Formulation and evaluation of a polyherbal suspension using popular antioxidant plants from tribal belts of Kandhamal, Odisha

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KEYWORDS

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ABSTRACT

The possibility of gastrointestinal problems, skin sensitivities, and in certain cases, an elevated risk of cancer, are the main drawbacks of long-term use of contemporary marketed antioxidant formulations. By using well-known antioxidant plants (like Cayratia trifolia, Sesbania grandiflora, Cordia dichotoma, and Teprosia purpurea) from tribal belts of Kandhamal, part of the eastern Ghat mountain range, and fenugreek mucilage as a natural suspending agent; our study seeks to develop and assess an appropriate polyherbal suspension that addresses the aforementioned problem. Eight batches (F1–F8) were made using varying concentrations of propylene glycol and natural suspending agent. Numerous criteria, including rate of flow, volume of accumulation/sediments, acidity/basicity, re-dispersibility, flow, effect of temperature, agglomeration/flocculation degree and viscosity; were examined to assess these suspensions. It was discovered that batches F6 and F7 have antioxidant qualities and were steady during our entire study period. It was noted that the viscosity of our formulation raised with increase in concentration of suspending agents, which decreases sedimentation and increases suspension stability. Folin Ciocalteu's reagent method, which measures the formulation's total phenolic contents, was used to study the antioxidant qualities.

INTRODUCTION

Nowadays, out of all the drug administration routes, delivery via oral route is the most widespread[1]. Oral pharmaceutical herbal suspensions have long been one of the best dose forms for patients who can't handle solid medication forms[2]. In comparison, other doses form suspension has higher rates of bioavailability[3]. But as this chosen dosage form is having issue regarding stability thermodynamically, A suspending agent must be added to slow down the rate of settling and facilitate the easy redispersion of any settled particle matter. This is accomplished by both improving the suspending medium's consistency and providing protective colloidal activity[4,5]. Suspending agents derived from natural resources are opted, particularly when taking potential side effects and consumer perception into account. This is because they are generally thought to be more biocompatible, less toxic, readily available, and frequently have a lower environmental impact while still providing effective suspension properties[6]. We have already aware about the excellent suspension enhancing properties found in nature such as gums of xanthane, Iranian gums, aloe elegans mucilages[7,8] karaya gums, alginates[9,10]. We have chosen fenugreek seeds mucilage especially cultivated from Daringibadi hill area of the same region by following previous research reports[11]. Our targeted antioxidant suspension was prepared from the most effective liver curing plants as claimed by local tribal of eastern ghat mountain ranges of Kandhamal district, Odisha like roots of *Cayratia trifolia*[12], flowers of *Sesbania grandiflora*[13], barks of *Cordia dichotoma*[14] and seeds of *Teprosia purpurea*[15]. The antioxidant potential of this formulated suspensions was determined by total phenolic contents as pyrochatechol by using FCR-Folin ciocalteu's reagent method[16].

MATERIALS AND METHODS

Extraction & finalization of suitable plant extracts for formulation

The finalized four plant parts were collected from Daringibadi and Amapani hill areas of Kandhamal district during rainy season after proper recognition and validation/certification by

taxonomist. These dried plant parts were powdered separately, and all the physical evaluations have been completed after which these were successively extracted with different solvents (Pet. ether, Ethyl acetate & methanol) according to polarity. All the extracts were separately investigated to establish folklore claim of liver curing property by following all *in vitro* antioxidant study protocol like Total phenolic content, DPPH, scavenging assay with NO_2, Superoxide radical scavenging assay etc. Based on all these *in vitro* studies, all ethyl acetate extracts of our selected plant parts (EACT, EASG, EACD, EACT) have shown optimum antioxidant potential which were further selected for our formulation study.

Natural suspending agent preparation (mucilage of fenugreek seeds)

The fenugreek seeds were collected from local tribal cultivators of Daringibadi hill area. These seeds were shade-dried, powdered and macerated using distilled water as solvents for overnight. After 12 hr, these seed powders along with water were boiled for some time. The supernatant portion of this slurry was collected after cooling to which acetone was added sufficiently with continuous stirring to allow precipitation of mucilage. This mucilage was air-dried and powdered and finally collected by passing through required seive for use as natural suspending agent for our study[17].

