Advances in Bioresearch
Adv. Biores., Vol 14 (5) September 2023: 307-312

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.5.307312

Advances in Bioresearch

ORIGINAL ARTICLE

Formulation Development and Evaluation of Polyherbal Suspension of *Matricaria chamomilla* Linn Flowers, *Emblica officinalis* Gaertn Fruits and *Withania somnifera* (L) Dunal Roots

Umesh Chandra Pandey*[1], Karunakar Shukla[2], Mohit Chaturvedi[3]
[1]*Research Scholar, Dr. A. P. J. Abdul Kalam University, Indore (M.P.)
[2]Professor & Principal, College of Pharmacy, Dr. A. P. J. Abdul Kalam University, Indore (M.P.)
[3]Professor, Institute of Pharmacy, Dr A.P.J. Abdul Kalam University, Indore (M.P.)
*Corresponding Author E-mail: umeshpharma576@gmail.com

ABSTRACT
Formulations restrain 2 or more than 2 herbs are called polyherbal formulation. Drug formulation in Ayurveda is based on 2 principles: Use as a single drug and use of more than one drug. The last is known as polyherbal formulation. The idea of polyherbalism is peculiar to Ayurveda even though it is tricky to explain in term of modern parameters. Matricaria chamomilla, Emblica officinalis and Withania somnifera are well known plants available throughout India and they are commonly used for the treatment of various diseases including anxiety. The anti-anxiety activity of the individual plant parts is well known, but the synergistic or combined effects are unclear. The concept of polyherbalism has been highlighted in Sharangdhar Samhita, an Ayurvedic literature dating back to 1300 AD. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. The present investigation was focused on the development and stability study of polyherbal suspension produced from alcoholic extracts of selected medicinal plant. Three suspensions Polyherbal formulation- PHLF1, Polyherbal formulation- PHLF2, Polyherbal formulation-PHLF3 of different concentration of sodium carboxy methyl cellulose 0.7%, 1.4%, 2.0% respectively formulated and evaluated to accelerated stability for 3 months. Polyherbal formulation-PHLF3 exhibited pleasant appearance and texture; there were no changes in sedimentation, flow rate, pH, viscosity and other physiochemical parameters. Quality control parameters like phytochemical test were also done on the developed polyherbal formulation. It reveals the presence of various phytoconstituents. All the quality control parameters in formulated suspension are stable and acceptable. It is concluded that suspension of ethanolic extracts of Matricaria chamomilla, Emblica officinalis and Withania somnifera formulated in combinational therapy could be effective and safe for use.
Keywords: Polyherbal formulation, *Matricaria chamomilla*, *Emblica officinalis, Withania somnifera,* Sodium carboxy methyl cellulose

Received 22.05.2023 Revised 11.06.2023 Accepted 26.08.2023

How to cite this article:
Umesh Chandra P, Karunakar S, Mohit C. Formulation Development and Evaluation of Polyherbal Suspension of *Matricaria chamomilla* Linn Flowers, *Emblica officinalis* Gaertn Fruits and *Withania somnifera* (L) Dunal Roots. Adv. Biores., Vol 12 (5) September 2023: 307-312.

INTRODUCTION

Plants are very useful to mankind. Many of them are used exclusively for medicinal purposes. According to the World Health Organization (WHO), a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. Such plants are in great demand by pharmaceutical companies for their active ingredients [1, 2]. The oral route of drug administration is one of the oldest methods of administrating drugs for systemic effects. In general, the parenteral route is not readily used for the self-administration of medicines. The majority of medicine used to produce systemic therapeutic effects is probably given by the oral route [3, 4]. Polyherbal formulations are the product of nature, they are comparatively cheaper, eco-friendly and readily available than modern drugs. Their better affordability and greater accessibility account for increasing demand globally, particularly in rural areas and some

developing countries, where costly modern treatments are not available. The scientific advancement carries with its developments in polyherbal formul ations through the study of diverse phytoconstituents and the discovery of helpful medicinal herbs combinations that work synergistically to exert a therapeutic effect. Almost, they bring out satisfactory effect and safety making them one of the highly selected drugs of choice [5, 6]. Ayurvedic herbal formulations were also administered preferentially by oral route. Liquid forms of drugs contain certain limitation, but public demand or expectations are tremendous for such formulations. Moreover, some formulations are more effective in a liquid form and are used commonly by young children's or the adult to overcome difficulty in swallowing solid oral dosage forms. Most of the orally administered herbal formulations belong to the liquid dosage form of drug or drug combination. Designing and developing oral liquid herbal formulations is to date a challenge in modern pharmaceutics.

