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Research Article

Formulation and Validation of Herbosomes Loaded Herbal Topical Preparation for Eczema.

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A B S T R A C T

Eczema, a common inflammatory skin condition, presents significant challenges in management due to its multifactorial etiology and diverse clinical manifestations. Characterized by pruritus, erythema, and eczematous lesions, eczema negatively impacts patients' quality of life and requires comprehensive treatment approaches. Conventional treatments include topical corticosteroids, moisturizers, and immunomodulators, which provide symptomatic relief but may be associated with adverse effects and limited long- term efficacy. In recent years, there has been growing interest in alternative therapies, including herbal medicines, probiotics, and phototherapy, for eczema management.Herbal remedies, such as Berberis Aquifolium, have shown promise in eczema treatment due to their anti-inflammatory and antimicrobial properties. Novel drug delivery systems, such as Herbosomes, offer innovative approaches to enhance the bioavailability and efficacy of herbal constituents for eczema management. In the present study, the Berberis Aquifolium herbosomal lotion was formulated and used for an analytical method that is Ultraviolet spectroscopy (UV) which is developed and validated. In the method development solvent used is distilled water. The method is validated for linearity, range, limit of detection, limit of quantification, precision, and robustness. The developed analytical method was found to be simple, reliable, precise, and robust. The validated UV Spectroscopy method was successfully applied for quantification of Berberis Aquifolium loaded Herbosomal lotion.

Keywords : Eczema, Inflammatory skin condition, Topical corticosteroids, Moisturizers, herbal medicines , Herbosomal lotion, UV spectroscopy, Validation.

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INTRODUCTION:

In recent decades, significant scientific research has been devoted to targeted drug delivery, aiming to develop novel drug delivery systems (NDDS). Herbal drugs offer numerous advantages including enhanced solubility and bioavailability, increased pharmacological activity, and protection from physical and chemical degradation [1]. There is considerable scope for the application of novel drug delivery systems to herbal drugs, which could significantly enhance their activity and overcome various challenges.

Achieving good bioavailability in natural products requires striking a balance between hydrophilicity (for dissolution in gastrointestinal fluids) and lipophilicity (for crossing lipidic bio membranes). Many phytoconstituents, such as polyphenolics, exhibit good water solubility but are poorly absorbed due to their large molecular size, incompatibility with passive diffusion processes, or poor miscibility with oils and other lipids [2].

One approach to enhancing the bioavailability of water-soluble phytoconstituents, particularly polyphenolics, is by converting them into lipid-compatible molecular complexes known as "Herbosomes." This technology, which is patented in the US, involves creating molecular associations that are more bioavailable than simple herbal extracts, owing to their enhancedcapacity to cross lipid bio membranes and circulate effectively[3,4]. Herbosomes form at the molecular level through various electrostatic forces, including ion-dipole interactions, dipole- dipole interactions, and hydrogen bonding[5].

Phospholipids are small lipid molecules characterized by a glycerol moiety bonded to two fatty acids, with the third hydroxyl typically bearing a phosphate group. These

molecules play a crucial role in the formation of Herbosomes and contribute to their enhanced bioavailability and efficacy.

Eczema, a chronic inflammatory skin disorder, poses significant challenges in management due to its multifactorial etiology and diverse clinical presentations. Traditional treatment modalities often involve corticosteroids and immunosuppressive agents, which may carry adverse effects and offer limited long-term efficacy. In recent years, there has been a growing interest in herbal remedies for eczema management due to their potential efficacy and favourable safety profiles[6].

Berberis Aquifolium, commonly known as Oregon grape or Mahonia aquifolium, is a medicinalplant with a rich history in traditional medicine for treating various skin conditions, includingeczema. This review aims to provide a comprehensive overview of the therapeutic potentialofBerberis Aquifolium in eczema treatment[7].