Quality control checks for isolated suspending agents

The evaluation of isolated mucilage was performed as per standard protocol with respect to the parameters such as swelling index[18], micrometric studies such as angle of repose, tap & bulk density[19, 20, 21, 22] etc. and found satisfactory results.

Preparation of antioxidant Herbal suspension

All the freeze-dried ethyl acetate extracts (2 gm each) along with other additives and fenugreek mucilage powers were taken to prepare different batches of suspension as per plan.

We prepared various batches of suspensions using the formula mentioned below. First, a paste was prepared by taking the most common preservative i.e. methyl and propyl paraben which were triturated with collected mucilage taking water. The lyophilized ethyl acetate extracts of each plant (2 gm each) along with glycerin were added slowly to above paste with occasional stirring and trituration for some time. Aspartame (sweetener), raspberry flavoring agent and Sunset yellow supra color were added to the prepared suspension base mixture and the volume of each batch were made up to 100 ml by adding water which was finally homogenized.

Table 1: composition of polyherbal suspension

Ingredients	Batch Code							
	F_1	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**
Herbal Extract (EACT, EASG, EACD, EACT 1:1:1:1	(500 x 4= 2000 mg) in each batch							
Mucilage (mg)	500	500	1000	1000	1500	1500	2000	2000
Glycerin (ml)	6	12	6	12	6	12	6	12
Prop.paraben	2.5 mg in each batch							
MethylParaben	5 mg in each batch							
Sweetner-Aspartame	100 mg in each batch							
Raspberry Flavor	2.5 mg in each batch							
Sunset Yellow Supra	0.5 mg in each batch							
water (purified)	Volume of each batch made up to 100 ml							

Quality control tests of formulated batches of suspensions

pH assessment:

pH of all the developed formulations was measured as per I.P. standards.

Volume of Sedimentation

Volume of sedimentation was calculated as per the formula $F = H_u/H_o$

Where, H_o - Original height & H_u - final height of sediments after settling of suspention

Re-dispursibilty/Redistributing capability

The final volume of each batch was placed in well-calibrated transparent tubes separately at fixed

room temperature (23^0C) at various intervening times (i.e. 24, 120, 240, 360, 480, 720 hr). During this period, tubes were shaken with force for facilitating proper re-distribution of sediments, if any which were noted properly[24].

Rate of flow

The exact time required for a pre-fixed volume of suspension to flow through orifice of pipette was studied. The rate of flow of all our developed batches were determined by following formula RF= distance covered (cm)/ Time of flow (sec)

Measurement of Viscosity through viscometer

Viscosity of all the formulated batches were individually determined by utilizing Brookfield's viscometer at 100 R.P.M. using spindle-4 at 25^0 C temperature. We have studied all batches in triplicate for better accuracy[25].

Level of flocculation

We have also studied the degree of flocculation of our formulated batches by following formula

$$\beta = \frac{(Vu)floc}{(Vu)defloc}$$

*(Vu)*floc: sedimentation volume in flocculated suspension

(Vu)defloc: sedimentation volume in deflocculated suspension.

Temperature Impact on formulations

We have also explored the temperature impact on viscosity of various formulations in 30^0 to 60^0 c temperature range.

Total content of drugs i.e. Total phenolic compound (Antioxidant)

Presence of TPC in all extracts collected from *Cayratia trifolia* root, flowers of *Sesbania grandiflora*, barks of *Cordia dichotoma* and Seeds of *Tephrosia purpurea* were investigated by using FCR-Folin ciocalteu's reagent method. The test was carried out by taking test extract (1ml methanolic sol^n) in 46 mL pure water. This test solution mixture was taken in Erlenmeyer flask to which 1 mL Folin's Ciocalteu's reagent by stirring after which it was kept for 180 sec 2% Na_2Co_3 (3000 microlitre) was mixed in this solution with occasional shaking of 120 min. Generally, FCR is used to change the phenolic content in test solution to blue colour. This absorbance was studied under UV at 760 nanometers and total phenolic content concentration was interpreted as per below mentioned formula from standard pyrocatechol graph.

$$Abs(absorbance\ of\ sample) = 0.001 \times pyrocatechol\ in\ \mu g + 0.0033$$

Measurement of particle size

We have taken compound microscope and approached through optical microscopy technique to find out exact particle size. After being spread out on a slide, the suspension was examined under this microscope. Twenty particle diameters were measured [26].