MATERIAL AND METHODS

Plant materials

The flowers of *M. chamomilla*, fruits *E. officinalis* and roots of *W. somnifera* of were collected from Bhopal Region, Madhya Pradesh, India. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number 252/Saif./Sci./Clg/Bpl, 253/Saif./Sci./Clg/Bpl and 254/Saif./Sci./Clg/Bpl respectively was kept in Department of Botany, Saifia College of Science, Bhopal for future reference. Fresh all three plants were used for pharmacognostical studies. Flowers, fruits and roots of plants were dried under shade and powdered to 60# separately and stored in airtight containers and used for phytochemical studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction

Plant material fattening

Flowers, fruits and roots powder of plants was produced after shade drying at room temperature. The shade-dried plant material was coarsely ground up and put through a petroleum ether extraction process utilizing soxhlet equipment. The extraction process was continued until the material had been sufficiently defatted.

Extraction by soxhlation process

Flowers, fruits and roots of plants that have been defatted were exhaustively extracted with ethanol as solvent by soxhletion method. The extract evaporated beyond their boiling points. The dried crude concentrated extract was weighed in order to calculate the extractive yield. When ready for analysis, it was then put into glass vials (6 x 2 cm) and stored in a refrigerator 4°C [7].

Preparation of polyherbal suspension dosage form

The dried lyophilized ethanolic extracts of *M. chamomilla*, *E. officinalis* and *W. somnifera* were taken for the preparation of 100 ml of Suspension. The formulae for preparing 100 ml of a suspension of extracts of *M. chamomilla*, *E. officinalis* and *W. somnifera* was as shown in Table 1. They were taken in the ratio of 1:1:1. Suspension was prepared by using various bioactive extracts of selected plant materials trituration method in mortar and pestle by using the suitable suspending agent of Tween 80 and Sodium carboxy methyl cellulose (CMC) along with other excipients. The dried extracts were mix in water and the additives likeTween-80, Sodium CMC. The suspending agent, sodium CMC in the aqueous medium containing selected preservatives was added in mortar and pestle along with ethanolic extracts of selected plant material with continuous triturating. Three possible formulations of Suspension viz. Polyherbal formulation PHLF1 Polyherbal formulation- PHLF2 and Polyherbal formulation- PHLF3 were prepared by using 0.7%, 1.5%, 2.0% aqueous Sodium C.M.C solution respectively. Finally, by addition of purified water by continuous trituration in suspension brought up to the final volume to get the uniform product. All three possible forms of suspension of extracts of *M. chamomilla*, *E. officinalis* and *W. somnifera* were then subjected to evaluation as per standards.

Table 1: Composition of polyherbal formulation (Suspension)

Ingredients	PHLF1	PHLF2	PHLF3
Matricaria chamomilla extract (AEMC)	1g	1g	1g
Emblica officinalis extract (AEEO)	1g	1g	1g
Withania somnifera extract (AEWS)	1g	1g	1g
Tween 80	0.1 %	0.1 %	0.1 %
Sodium CMC	0.7 %	1.5 %	2 %
Methyl paraben	0.10 %	0.10 %	0.10 %
Glycerine	2 ml	2 ml	2 ml
Lemon oil	0.10%	0.10%	0.10%
Purified Water (up to)	100 ml	100 ml	100 ml

Phytochemical investigation of the polyherbal formulation

Various phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were analysed qualitatively in the polyherbal formulation [8, 9].

Quality parameters of polyherbal suspension [10-12]

The organoleptic characters of the polyherbal suspension were evaluated by using the following parameters colour, odour, taste and texture etc.

Accelerated stability studies

The accelerated stability studies were carried out for polyherbal formulations (PHLF1 to PHLF3) of bioactive constituents at 8°C, room temperature and 45°C±2 at 75%±5 humidity. The stability of polyherbal suspension was studied for three months. The different parameters such as pH, sedimentation volume, re-dispersibility were studied for all the formulation at 1st, 2nd and 3rd months.

Sedimentation volume

The sedimentation volume is the ratio of the ultimate height of the sediment to the initial height of the total suspension as the suspension settles in a cylinder under appropriate standard conditions. It was evaluated by keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain period and note that the volume of the sediment is expressed as ultimate height.

Redispersibility

The suspension was allowed to settle in a measuring cylinder. The mouth of the cylinder was closed and was inverted through 180º and the number of inversions necessary to restore a homogeneous suspension was determined.

Rheology

The time required for each suspension sample to flow through a 10 ml pipette was determined by the apparent viscosity by using the equation.

Flow rate = Volume of Pipette (ml)/ Flow rates of (seconds)

pH

The pH of the suspension was determined by using a pH meter (Eutech).

