2							14.20%
F3							24.60%
F4							27.10%
F5							29.12%
F6							31.97%
F7	Brown	Homogenous	Smooth	7100±10	6.9±0.12	25±5	18.76%
F8	Brown	Homogenous	Smooth	7750±10	6.5±0.12	40±5	15.86%

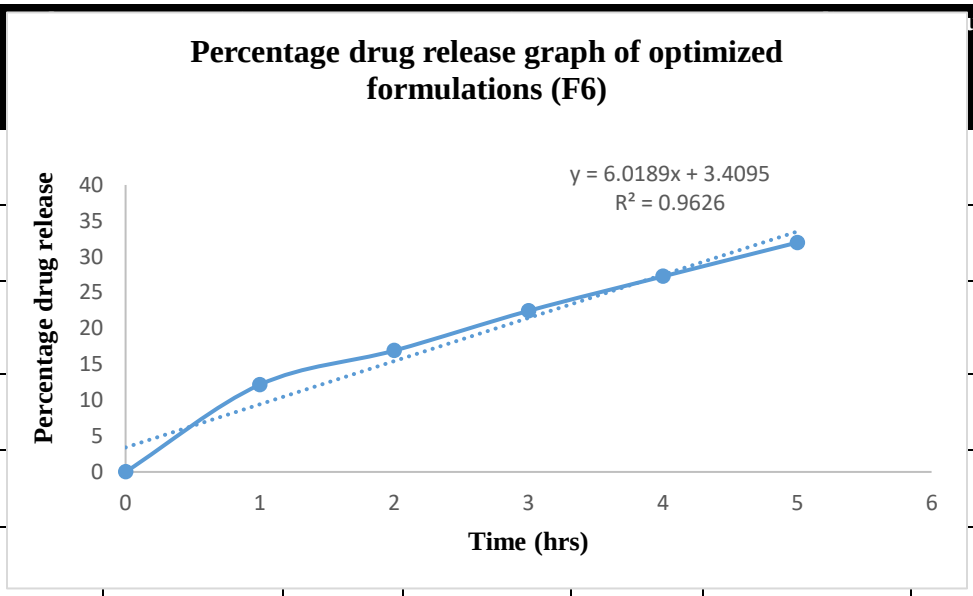

Figure 2: Percentage drug release graph of optimized formulation (F6)

Table 6: Drug release kinetics of various formulations

Formulations	Kinetics of drug release

	Cumulative drug release (%)	**Zero order model**	**First order model**	**Higuchi model**	**Peppas parameters**
F6	**40**	**0.935**	**0.96**	**0.90**	**0.97**

Table 7: Results of stability studies

Formulation	Percentage drug content after stability	pH measurement after stability
F1	17.92	6.0
F2	14.20	6.1
F3	24.60	7.1
F5	29.12	7.3
F6	31.97	7.2

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Ethical Statement: No research work carried out on animals.

Conclusion

SB extract has great nutritional and therapeutic values. It is a plant that is frequently used in traditional medicine for a range of clinical issues. Its pharmacological activities have been scientifically examined, and include antiulcerogenic, in vitro and in vivo antioxidant, cardiac disease, antiatherogenic, radio protecting, and positive effects on experimental injury and clinical liver conditions, as well as the prevention of platelet aggregation. The bioactive and antioxidant qualities of sea buckthorn are due to a variety of phenolic components in the plant, including phenolic acids, flavonoids, and hydrolysable tannins. Thus, SB extract loaded herbal gel for good oral hygiene can be prepared. Herbal formulations are mostly preferred by consumers with a belief that they are free from any kind of side effects.