Berberis Aquifolium contains bioactive compounds such as berberine, which exhibit anti- inflammatory, antimicrobial, and antioxidant properties, making it a promising candidate for eczema management. Preclinical and clinical studies have shown significant improvements in eczema symptoms with topical formulations containing Berberis Aquifolium extract[8].However, further research is needed to elucidate its mechanisms of action, optimal dosage regimens, and long-term safety and efficacy. Berberis Aquifolium offers a promising natural therapeutic option for eczema management, providing an alternative or adjunct to conventional treatments[9].

Herbal lotions have gained popularity as natural alternatives for skincare, offering potential benefits such as hydration, soothing effects, and antioxidant protection. In recent years, there has been increasing interest in exploring the therapeutic properties of botanical extracts in skincare formulations. This review provides an overview of the efficacy and safety of herbal lotions in promoting skin health[10].Herbal lotions are formulated using a combination of plant-based ingredients such as extracts, essential oils, and carrier oils. These ingredients often possess anti-inflammatory, antioxidant, antimicrobial, and moisturizing properties, making them suitable for treating various skin conditions, including eczema, acne, and dryness.herbal lotions offer advantages such as minimal risk of adverse effects, making them suitable for sensitive skin types. Their natural composition and gentle formulation make them appealing to consumers seeking safer and sustainable skincare options[11,12].

Pharmaceutical analysis is a branch of practical chemistry which involves a set of procedures to classify, determine, quantify, purify a substance, separate the compounds of a solution or mixture, or determine the structure of chemical substances, atomic spectroscopy, UV-visible spectroscopy is most frequently used techniques in pharmaceutical analysis. Validation of the method is the mechanism used to ensure that the analytical technique employed for a specific test is appropriate for its intended use. Method validation can be used to assess the quality, reliability, and consistency of the analytical results and it is an essential part of good analytical practice. Different parameters of analytical validation are system suitability test, specificity, accuracy, range, linearity, precision, etc. The aim of this research was to validate a Herbosomal lotion of Berberis Aquifolium extract.

MATERIALS AND METHODS:

Materials :

Zyrex Ayurveda India, has given Berberis Aquifolium, Karanj seed and Aloe vera extracts as a gift sample and Shivabiochem industries Maharashtra, India has given Soya lecithin as gift sample. Dichloromethane , N-Hexane, Stearic acid, Cetyl alcohol, liquid paraffin (mineral oil), triethanolamine (TEA), Glycerine, Preservative (Methyl paraben),bentonite other chemical and solvents were analytical grade and procured from laboratory.

Methods:

Formulation of Herbosome[13-14]:

- **Rotary Evaporation Technique**

Specified amount/quantity of herb extract mixed with phospholipid in the ratio of 1:1 dissolved in 20ml of Dichloromethane and transfer it into the rotary evaporator flask reflux it till the formation of thin film occurs at temp 40^{0c} and then hydrate the film with 20 ml of n Hexane by vigorous shaking and then transfer the suspension into the beaker followed by stirring on magnetic stirrer for 30minand at last centrifuge the suspension for 15 min at 3000rpm and then observe the herbosomes under the microscope. The precipitate obtained was collected, placed in an amber coloured glass bottle and stored at room temperature.

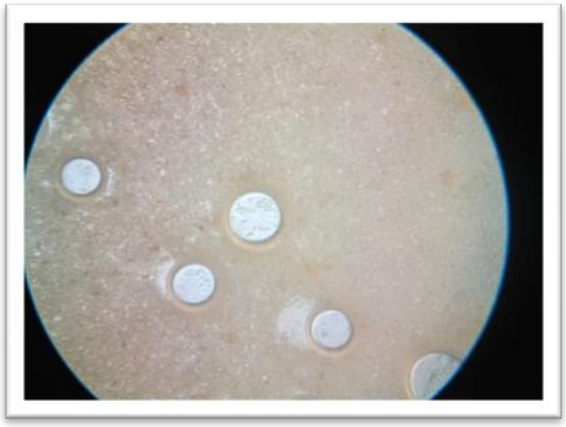

Figure 1: Microscopic image of Herbosomes

Formulation of Lotion[15] :

