ISSN No:-2456-2165

Formulation and Evaluation of Novel HerbalThroat Spray Containing Triphala and Liquorice Extracts

Aakanksha M. Gupta[1]
Dhvani J. Patel[1*]
Monali T. Patel[1]
Riddhi M. Patel[1]
Geetha M[2]
[1]Research Scholar, [2]Professor
Anand Pharmacy College, Anand, Gujarat

Corresponding Author:- Dhvani J. Patel[1]

Abstract:- Acute pharyngitis is a highly prevalent community-acquired infection, characterized by a sore throat. Oral throat spray preparations have several benefits compared to standard topical preparations, it gives a uniform drug distribution and dose, increased bioavailability, and lower incidence of irritation. Throat spray formulation comprises polymers and excipients that improve formulation characteristics and enhance the stability of active substances. There is evidence that natural product has the potential to suppress sore throat infection. The present study aimed to develop an oral herbal throat spray containing Triphala and Liquorice. Four batches (F1 to F4) of spray solution were formulated with selected chief ingredient, eudragit EPO as polymer and propylene glycol as excipients, and evaluated. The evaluation parameter such as pH, spray pattern, spray angle, particle size, and evaporation time was performed to find out the difference between the formulation. The results indicated that formulation(F2) was hazy and slightly yellow in appearance, stable at pH 7, had less evaporation time, spray particle was spherical and uniform in size, spray angle is 75.42º, droplet size is 10µm, and contain a total of 88 percent of drug content. The spray is convenient to apply so, improves patient compliance and acceptance.

***Keywords*:-** *Herbal Throat Spray, Triphala, Liquorice, Eudragit EPO.*

I. INTRODUCTION

Acute pharyngitis is a highly prevalent community-acquired infection characterized by a sore throat. 1 in 10 people is affected by a sore throat every day. Oral throat spray preparations have several benefits compared to standard topical preparations. There is evidence that natural products have the potential to suppress sore throat infections. Many viruses and bacteria can cause acute pharyngitis. For example, *Streptococcus pyogenes*, also called group *A. Streptococcus* (group A strep), causes acute pharyngitis known as strep throat. The main cause of a sore throat is irritation or inflammation of the throat mucosa. This causes pain and discomfort, which is worsened by swallowing. Throat sprays are directly a localized painkiller for the throat, which a person sprays directly onto their throat to offer instant relief.[1]

Viruses are the most common cause of pharyngitis in all age groups. However, experts estimate that group A strep, the most common bacterial cause, causes 20% to 30% of pharyngitis episodes in children. Likewise, experts estimate it causes approximately 5% to 15% of pharyngitis infections in adults [2]. The annual incidence of sore throat was estimated to be 100 per 1000 person-years. A bacterial or presumed bacterial etiology was clinically diagnosed in 64% of the patients, and a viral or presumed viral etiology in 30% of cases. India is a rich heritage of herbal drugs. Herbal drugs constitute a major share of all the officially recognized health systems in India, viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy. [3] We, therefore, decided to work on formulations that are safe for human consumption. The cost of formulation developed using indigenous material is expected to be low. Therefore, a more significant market share can be attained if the price is less and the product is efficacious. Triphala is an herbal preparation that has long been used in Ayurveda, a healing system that originated thousandsof years ago in India. It is widely used as an anti-inflammatory, anti-viral, and anti-bacterial. [4], [5] Liquorice extract and its principal component, glycyrrhizin, have been extensively used in the treatment of various diseases as such anti-inflammatory, anti-ulcer, and anti-viral.[6]

A suitable dosage form is convenient to use by patients. However, some liquid and semisolid products are to be applied by hand on the affected parts, which causes irritation. We, therefore, planned to develop a spray formulation. Upon actuation of liquid from a non-aerosolized container, the solution is sprayed on the affected part, giving faster onset of action.