Particles size analysis

The distribution of particle size in suspension is an important aspect of its stability. Particle size distribution was carried out by using optical microscopy in dilute suspensions.

Determination of microbial limit test

A microbial limit test was performed as per I.P 2014. Take a four-petri plate and label two plates for bacteria and remaining two for fungi count. Transfer 1ml quantity of each pretreated dilution sample solution to each of four petri plates. Add 15 ml of sterile liquefied SCDA at not more than 45°C, into two plates labelled for bacterial count [13].

RESULTS AND DISCUSSION

It was observed that all these three formulation PHLF1 Polyherbal formulation- PHLF2 and Polyherbal formulation- PHLF3 have similar organoleptic characteristics such as liquid in nature, Brown black - dark brown in colour, slightly bitter taste Table 2. In Polyherbal formulation- PHLF1 it was observed that, sedimentation volume ranging from (2.21-2.38), pH slightly alkaline pH (7.41-7.60), viscosity (45.2-52.6) rapid flow rate per 5 ml of formulation and particle size observed around (21.20-21.91µm) Table 3. In polyherbal formulation PHLF2, it was observed that sedimentation volume reduced to ranging from (1.51-1.61) as compared to polyherbal formulation- PHLF1 due to the increase in the concentration of Sodium C.M.C. it also affects the viscosity (50.8 centipoise to 54.9 centipoise), alkaline pH (6.82-7.10), increase in viscosity decrease the flow rate of formulation (30sec-41sec) per 5 ml of formulation and particle size

observed around (19.20-20.94 µm) Table 4. Polyherbal Formulation- PHLF3 formulation appears like dark brown shade in colour with characteristic odour and texture at room temperature (RT) and 45ºC. The suspension had a pleasant appearance and texture at different temperature and did not exhibit any change. As shown in the resulting pH of the suspension is 6.50 throughout storage, it does not show any appreciable changes. Viscosity centipoise and flow rate 56.9 and 61 seconds per 5 ml indicating satisfactory rheological behaviour of formulated suspension. There were no noticeable changes in sedimentation volume as time increases because it is near to 1 which is the acceptable limit (Table 5). To assess the standard and shelf life of the herbal formulation total aerobic bacterial count was performed. Unintentional contamination, like fungal contamination throughout the production stage, may cause deterioration in safety and quality as the risk of mycotoxin production, particularly aflatoxin, could arise mutagenic, carcinogenic, teratogenicity, neurotoxic, nephrotoxic, and immunosuppressive activities. For the evaluation of microbial contamination, total aerobic count, total fungal count, *Escherichia coli, Candida albicans* and Salmonella spp. the count was determined as per Indian Pharmacopoeia. It was observed that polyherbal formulation PHLF1 to PHLF3 there is absence of *Escherichia coli, Salmonella* and *Candida albicans* which is within limits of standardization parameters. Polyherbal formulation- PHLF3 were found to have excellent redispersibility, good flow rate, Ph and viscosity Table 6. The present investigation revealed the presents of bioactive compounds such as carbohydrates, tannins and phenolics, amino acids and proteins, flavonoids, fixed oils and fats, alkaloids, glycosides and phytosterols in the polyherbal formulation Table 7.

Table 2: Organoleptic characters of the polyherbal formulation

Parameter	PHLF1	PHLF2	PHLF3
Nature	Liquid suspension	Liquid suspension	Liquid suspension
Colour	Brown black	Brown black	Dark brown
Odour	Characteristics	Characteristics	Characteristics
Taste	Slightly bitter	Slightly bitter	Slightly bitter

Table 3: Accelerated stability study of PHLF1 at RT, 8^{0}C, 45^{0}C

Parameter	First month	Second month	Third month
Redispersibility	Poor	Poor	Poor
Particle size	21.20µm	21.52µm	21.91 µm
Flow rate	5 ml/ 26sec	5 ml/ 27sec	5 ml/ 28sec
pH	7.41	7.52	7.60
Sedimentation volume	2.21	2.29	2.38
Viscosity Cp	45.2	49.1	52.6

Table 4: Accelerated stability study of PHLF2 at RT, 8^{0}C, 45^{0}C

Parameter	First month	Second month	Third month
Redispersibility	Good	Good	Good
Particle size	19.20µm	20.12µm	20.94 µm
Flow rate	5 ml/ 30sec	5 ml/ 37sec	5 ml/ 41sec
pH	6.82	6.92	7.10
Sedimentation volume	1.51	1.55	1.61
Viscosity Cp	50.8	53.6	54.9