Cetyl alcohol, steric acid and mineral oil (liquid paraffin) was taken in first beaker, then heat on a water bath for uniform mixing. After few minutes oil phase was formed. Aloe vera extract, Karanj seed extract,Bentonite, Methyl Paraben, Glycerine, Triethanolamine and water was taken in second beaker. Mixing all the ingredients by heating on a water bath, the aqueous phase was formed. Oil phase was added into aqueous phase.

Table1: List of Ingredients used in the formulation

S/N	Ingredients	Category	Quantity
Water Phase			
1	Karanj Seed Extract	Antiseptic	1gm
2	Aloe Vera Extract	Moisturiser	1gm
3	Bentonite	Thickening Agent	1.25 gm
4	Methyl Paraben	Preservative	0.05 gm
5	Glycerine	Humectant	1.5 ml
6	Triethanolamine	Neutralizer	0.45 ml
7	Water	Diluent	Q.S.
Oil Phase			
8	Cetyl Alcohol	Co-emulsion	1gm
9	Steric Acid	Emulsifier	1.25 gm
10	Mineral oil (liquid Paraffin)	Occlusive	2.5 ml

Incorporation of Herbosomes in Formulation (Lotion):

Herbosome containing drug was mixed in to lotion by an electrical mixer 25rpm/2 min, with the concentration of herbosome in lotion being 1% (w/w Herbosome suspension/total)

METHOD DEVELOPMENT AND VALIDATION:

Instrument: Double beam UV Visible Spectrophotometer (UV SHIMADZU 1800)

Derivative UV spectroscopy has been widely used as a tool for quantitative analysis and for quality control. This technique has various advantages over the conventional absorbance methods such as the discrimination of the sharp spectral features over the large bands and the enhancement of the resolution of the overlapping spectra.

Method

In order to ascertain the wavelength of maximum absorption ofBerberis Aquifolium, stock solution of 10 mg/ml was prepared by taking 10 mg of drug in 10 ml of Water. Different solution of drugs in water were scanned using spectrophotometer within the wavelength region of 400-200 nm against Water as blank. The resulting spectra were shown in Fig. 3 & absorption curve showed characteristic absorption maxima at 260nm for drug.