II. MATERIALS AND METHODS

The Triphala powder was bought from a local market having Manufacturing Lic.no: 288Ayu; Batch no -22227. The sample formulation has been deposited in the Department of Pharmacognosy, Anand Pharmacy College, Anand for future reference. The reference standard of Gallic

acid and Glycyrrhizin were bought from Loba Chemi. Pvt. Ltd. Mumbai and Yucca Enterprises, Mumbai-37, India. Eudragit EPO was bought from Evonik India, Pvt. Ltd. Mumbai

➢ *Physicochemical Properties*

Total ash value, Acid insoluble ash, water-soluble ash, and extractive value for Triphala and Liquorice powder were performed. The qualitative analysis of phytochemicals was determined following the standard protocols [7,8].

Tests for alkaloids, flavonoids, protein and amino acids, phenolic compounds, Terpenoids, carbohydrates, and saponins were performed.

- *Colorimetric Analysis:*

➢ *Estimation of Total Phenolic Content*

The phenolic content in the methanolic extract of Triphala churna was weighed according to the reported method. [7]

- *Preparation Standard Stock Solution*:

100 µg/ml Gallic acid standard stock solution was prepared by 10mg Gallic acid dissolved in methanol and makeup volume up to 100 ml with methanol in a volumetric flask.

- *Preparation of Reagent***:**

Folin Ciocalteu reagent: Folin Ciocalteu reagent: distilled water (1: 2) solution was prepared.

- *20% Sodium Carbonate Solution:*

20 gm of anhydrous sodium carbonate was dissolved in 100 ml of distilled water. *Procedure for standard solution***:** From the stock solution, 0.1,0.2,0.3,0.4, and 0.5ml were transferred into a 10ml volumetric flask which give1,2,3,4, and 5µg/ml concentrations, respectively. In addition, 0.5ml of Folin- Ciocalteu reagent and 1.5ml of 20% sodium carbonate solution were added. Volume was made up to 10ml with distilled water. These mixtures were kept for 30min. and absorbance of the blue color was measured at 720nm. The percentage of total phenolic was calculated from the calibration curve of Gallic acid plotted using the above procedure, and total phenolic was expressed as a percentage of Gallic acid.

- *Procedure for Test Solution***:**

The reaction mixture consists of 1 ml of extract. 5ml of distilled water was taken in a volumetric flask, and 0.5ml of Folin Ciocalteu reagent was treated with a mixture and shaken it. After 5minute, 1.5ml of 20%Na_2CO_3 solution is treated to a mixture, making up the volume with 10ml distilled water.

➢ *UV Analysis for Liquorice Extract [9]*

- *Selection of Solvent:*

After assessing the solubility of marker and extract in various solvents Phosphate Buffer (pH-6.8): ethanol in 70:30 extents have been chosen as a solvent for spectrophotometric assessment.

- *Selection of Wavelength:*

The dilution was obtained to the concentration of 5µg/ml and was scanned in the UV range (200–400 nm) in a 10mm cell against solvent blank. The study of the spectrum revealed that glycyrrhizin shows λ max at 254nm. Thus, 254 nm wavelength was selected for spectrophotometric evaluation.

- *Preparation of Standard Stock Solution:*

A stock solution of glycyrrhizin standard was prepared by dissolving an accurately weighed 1mg of glycyrrhizin in 10ml of methanol in the volumetric flask. From this solution, various concentration of the standard solution was prepared in 10 ml of methanol in the volumetric flask to obtain concentrations 4, 8, 12, 16, 20, and 24 µg/ml. UV-Spectrum of 24 µg/ml stock solution of glycyrrhizin. Standard was taken to determine its λmax value. The absorbance of the resulting solutions was estimated at 254 nm. A calibration curve as concentration versus absorbance was developed.

- *Preparation of Test Solution:*

Accurately weighed 10 mg of herbal hydroalcoholic extract of Liquorice and was transferred to 10 mL volumetric flask and dissolved Phosphate Buffer (pH-6.8): methanol in 70:30 proportions and final volume was adjusted with the same solvent in 10 mL volumetric flask. The sample solution was then filtered through Whatman filter paper No.41. From the above solution, 0.1 mL of the solution was taken and diluted to 10 mL with Phosphate Buffer (pH-6.8): methanol in 70:30 proportions to get the final concentration containing 10µg/mL of glycyrrhizin.

➢ *FTIR Spectroscopic Analysis [10]*

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc. The powdered sample of each plant specimen was loaded in FTIR Spectroscope, with a scan range from 400 to 4000cm^{-1} with a resolution of 4cm^{-1}.