Studies on in vitro dissolution

Formulated suspensions (n = 6) were dissolved in 500 ml of water for 30 minutes at 37 ± 0.50C and 50 rpm using a USP type II dissolution test apparatus (TDT 08 L, Electrolab, Jaipur, India). Despite being primarily made for tablets and capsules, the USP type II dissolution test apparatus has also been utilized by several researchers to examine the dissolution behavior of suspensions[27,28].

The dissolution vessels were filled with 10 mililiter of herbal suspensions. 10ml aliquots were withdrawn in 30minutes. Repeat The same procedures were repeated for all developed suspensions. After passing through Whatman filter paper, the aliquots were examined at the appropriate wavelength using a UV visible spectrophotometer. (Shimadzu Ultraviolet Spectrophotometer Double Beam UV 1900i)

RESULTS AND DISCUSSION

Evaluation of suspension

Formulation $F_1$and F_2 were found not up to the common standards (low viscosity) so we have discarded.

Determination of pH

All our formulated batches were found to be in between 7.00 to 7.52 pH range.

Table 2. pH description of all suspension batches taken

Formulations	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
pH	7.02	7.06	7.20	7.26	7.31	7.42	7.33	7.52

Sedimentation Volume

After a week, we observed that the sedimentation volume had decreased (<1). We observed after 1 month that batches F6 and F7 were stable and properly distributed. In suspensions with smaller concentrations of the suspending agent than those with larger concentrations, the dispersed particle was sediment more quickly.

Re-dispersibility

To provide a more consistent dosage delivery of medication following shaking, the suspension must be radially dispersible due to sedimentation during storage. If sediment is still present after vigorous shaking, the suspension is referred to as caked. After 30 days, it was discovered that all the suspension was readily redistributable after a maximum of 13 shakings. In comparison to higher concentrations, re-dispersibility was observed to be faster for suspensions containing fewer suspending agents. This could be because these suspensions have a higher viscosity at increasing concentrations.

Rate of flow

It was noticed that whenever we increase the viscosity & concentration of natural mucilages, it affects the flow rate. The results were in-between range 0.06- 0.1 (Table-5).

Measurement of Viscosity through viscometer

The sheer thinning of the suspension was demonstrated by the fact that the viscosity of all formulations reduced as the rpm of viscometer increased. Table 5 contains the viscosity values for each batch.

Level of flocculation

Using varying concentrations of natural mucilage, the degree of flocculation was assessed for each prepared suspension. Table 5 lists the detailed result for each formed suspension, which is observed to rise with increasing suspending agent concentration. This is because increasing concentrations cause the suspension to become more viscous, which eventually lessens sedimentation.

Temperature Impact on formulations

It was observed that the increase in temperature leads to reduced viscosity in all our developed formulations.

Content of active constituents (i.e. Total phenolic content)

Total phenolic content as pyrocatechol for all batches was found to be in the range of NLT 80% in trial 6&7 (refer table-3)

Table 3. Drug content as total phenolic compound for all batches of suspensions

Batch	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Total content of drug (TPC as pyrocatechol)	45%	42%	50%	55%	70%	85%	84%	68%

Dissolution study Result

It was observed that out of all our tested batches, F_5 & F_6 able to release 75 per cent of drug after 30 min gap (Table 4).

Table 4. Comparative Dissolution profile for all batches of suspensions

Tested Batches	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**
Release of drug (Total phenolic compound as pyrocatechol)	38%	39%	44%	53%	62%	78%	75%	60%

Measurement of size of particle

20 particles from each tested batch were determined and measurements are mentioned in the table below (Table 5).