Table 5: Accelerated stability study of PHLF3 at RT, 8^{0}C, 45^{0}C

Parameter	First month	Second month	Third month
Redispersibility	Excellent	Excellent	Excellent
Particle size	16.20µm	16.72µm	16.94 µm
Flow rate	5 ml/ 50sec	5 ml/ 57sec	5 ml/ 61sec
pH	6.22	6.42	6.50
Sedimentation volume	1.17	1.13	1.11
Viscosity Cp	53.8	54.6	56.9

Table 6: Microbial limit test for polyherbal formulation

Parameters	PHLF1	PHLF2	PHLF3
Total aerobic count (cfu/ml)	2.5 X 10^2	2.45 X 10^2	2.4 X 10^2
Total fungal count (cfu/ml)	4.0 X 10^2	3.5 X 10^2	3.2 X 10^2
E coli (per ml)	absent	absent	absent
Salmonella species (per ml)	absent	absent	absent
Candid albicans (per ml)	absent	absent	absent

Table 7: Phytochemical Investigation of the polyherbal formulation

Phytoconstituents	PHLF1	PHLF2	PHLF3
Carbohydrates	+	+	+
Tannins and Phenolics	+	+	+
Amino acids and Proteins	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Fixed oils and Fats	+	+	+
Alkaloids	+	+	+
Glycosides	+	+	+
Phytosterols	+	+	+

CONCLUSION

The present investigation revealed the presents of bioactive compounds such as carbohydrates, tannins and phenolics, amino acids and proteins, flavonoids, fixed oils and fats, alkaloids, glycosides and phytosterols in the polyherbal formulation. Liquid dosage forms have the upper hand over solid dosage form in children and elder people due to them overcome the problem of swallowing. In Ayurveda, most of the formulations are developed in liquid form and mostly in combination with more than two crude drugs. Pharmaceutical suspension is one of the most trusted and acceptable formulations among another oral dosage form because of flexibility, ease of administration, easy swallowing in the administration of the drug. The polyherbal suspension was prepared by using lyophilized ethanolic extracts of selected plants by trituration method using a suitable suspending agent and other excipients. There are noticeable changes were observed in sedimentation, viscosity and other physicochemical parameters after performing stability studies at variable temperature with different concentration of Sodium CMC. As per the result of accelerated stability studies of polyherbal suspension PHLF1 to PHLF3. It concludes that as we increase the concentration of sodium CMC gradually there is an increase in viscosity of the formulation, decreases the flow rate of formulation simultaneously. It also affects sedimentation volume and pH of the suspension. There were no noticeable changes in the organoleptic and physicochemical properties of the polyherbal formulation. In polyherbal formulation PHLF3 all the stability parameters are stable acceptable, and optimum at variable temperature.

REFERENCES

1. Huai H.(2010). Ethnomedicinal analysis of toxic plants from five ethnic groups in China. Ethnobot Res Appl; 8:169-79.
2. Husain SZ, Malik RN, Javaid M, Bibi S. (2008). Ethonobotanical properties and uses of Medicinal plants of Morgah Biodiversity Park, Rawalpindi. Pak J Bot; 40:1897-911.
3. Ansel HC, Allen LV. (2000). Pharmaceutical dosage forms and drug delivery systems, 7th edition, Lippincott, 347-56.
4. Aulton ME, (1996)., Pharmaceutics: The Science of Dosage form. Churchill Livingstone, 304.
5. Subramani P, Gan ST, Sokkalingam A, Dhanaraj. (2014). Polyherbal Formulation: Concept of Ayurveda. Pharmac Rev. 8(16): 73–80.
6. Shinde JS, Khurde SS, Suchita LS, Chavan SS. Hulmajge SB. (2016). Need of polyherbal formulations and its standardization: A Review. World J Pharm Pharmac Scien. 5(11):526-533.
7. Jain DK, Gupta S, Jain R, Jain N. (2010). Anti-inflammatory Activity of 80% Ethanolic Extract of Acorus calamus Linn. Leaves in Albino Rats. Research J. Pharm. and Tech; 3 (3): 882-884.
8. Dutta R, Sharma MK, Khan A, Jha M. (2020). Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. Journal of Advanced Scientific Research. 11(03):176-82.
9. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. (2019). Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of Salmalia malabarica. Pharmacologyonline. 1:21-6.
10. Jain NK, Sharma SN. (1994). Textbook of Professional Pharmacy, Vallabh Prakashan, New Delhi, 3rd ed, 228.

11. Stability studies an overview of ICH guidelines for drug products: Natalie McClure, Matrix Pharmaceutical Inc. 1997.
12. Mahato RI. (2007), Pharmaceutical dosage forms and drug delivery. Boca Raton: CRC Press, Taylor & Francis group. 123-133.
13. Microbial contamination in non-sterile products, (2.2.9), Volume I, Indian Pharmacopoeia, 2014; 38-50.