- *Formulation of Spray:*

The spray was formulated with Triphala extract and Eudragit EPO dissolved in an organic solvent. Glycyrrhiza dissolved in an aqueous solvent. The polymeric spray is prepared by incorporating the polymer, Ethanol used to increase permeation and plasticizer in a solvent system. Accurately weighed, Triphla was dissolved in the solvent. Next, the accurately weighed Eudragit EPO dissolved in an organic solvent; then Liquorice extract dissolved in an aqueous phase. Then the aqueous phase was slowly added to the organic phase with continuous stirring with the help of a magnetic stirrer for 1 hour. The mixture was carefully filled in the bottle to minimize the evaporation of the solvent. Finally, the resulting solution was filled in a container that

could be used.

Table 1 Formulation Composition

Name of Component (mg/ml)	F1	F2	F3	F4
Triphala extract	150	150	15	150
Liquorice extract	120	120	120	12
Eudragit EPO	100	50	50	50
Propylene glycol	3	3	3	3
Ethanol	15	20	15	5
Water	15	10	15	25
Glycerin	-	-	0.5	0.5

III. EVALUATION PARAMETER

➢ *Appearance*:
The appearance of the spray solution was evaluated visually, for clarity and color [11]

➢ *pH*:
The pH of the optimized spray solution was calculated using the digital pH meter. The pH meter was adjustedusing a phosphate buffer of different pH values (4.0 and 7.0) before calculating the pH of the optimized formulation. Finally, the pH was determined for the spray solution. [11], [12]

➢ *Evaporation Time*:
Evaporation time is needed to dry the spray. It was measured by spraying the formulation on a glass slide and the drying time was noted using a stop clock. [11]

➢ *Spray pattern*:
The formulation was sprayed on pH-sensitive paper. The distance separating the container from the target was kept constant at 5cm. Then, the spray pattern was evaluated by spraying the concentrate in vertical and horizontal positions. Finally, the sprayed diameter was noted. The maximum diameter (Dmax) and minimum diameter (Dmin) was noted. The diameter ratio was calculated by ratio Dmax and Dmin. [13]

Diameter ratio=Dmax / Dmin

Where Dmin and Dmax are the maximum and minimum diameters of the spray pattern respectively.

➢ *Spray Angle*:
The two parameters were correlated with the polymer concentration and viscosity of the formulation. The spray angle should be less than 85º for easy actuation of drug solution from the container and to cover the maximum surface area. The sprays were actuated horizontally onto a white paper mounted 10 cm from the nozzle. [11], [12]

Spray angle (θ) = tan-1 (l/r)

➢ *The Volume of each Spray*:
The volume of each spray was calculated using the equation given below. An average of five sprays was calculated. [11], [12]

(Vs) = (Wt. –Wo)/D

Where Vs is the volume of sprayed; Wt. is the weight of solution after spray; Wo is the initial weight of solution in the container; D is density.

➢ *Viscosity*:
The viscosity of the solution was determined by the Oswald viscometer. 20ml solution was filled in Oswald viscometer. The flow of solution was measured in time from A to B point in the viscometer. Reading was taken at least three times. The viscosity of the spray solution was measured against the viscosity of water. The viscosity of the solutionwas evaluated visually and rated as low (water-like), medium (glycerol-like), or high (syrup-like). [13]

The viscosity of the formulation was determined using the following formula:

$$\eta Y = \eta w \frac{dY\, tY}{dw\, tW}$$

Where, ηw = Viscosity of water; ηY = Viscosity of tested liquid; d w= Density of water; dY = Density of tested liquid; tW = Timing of runoff of water; tY = Timing of runoff of tested liquid

➢ *Droplet Size*:
Microscopic method is generally employed for the measurement of particle size the in range of 0.2 to 100µm. The formulation was sprayed on a clean glass slide and at least sizes of 10 particles were measured under amicroscope. The droplet size was calculated using micrometers under low magnification. The droplet size was characterized once the spray was fully developed. Fine particles should be as low as possible to avoid droplet deposition in the lower airways. [13]