Table 5: Evaluation of suspension

Parameter	**Developed trial formulation batches**							
Batch	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**
Particle size	22.22	22.02	22.52	21.63	23.45	22.33	23.67	22.52
Degree of flocculation	2.60	2.62	2.89	2.99	2.55	3.12	3.54	3.39
Rate of flow	0.12	0.11	0.087	0.082	0.069	0.070	0.068	0.060
Number of shakes required for complete dispersion	05	05	07	08	09	8	9	12
Viscosity (Poise)	1.16	1.16	2.01	3.48	3.92	5.92	6.72	7.13

Apart from the above evaluation parameters, we have also performed stability investigation in accelerated format[29] by maintaining ideal condition of 75% RH & 40 degree centigrade with time points i.e 0 day, 30 days, 90 days & 180 days in HDPE bottle for the trail batch 6, as both trial batch 6&7 results are found satisfactory. The stability study for trail batch 6 found satisfactory below (table-6)

Table-6 Stability investigation in accelerated mode at time interval 0 day, 30 days, 90 days & 180 days in HDPE bottle[30].

Test	specification	Results	Results	Results	Results
	As per I.P. Standard	**0 day**	**30 days**	**90 days**	**180 days**
Description	Orange color suspension has pleasant odor	complies	complies	complies	complies
pH	5.0 to 8.0	7.42	7.4	7.39	7.30
Assay	NLT 80%	85%	84%	84%	83%
Microbiological study					
Total Aerobic Microbial Count	NMT1000 C.F.U. per gm	<10cfu/g		<10cfu/g	Less than 10 C.F.U./gm
Total Yeast and Mould Count	NMT 100 C.F.U. per gm	<10cfu/g	Not required	<10cfu/g	Less than 10 C.F.U./gm
E.Coli	Should absent	Absent	to perform at 1M	Absent	Absent

CONCLUSION

Our formulated polyherbal suspension with *Cayratia trifolia*, *Sesbania grandiflora*, *Cordia dichotoma* and *Teprosia purpurea* plant parts along with natural suspending agent exhibit improved stability with time containing the desired antioxidant property i.e. total polyphenol contents. It was observed that raising concentration by taking suspending agents from our selected plants increased thickness of developed formulations, reducing sedimentation rates thus enhancing overall stability. This developed polyherbal suspension can be further studied preclinical and subsequent clinical evaluation for suitable alternatives in marketed antioxidant formulations after regulatory protocols.

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REFERENCES

1. Lou J, Duan H, Qin Q, Teng Z, Gan F, Zhou X, Zhou X. 2023. Advances in Oral Drug Delivery Systems: Challenges and Opportunities. Pharmaceutics, 15,2, 484.
2. Khan D, Kirby D, Bryson S, Shah M, Mohammaed AR. 2022. Paediatric specific dosage forms: Patient and formulation considerations. International Journal of Pharmaceutics, 616, 121501.
3. Arora K, Vats V, Verma PK. 2022. A Review on Pharmaceutical Suspension and its Advancement. Annals of Clinical Case Reports, 7, article 2321.
4. Mali SS, Salunkhe SS, Kamble PM, Nadaf SJ. 2014. Formulation and evaluation of ciprofloxacin suspension using natural suspending agent. International Journal of Pharma Science, 5,3, 63-70.
5. Kumar R, Patil MB, Patil SR, Paschapur MS, 2009. Evaluation of Abelmoschus Esculentus Mucilage as Suspending Agent in Paracetamol Suspension, International Journal of Pharmaceutical Research, 1,3, 658-665.
6. Prajapati VD, Jani GK, Moradiya NG, Randeria NP. 2013. Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydrate Polymers, 92, 2, 1685-1699.
7. Deb PK, Kokaz SF, Abed SN, Paradkar A, Tekade RK, 2019. in Basic Fundamentals of Drug Delivery, Pharmaceutical and Biomedical Applications of Polymers. 203-267.
8. Das A, Ringu T, Ghosh S, Pramanik N. 2022. A comprehensive review on recent advances in preparation, physicochemical characterization, and bioengineering applications of biopolymers. Polymer Bulletin, 80, 7247-7312.
9. Kulkarni VS, Shaw C. 2016. Use of Polymers and Thickeners in Semisolid and Liquid Formulations. In: Essential Chemistry for Formulators of Semisolid and Liquid Dosages, 43-69.
10. Mann AS, Jain NK, Kharya MD. 2007. Evaluation of the Suspending Properties of Cassia tora Mucilage on Sulphadimidine Suspension, Asian Journal Experimental Science, 21,1, 63-67.
11. Senthil V, Sripreethi D. 2011. Formulation and Evaluation of Paracetamol Suspension from Trigonella Foenum Graecum Mucilage. Journal of Advanced Pharmacy Education & Research, 1,5, 225-233.
12. Perumal PC, Sophia D, Raj CA, Ragavendran P, Starlin T, Gopalakrishnan VK. 2012. In vitro antioxidant activities and HPTLC analysis of ethanolic extract of Cayratia trifolia (L.). Asian Pacific Journal of Tropical Disease, 2,2, 5952-5956.
13. Liaotrakoon W, Liaotrakoon V. 2017. Antioxidative Properties of White and Red Flowered Agathi (Sesbania grandiflora) Tea and Tea Extracts. Walailak Journal of Science & Technology, 16,11, 831-839.
14. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal

of Pharmaceutical Negative Results. 2022 Dec 31:9189-98
15. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
16. Stankovic MS. 2011. Total phenolic content, flavonoid concentration and antioxidant activity of marrubium peregrinum l. extracts; Kragujevac Journal of Science. 33, 63-72.
17. Lindi AM, Gorgani L, Mohammadi M, Hamedi S, Darzi GN, Cerruti P, Fattahi E, Moeini A. 2024. Fenugreek seed mucilage-based active edible films for extending fresh fruit shelf life: Antimicrobial and physicochemical properties. International Journal of Biological Macromolecules. 269, 2, article: 132186.
18. Kumar D, Singhal A, Bansal S, Gupta SK. 2024. Extraction, isolation and evaluation trigonella foenum-graecum as mucoadhesive agent for nasal gel drug delivery.Journal of NPA, 37, 40-45.
19. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
20. A.K. Nayak, D. Pal, J. Pradhan, T. Ghorai, 2012. The potential of Trigonella foenum-graecum L. Seed mucilage as suspending agent, Indian j. Pharm. Edu. res. 46(4) (2012) 312-317.
21. Richardson JC, Dettmar PW, Hampson FC. 2004. Oesophageal bioadhesion of sodium alginate suspension: particle swelling and mucosal retention, European Journal Pharmaceutical Science. 3, 49-56.
22. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91.

23. Khandelwal KR. 2002. Practical Pharmacognosy, Techniques and Experiments, 9th edition, Nirali prakashan, pp. 149-156.
24. Libermann HA, Lachmann L, Joseph BS, Kanig JL. 1987. Compression and consolidation of powdered solids. In: The Theory and Practice of Industrial Pharmacy; third ed., Varghese Publishing House, Mumbai, pp. 67–71.
25. Lieberman HA, Lachman L, Joseph BS, Kanig JL. 1987. Preformulation. In: The Theory and Practice of Industrial Pharmacy; third ed., Varghese Publishing House, Mumbai. 183–184.
26. Pal D, Nayak AK, Kalia S. 2010. Studies on Basella alba Leaves mucilage: Evaluation of suspending properties, International Journal of Drug Discovery Technology. 1,1, 15-20.
27. Strum JD, Colaizzi JL, Goehl TJ, Jaffe JM, Pitlick WH, Shah VP, Poust RI. 1978. Bioavailalivity of sulfonamide suspensions I: Dissolution profiles of sufamethizole using paddle method, Journal Phamaceutical Science. 67, 1399-1402.
28. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-98.
29. Anonymous, 2003.Q1A(R2) Stability Testing of New Drug Substances and Products. File No. 03-118437-914.
30. Anonymous, 2016.USP <61>Microbial Enumeration Tests for Nonsterile Products & USP<62> Tests for Specified Microorganisms in Nonsterile Products, Sixth Interim Revision Announcement, 34(6), 1-9.