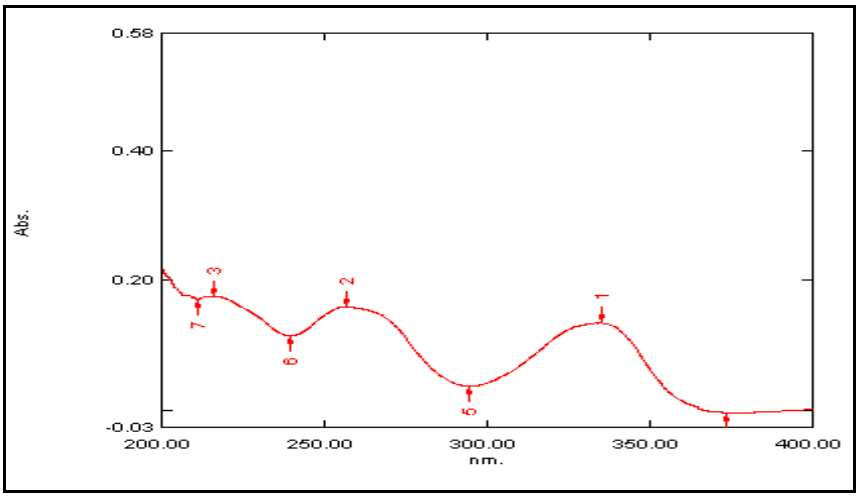

Figure: 2. UV Spectrum of Berberis Aquifolium in water

Table 2: Standard Calibration Curve of Berberis Aquifolium Extract at 256nm

Concentration	Absorbance
0.5	0.30
1	0.44
1.5	0.60
2	0.76
2.5	0.91

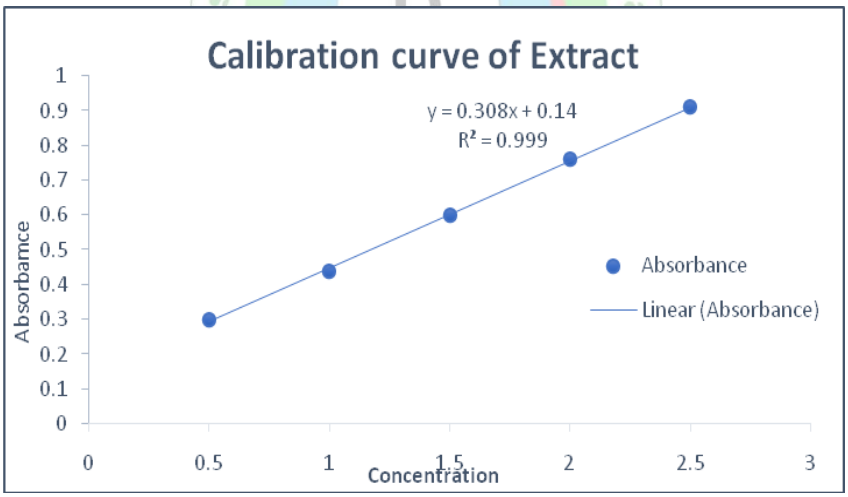

Figure 3: Standard Calibration Curve of Linearity

Preparation of standard stock solution of Berberis Aquifolium

The standard stock solution was prepared with weighed amount of Berberis Aquifolium (10 mg). The stock solution was dissolved separately in 10mL of water in a volumetric flask. A series of dilutions of 0.5, 1, 1.5, 2 and 2.5 were prepared, and absorbance was measured at 260nm. These diluted solutions were analyzed for Linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Validation Parameters of Herbosomal Lotion

LINEARITY [16]:

The linearity of this method was determined at concentration levels ranging between 0.5 mg/ml and 2.5mg/ml. The plot of absorbance v/s concentration of Berberis Aquifolium lotion was found to be linear in the range Beer's law was obeyed over this concentration range.

Table 3: Standard Calibration Curve of Herbosomal Lotion at 256nm

Concentration	Absorbance
0.5	0.41
1	0.63
1.5	0.82
2	0.99
2.5	1.26

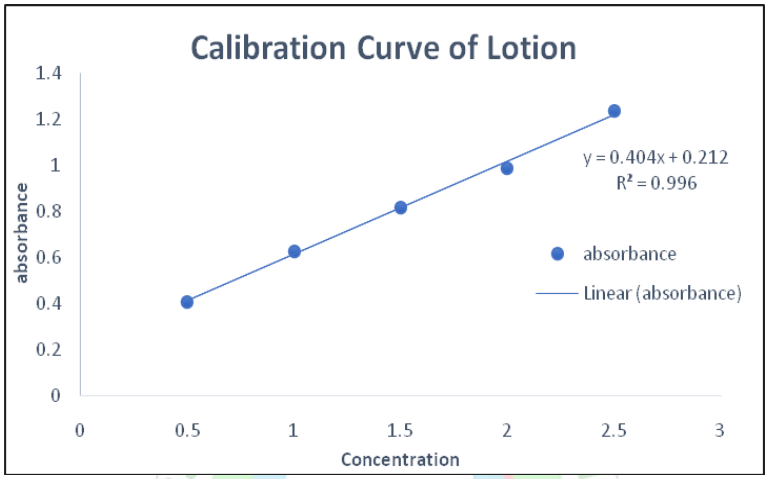

Figure 4: Standard Calibration Curve of Linearity

The precision of the method was assessed by repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was determined by analyzing 10 µ/ml of Berberis AquifoliumLotion for three times within the day and average % RSD was calculated. Inter-day precision was determined by analysing the same concentration of solutions for three days and average % RSD was calculated.