➢ *Drug Content*:
Formulation was determined by mixing formulation with appropriate solvent for complete drug extraction. The solution then be filtered through a 0.45µm membrane filter and subjected to colorimetric analysis. Drug content was calculated from a linear regression equation using the standard curve. Sample from the drug-free spray will use as a blank solution during analysis.[11]– [13]

ISSN No:-2456-2165

➢ *Stability Study*:

The short-term stability studies of spray solutions were conducted at 25˚C ± 2˚C and 60%RH ± 5%RH for a duration of one month. [11], [12]

IV. RESULT AND DISCUSSION

Table 2 Physiochemical Properties of Triphala and Liquorice

Physiochemical Properties	Triphala (%)	Standard value (%)[7]	Liquorice (%)	Standard value (%)[8]
Total ash value	5.56 ± 0.04	NMT 7	2.95 ± 0.64	NMT 10
Acid insoluble ash	1.32 ± 0.5	NMT 1	1.56 ± 0.8	NMT 2.5
Water soluble ash	1.44 ±0.07	NMT 2	1.21± 0.5	NMT 2
Extractive value	8.56 ± 0.8	NMT 8	7.6 ± 0.04	NMT 10

NOTE: NMT= Not more than, NLT= Not less than

Table 3 Chemical tests for Triphala and Liquorice extract

Tests	Triphala extract	Liquorice extract
Alkaloid	+	-
Flavonoid	-	+
Carbohydrate	+	+
Protein and amino acid	-	-
Phenolic compound	+ +	-
Saponin	-	+
Terpenoid	-	+

NOTE: "+" Indicate presence of the compound "-" Indicate absence of the compound

➢ *TLC Analysis for Triphala Extract [7]*

- *Stationary phase: Silica gel G*
- *Solvent system: Toluene: Ethyl acetate: formic acid: methanol (3:3:0.8:0.2)*
- *Chamber saturation: 30 min.*
- *Standard solution: Standard Gallic acid dissolved in methanol.*
- *Test solution: Methanolic extract of Triphala churna*
- *Detection: detection was carried out with the Spraying with 5%$FeCl_3$ reagent at 254nm*
- *Rf Value: S1 = 0.50, S2 = 0.56*
- *Standard Rf value: Chebulagic acid = 0.48(Pale blue), Chebulinic acid = 0.58(Dark blue) [14]*

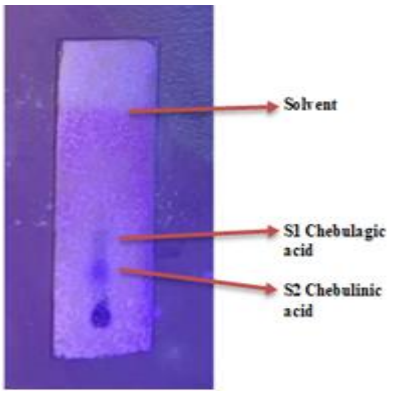

Fig 1 TLC Analysis of Triphala

➢ *TLC Analysis for Liquorice Extract [8]*

- *Stationary phase: Silica gel G*
- *Solvent system: Butanol: Water: Glacial acetic acid (7:2:1)*
- *Chamber saturation: 30 min.*
- *Standard solution: Standard Glycyrrhizin dissolved in ethanol.*
- *Test solution: Methanolic extract of Glycyrrhiza glabra*
- *Detection: Detection was carried out under UV light at 254nm*
- *Rf Value: 0.71*
- *Standard Rf value: Glycyrrhizin Rf = 0.70 [8]*

➢ *Colorimeter Analysis of Triphala Extract*

The linearity range for gallic acid is 1-5 µg/ml concentration at the selected wavelength. The unknown concentration was found to be 1.6 µg/ml. The coefficient of correlation for gallic acid at 720nm is 0.996. The drug shows good regression value at 720nm wavelength.