References

1. Luntraru CM, Apostol L, Oprea OB, Neagu M, Popescu AF, Tomescu JA, Mulțescu M, Susman IE, Gaceu L. Reclaim and Valorization of Sea Buckthorn (Hippophae rhamnoides) By-Product: Antioxidant Activity and Chemical Characterization. Foods. 2022 Feb 4;11(3):462.
2. Upadhye K, Charde K, Dixit G, Bakhle S. Formulation and evaluation of herbal gel for management of mouth ulcers. *Indian J Pharm Pharmacol* 2021;8(3):226-230.
3. Jaśniewska A, Diowksz A. Wide spectrum of active compounds in sea buckthorn (Hippophae rhamnoides) for disease prevention and food production. Antioxidants. 2021 Aug 12;10(8):1279.
4. Ciesarová Z, Murkovic M, Cejpek K, Kreps F, Tobolková B, Koplík R, Belajová E, Kukurová K, Daško Ľ, Panovská Z, Revenco D. Why is sea buckthorn (Hippophae rhamnoides L.) so exceptional? A review. Food Research International. 2020 Jul 1; 133:109170.
5. Charde K, Upadhye KP, Gholse YN, Chaple DR. Current updates on recurrent aphthous stomatitis: etiology, pathogenesis and management. *World J Pharm Sci*. 2020;9(5):448–462.
6. Sing R, Bansal S, Mishra MK. Formulation and evaluation of herbal oral gel containing extracts of powdered Psidium guajava linn leaves with Curcuma longa linn rhizomes to treat mouth ulcer. International Journal of Drug Development and Research. 2020;12(2):150.
7. Sharma PC, Kalkal M. Nutraceutical and Medicinal Importance of Seabuckthorn (Hippophae sp.). Therapeutic, Probiotic, and Unconventional Foods. 2018 Jan 1:227-53.
8. Thombre KP, Sharma D, Lanjewar A. Formulation and evaluation pharmaceutical aqueous gel of powdered Cordia dichotoma leaves with guava leaves. Am. J. PharmTech Res. 2018; 8:268-77.
9. Upadhye K, Charde K, Dixit G, Sheikh S. Studies on Inorganic Materials Based Antiulcer
10. Pharmaceutical Gel for Oral Cavity. Formulation and Evaluation. Acta
11. Scientific Pharmaceutical sciences. *Acta Sci Pharm Sci*. 2018;2(7):38–44.
12. Shaikh S, Shete A, Doijad R. Formulation and evaluation pharmaceutical aqueous gel of powdered guava leaves for mouth ulcer treatment. Pharma Tutor. 2018 Apr 1;6(4):32-8.
13. Patel NA, Patel M, Patel RP. Formulation and evaluation of polyherbal gel for wound healing. International Research journal of pharmaceuticals. 2011;1(1):15-20.
14. Mishal ganesh., dixit Gouri., gulkari vijay., 2012 formulation and evaluation of herbal gel.3(4):501-505.
15. Goyal S, Sharma P, Ramchandani U, Shrivastava SK, Dubey PK. Novel anti-inflammatory topical herbal gels containing Withania somnifera and Boswellia serrata. International Journal of Pharmaceutical and Biological Archives. 2011;2(4):1087-94.
16. Yusufoglu HS. Topical anti-inflammatory and wound healing activities of herbal gel of Ziziphus nummularia L. (F. Rhamnaceae) leaf extract. International Journal of Pharmacology. 2011;7(8):862-7.
17. Ray A. Diabetic foot ulcer: a serious problem for diabetic patient. Pharma Tutor. 2014 Oct 1;2(10):23-6.
18. Hurler J, Žakelj S, Mravljak J, Pajk S, Kristl A, Schubert R, Škalko-Basnet N. The effect of lipid composition and liposome size on the release properties of liposomes-in-hydrogel. International journal of pharmaceutics. 2013 Nov 1;456(1):49-57.

19. Karodi R, Jadhav M, Rub R, Bafna A. Evaluation of the wound healing activity of a crude extract of Rubia cordifolia L. (Indian madder) in mice. International Journal of Applied Research in Natural Products. 2009 Jun 2;2(2):12-8.
20. Williams A. Transdermal and topical drug delivery from theory to clinical practice. Pharmaceutical press; 2003.
21. Suryakumar G, Gupta A. Medicinal and therapeutic potential of Sea buckthorn (Hippophae rhamnoides L.). Journal of ethnopharmacology. 2011 Nov 18;138(2):268-78.
22. Zeb A. Important therapeutic uses of sea buckthorn (Hippophae): a review. Journal of biological sciences. 2004;4(5):687-93.
23. Saroha K, Singh S, Aggarwal A, Nanda S. TRANSDERMAL GELS-AN ALTERNATIVE VEHICLE FOR DRUG DELIVERY. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2013 Jul 1;3(3).
24. Triantafillopoulos N. Measurement of fluid rheology and interpretation of rheograms. Kaltec Scientific Inc, Michigan. 1988.
25. SB. Ramane., VN. Syed. and KR. Biyani., 2013 Evaluation of Wound Healing Activity of Polyherbal Gel – A Novel Herbal Formulation. 4 (3): 788-794.
26. Trivedi V, Rathore RP, Goyal M, Singh N. Rheological Study of Diclofenac Gel Containing Different Concentration of Carbapol 940. International Journal of Research in Pharmacy & Science. 2013 Jan 1;3(1).
27. Negi A, Sharma N, Singh MF. Formulation and evaluation of an herbal anti-inflammatory gel containing Eupatorium leaves extract. Journal of Pharmacognosy and Phytochemistry. 2012 Nov 1;1(4):112-7.
28. Loveelen K, Guleri K. Topical gel: A recent approach for novel drug delivery Asian. J. Bio. Pharm. Sci. 2013; 3:1-5.
29. Reddy MS, Mutalik S, Rao GV. Preparation and evaluation of minoxidil gels for topical application in alopecia. Indian journal of pharmaceutical sciences. 2006;68(4).
30. Si S, Swain S, Kanungo SK, Gupta R. Preparation and evaluation of gels from gum of Moringa oleifera. Indian journal of pharmaceutical sciences. 2006;68(6):777.
31. Charde MS, Fulzele SV, Satturwar PM, Joshi SB, Kasture AV. Wound healing and anti-inflammatory potential of madhu ghrita. Indian journal of pharmaceutical sciences. 2006;68(1):26.
32. Rupal J, Kaushal J, Mallikarjuna SC, Dipti P. Preparation and evaluation of topical gel of valdecoxib. International Journal of Pharmaceutical Sciences and Drug Research. 2010;2(1):51-4.
33. Mekkawy A, Fathy M, El-Shanawany S. Formulation and in vitro evaluation of fluconazole topical gels. British Journal of Pharmaceutical Research. 2013;3(3):293-313.
34. Das S, Haldar PK, Pramanik G. Formulation and evaluation of herbal gel containing Clerodendron infortunatum leaves extract. International Journal of PharmTech Research. 2011;3(1):140-3.
35. Shinde AJ, Khade KM, Kadam AR, More HN. Development and in vitro evaluation of chitosan gel for wound healing activity. Int J Res Ayurveda Pharm. 2011;2(1):271-4.
36. Mundada M, Wankhede S, Patwardhan S, Avachat A. Formulation and evaluation of topical gel of lornoxicam using a range of penetration enhancers. Indian J Pharm Edu Res. 2012; 47:168-71.
37. Kumar RV, Kumar S. Formulation and evaluation of Mimosa pudica gel. Int J Pharm Sci. 2011;3(1):55-7.
38. Saiesh Phaldesai., Shabaraya A.R., D. Shripathy., Soman Leyana,2013 Topical Gel Containing Anti-Fungal Agent.2(5):236-234.
39. Bagade B Varsha., M Varsha. Jadhav and Vilasrao. J. kadam, 2013 Study on Antimicrobial Activity of Herbal Formulation.4 (11):3099-3104.

40. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000 Apr 3;65(3):403-18.
41. Nimisha N. Development and Evaluation of herbal Cosmaceutical for skin care. Int J Pharm Bio Sci. 2013;4(2):86-2.
42. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and applied pharmacology. 2010 Mar 1;243(2):198-216.
43. Kesarwani K, Gupta R. Bioavailability enhancers of herbal origin: An overview. Asian Pacific journal of tropical biomedicine. 2013 Apr 1;3(4):253-66.
44. Geetha S, Ram MS, Singh V, Ilavazhagan G, Sawhney RC. Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides)—an in vitro study. Journal of Ethnopharmacology. 2002 Mar 1;79(3):373-8.
45. Saggu S, Divekar HM, Gupta V, Sawhney RC, Banerjee PK, Kumar R. Adaptogenic and safety evaluation of seabuckthorn (Hippophae rhamnoides) leaf extract: a dose dependent study. Food and Chemical Toxicology. 2007 Apr 1;45(4):609-17.
46. Geetha S, Jayamurthy P, Pal K, Pandey S, Kumar R, Sawhney RC. Hepatoprotective effects of sea buckthorn (Hippophae rhamnoides L.) against carbon tetrachloride induced liver injury in rats. Journal of the Science of Food and Agriculture. 2008 Jul;88(9):1592-7.
47. Maheshwari DT, Kumar MY, Verma SK, Singh VK, Singh SN. Antioxidant and hepatoprotective activities of phenolic rich fraction of Seabuckthorn (Hippophae rhamnoides L.) leaves. Food and Chemical Toxicology. 2011 Sep 1;49(9):2422-8.
48. Singh AK, Paul AD, Naved T. Boiactivity guided extraction of Seabuckthorn (Hippophae rhamnoides L.ssp. turkestanica) leaves. Journal of Scientific and Industrial Research 2012; 72: 307-11.10.
49. Kushwaha SK, Kushwaha N, Maurya N, Rai AK. Role of markers in the standardization of herbal drugs: a review. Archives of Applied Science Research. 2010;2(1):225-9.
50. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965 Jan 1;16(3):144-58.
51. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials. 2000 Sep 1;21(18):1879-85.
52. Misal G, Dixit G, Gulkari V. Formulation and Evaluation of Herbal Gel. Indian Journal of Natural Products & Research 2012; 3(4): 501-05.14.
53. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: reviewing three decades of research. International journal of pharmaceutics. 2007 Mar 6;332(1-2):1-6.
54. Irfan M, Verma S, Ram A. Preparation and characterization of ibuprofen loaded transferosome as a novel carrier for transdermal drug delivery system. Asian journal of pharmaceutical and clinical research. 2012 Jul;5(3):162-5.
55. Dave V, Kumar D, Lewis S, Paliwal S, Ethosome for Enhanced Transdermal Drug Delivery of Aceclofenac. International Journal of Drug Delivery 2010; 2:81-92.
56. Olas B, Skalski B, Ulanowska K. The anticancer activity of sea buckthorn [Elaeagnus rhamnoides (L.) A. Nelson]. Frontiers in pharmacology. 2018 Mar 15; 9:232.