Intraday precision (on same day) data

Table 4: Intraday precision data

Sr.No	Concentration (µg/ml)		Absorbance	Mean	SD	% RSD
1.	10		0.71			
2.	10	Morning	0.73	0.72	0.01	1.39%
3.	10		0.72			
4.	10		0.61			
5.	10	Afternoon	0.60	0.6	0.01	1.67%
6.	10		0.59			
7.	10		0.88			
8.	10	Evening	0.87	0.87	0.01	1.15%
9.	10		0.86			

Interday precision (on different day) data

Table 5: Interday precision data

Sr.No	Concentration		Absorbance	Mean	SD	% RSD
1.	10		0.60			
2.	10	Day 1	0.59	0.6	0.01	1.67%
3.	10		0.61			
4.	10		0.55			
5.	10	Day 2	0.56	0.56	0.01	1.79%
6.	10		0.57			
7.	10		0.77			
8.	10	Day 3	0.78	0.77	0.01	1.3%
9.	10		0.76			

% RSD should not be more than 2 %

ACCURACY[16]:

Recovery studies were carried out by measuring the absorbance of derivative spectra at the specified wavelength of the added standard drug to pre-analysed sample solution at three different levels: 80, 100, and 120% at 256 nm to check the accuracy of the method. The resulting solutions were reanalysed and % recovery was calculated.

Table 6 Accuracy Determination Data

Sr. No	Concentration (%)	Original level (µg/ml)	Amount added (µg/ml)	Recovery (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	80	1.2	1.28	1.232	96.8%		
	80	1.2	1.28	1.241	96.1%	96.7%	0.576%
	80	1.2	1.28	1.247	97.2%		
2	100	1.2	1.60	1.578	99.2%		
	100	1.2	1.60	1.557	98.4%	98.2%	1.02%
	100	1.2	1.60	1.590	97.2%		
3	120	1.2	1.92	1.526	97.3%		
	120	1.2	1.92	1.526	97.3%	96.4%	1.56%
	120	1.2	1.92	1.500	94.7%		

LOD and LOQ [16]:

Limit of detection (LOD)

LOD is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD value was calculated from the calibration curve by using the equation.

LOD = 3.3 S/M
Where, S is the standard deviation of the absorbance of the sample and
M is the slope of the calibrations curve.

Limit of quantitation (LOQ)

LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. LOQ value can also be calculated from the calibration curve using the equation .

LOQ= 10 S/M
Where, S is the standard deviation of the absorbance of the sample and
M is the slope of the calibrations curve.

RESULT:

The standard calibration curve of Extract(= 0.308x + 0.14 R^2 = 0.9995) were essentially linear with good correlation coefficient. These equations were used to extrapolate the amount of drug from measured absorbance when required(Figure3.).

Linearity: The analysis of Herbosomal Lotion formulation containing Berberis Aquifolium extract revealed strictly linear relationship with absorbance in the studied concentration range (Figure 4).

Precision: Intra-day and inter-day precision experiments were performed. The %RSD values for precision were less than 2,

there by indicating that the method was sufficiently precise (Table 4,5).

Accuracy: The values of recovery were in between 95– 100% and percent relative standard deviation was less than 2 indicating that the method is accurate.(Table 6)

Limit of detection (LOD) and Limit of quantitation (LOQ):

The value of LOD and LOQ was found to be 1.311µg/ml and 3.973µg/ml respectively.

CONCLUSION:

The developed method was found to be simple, sensitive, accurate, precise, reproducible and most importantly cost effective. The proposed method is specific in estimating commercial formulations without excipient interference.

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