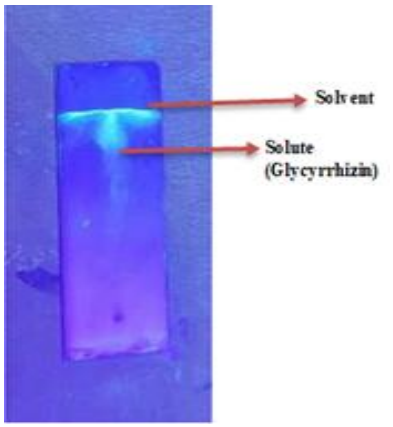

Fig 2 TLC Analysis of Liquorice

ISSN No:-2456-2165

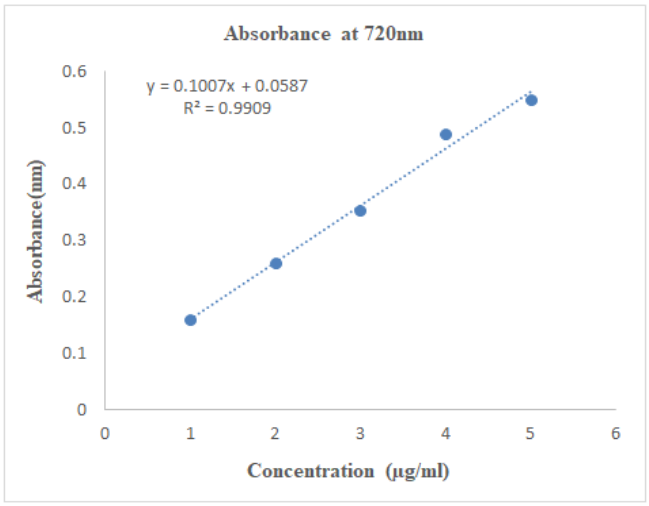

Fig 3 Calibration Curve of Triphala

➢ *UV Analysis of Liquorice Extract*

The linearity range for glycyrrhizin is 4-24 µg/mL concentration at the selected wavelength. The coefficient of correlation for glycyrrhizin at 254nm is 0.9972. The drug shows a good regression value at 254nm wavelength.

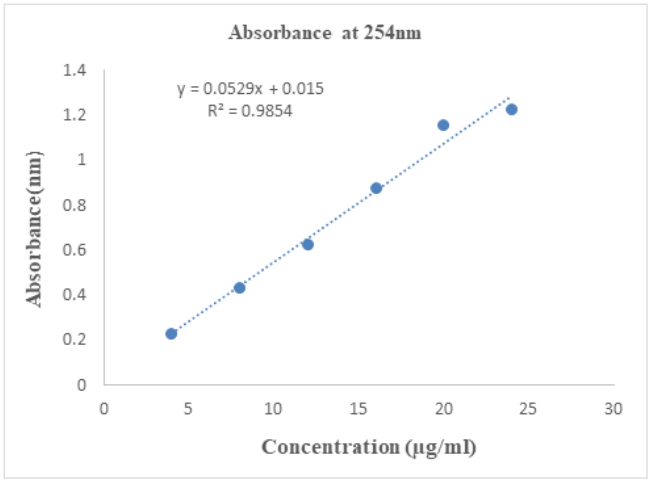

Fig 4 Calibration Curve of Liquorice

➢ *FTIR Spectroscopic Analysis for Triphala Extract*

FTIR spectra of Triphala extract show a peak at 3326.59 cm^{-1} (N-H) which indicates the presence of an amine. Peaks between 3000-2800 cm^{-1} indicate the presence of the C-H stretch. At the 1719.43 $cm^{-1,}$ there is a C=O stretch which showed the presence of esters and saturated aliphatic functional groups. Extract overlay has exhibited the presence of alkenes, hydroxyl, and aromatic groups.

➢ *FTIR Spectroscopic Analysis for Liquorice Extract*

FTIR spectra of Liquorice extract show a peak at 3361.0.4 cm^{-1} (N-H) which indicates the presence of an amine. Peaks between 2928.23 cm^{-1} indicate the presence of the C-H stretch. At 1600-1400 cm^{-1} there is a C=C stretch which showed the presence of alkenes.

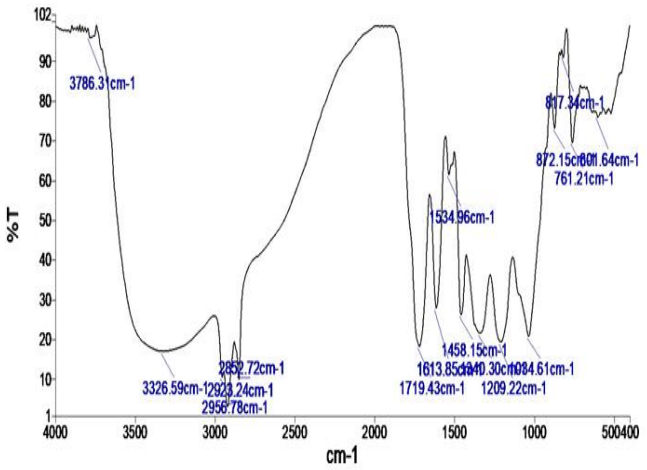

Fig 5 FTIR Analysis for Triphala Extract

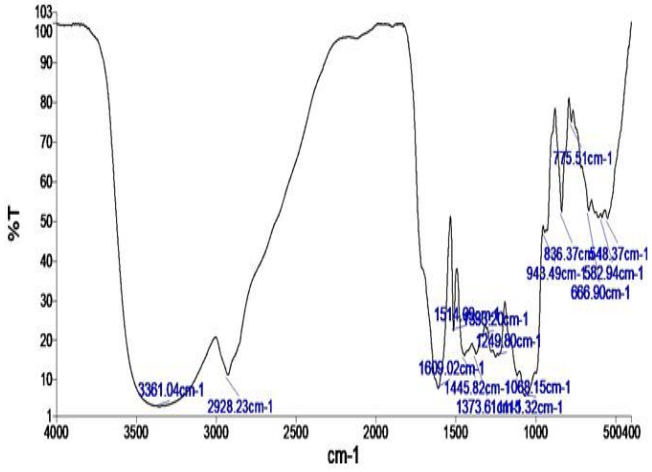

Fig 6 FTIR Analysis for Liquorice Extract

Table 4 Results of evaluation parameter

Parameter	F1	F2	F3	F4
Appearance	Hazy and yellowish-brown color solution	hazy and light-yellow color solution	Hazy and yellowish-brown color solution	Hazy and yellowish-brown color solution
pH	6.5	7.0	6.8	7.5
Evaporation time	3min 20sec	1min 20sec	2min 10sec	2min 30sec
Spray pattern	19.2mm	12.8mm	21.5mm	18.6mm
Spray angle	69.43 º	75.42 º	72.50 º	70.19 º
Volume of each spray	50 µl	80µl	80µl	80µl
Viscosity	4.6cps	3.4cps	2.1cps	2.7cps
Droplet size	12.5 ± 0.5µm	10.2 ± 0.7µm	15.0 ± 0.3µm	17.5 ± 0.6µm
Drug content	59%	88%	71%	75%

ISSN No:-2456-2165

V. CONCLUSION

The oral throat spray formulation is rich in phenolic contents such as Chebulinic acid, Chebulagic acid, and Glycyrrhiza glabra containing Glycyrrhin, and isoflavones. Colorimetry analysis, TLC, and UV analysis were used to confirm the phytochemicals. A total of four formulations of herbal spray have been made. Experimental studies have confirmed that the formulation F2 is better as compared to the other formulations. F2 formulation is hazy and slightly yellow in appearance, stable at pH 7, less evaporation time, spray particle is spherical and uniform in size, spray angle is 75.42º, droplet size is 10µm, and contains a total of 88 percent of drug content. Additionally, FTIR spectroscopy was used to identify the functional group. Further investigation will require to find out the detailed biological activity of sore throat spray at a preclinical and clinical level.

ACKNOWLEDGMENT

We are thankful to Dr. Tejal Gandhi, Principle at Anand Pharmacy College, Anand to give us the opportunity to perform the work.

REFERENCES

[1] M. Akram, M. Asif, and A. Sami, "Pharyngitis and sore throat: A review Logic connectives in fuzzy (higher) logic view project dose-dependent, antidepressant, and anxiolytic effects of a traditional medicinal plant for the management of behavioral dysfunctions in animal models view project," Article in African journal of biotechnology, vol. 13, pp. 663, 2011, doi: 10.5897/AJB10.2663.

[2] S. T. Shulman, A.L. Bisno, H.W. Clegg, M.A. Gerber, E.L. Kaplan, G. Lee, J. M. Martin, C.V. Beneden "Clinical practice guideline for the diagnosis and management of group a streptococcal pharyngitis: 2012 update by the infectious diseases society of America," IDSA Guideline, Clinical Infectious Diseases, vol. 55, no. 10, Nov. 2012, doi: 10.1093/cid/cis629.

[3] M. M. Pandey, S. Rastogi, and A. K. S. Rawat, "Indian traditional ayurvedic system of medicine and nutritional supplementation," Evidence-based complementary and alternative medicine, vol. 2013. doi: 10.1155/2013/376327.

[4] N. S. Kumar, A. S. Nair, A. M. Nair, and M. Murali, "Pharmacological and therapeutic effects of Triphala-A literature review," 23 Journal of Pharmacognosy and Phytochemistry, vol. 5, no. 3, 2016.

[5] R. Lakhan Singh Ram Manohar, N. Dwivedi, R. Lakhan Singh, and R. Gupta, "A review on antimicrobialactivities of Triphala and its constituents assessment of impacts on Human health from E-waste recycling sites. A review on antimicrobial activities of Triphala and its constituents," World journal of pharmacy and pharmaceutical sciences, vol. 5, no. 4, pp. 536, 2016, doi: 10.20959/wjpps20164-6341.

[6] D. Bisht, Rashid M, Arya RKK, "Revisiting Liquorice (*Glycyrrhiza glabra L.*) as anti-inflammatory, antivirals and immunomodulators: Potential pharmacological applications with mechanistic insight.," Phytomedicine Plus, vol. 2, no. 1. Elsevier B.V., Feb. 01, 2022. doi: 10.1016/j.phyplu.2021.100206.

[7] G. Venkateswarlu, S. Ganapaty, and A. M. S. Sudhakar, "Preparation of Triphala churna using the ingredients obtained from local market and comparative standardization," Pharmacognosy Journal, vol. 11, no. 1, pp. 102–111, 2019, doi: 10.5530/pj.2019.1.18.

[8] Chauhan S, Gulati N, Nagaich U. Glycyrrhizic acid: extraction, screening, and evaluation of anti-inflammatory property. Ars Pharmaceutica 2018, vol. 59, no. 2, pp. 61-67 Jun. 2018, doi: 10.30827/ars.v59i2.7513.

[9] I. Hyder, A. Naseer, and A. Ahmad, "Development and validation of ultraviolet-visible spectrophotometric method for estimation of glycyrrhizin in methanolic extract of glycyrrhiza glabra l," Asian journal of pharmaceutical and clinical research vol. 14, pp. 94-97, 2021, doi: 10.22159/ajpcr.2021v14i6.40401.

[10] M. Pakkirisamy, S. K. Kalakandan, and K. Ravichandran, "Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of Curcuma caesia roxb (black turmeric)," Pharmacognosy Journal, vol. 9, no. 6, pp. 952–956, 2014, doi: 10.5530/pj.2017.6.149.

[11] V. Wayal, K. Nagasree, and B. A. Vishwanath, "Design, Development and Evaluation of Silk Based Film Forming Spray for Wound Healing," Journal of Drug Delivery and Therapeutics, vol. 11, no. 3-S, pp. 15–18, Jun. 2021, doi: 10.22270/jddt.v11i3-s.4812.

[12] H. M. Patel, A. Gangat, U. B. Patel, and B. Akbari, "Fabrication and Characterization of Luliconazole Film-Forming Topical Spray for the Treatment of Fungal Infections," Manipal journal of pharmaceuticalscience vol. 6, 2020, pp. 52-64

[13] I. N. Pawar, R. R. Rajput, S. SVaghani, and B. V Katara, "Formulation and Evaluation of Topical Spray Containing Anti Acne Agent," Asian Journal of Pharmaceutical Technology & Innovation, no. 24, pp. 14– 28,2017 available on : www.asianpharmtech.comwww.asianpharmtech.com www.asianpharmtech.com

[14] K. Krishna, K. Gouri Sankar, S. Vardhan, and T. Tamizhmani, "Simultaneous Estimation of Chebulagic acid and Chebulinic acid in Marketed Polyherbal Formulations by HPTLC." International journal of pharmTech research Vol. 5,2013, pp. 1554-1560