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Development and standardization of polyherbal formulation for the management of tuberculosis

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Abstract
Tuberculosis also called TB is currently a major health hazard due to drug-resistant forms of bacilli. Global efforts are underway to eradicate TB using new drugs with new modes of action, higher activity and fewer side effects in combination with vaccines. To develop drugs from these new sources, additional work is required for preclinical and clinical results. Since ancient times, different plant part extracts have been used as traditional medicines against diseases including tuberculosis. This knowledge may be useful in developing future powerful drugs. The present study deals with the development of the polyhedral formulation comprising of the aqueous extracts of *Oscimum sanctum, Alpinia galanga, Adhatoda vasica* and *Long pepper*. The Preformulation parameters and parameters for finished product (hard gelatin capsule) include uniformity of weight, disintegration time, moisture content, pH, phytochemical estimation were performed. The anti-tuberculosis activity of the finished product was performed using Cup and Bore method on *Mycobacterium smegmatis*. The prepared finished product showed significant anti-tuberculosis activity against *Mycobacterium smegmatis.*

Keywords: tuberculosis, polyherbal formulation, hard gelatin capsule

1. 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].

Plants have been used worldwide in traditional medicines for the treatment of various diseases and it is estimated that even today approximately 65-75% of the World's population rely only on medicinal plants as their primary source of medicines4. India is one of the few countries in the World which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases [3, 4].

Herbal medicine is the oldest form of health care known to mankind. It is an integral part of the development of modern civilization. In herbal medicine plant based formulation are used to alleviate diseases. But the most important challenges faced by these formulations arise because of their lack of complete evaluation. So evaluation is necessary to ensure the quality and purity of the herbal product. It is very important to establish a system of evaluation for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous.

Tuberculosis (TB) is one of the leading infectious disease and health burden in the world [5]. It has been estimated that, one third of world's population including 40% from India estimated to be infected with tuberculosis [6]. Current tuberculosis treatment is a long course of combination of 3-4 antibiotic drugs, which have one or the other toxic side effects and led to poor patient compliance. Antitubercular drugs such as isoniazid (INH), rifampicin (RIF), pyrazinamide, ethambutol, streptomycin etc have been a mainstay in the treatment of tuberculosis [7].The global emergence of multidrug resistance (MDR) and extensively drug resistant (XDR) strains of Mycobacterium tuberculosis and more recently the reports of totally drug resistant tuberculosis [8] has become a common phenomenon, which cause drugs to be ineffective.

In traditional systems of medicine, many plants have been documented to be useful for the treatment of various systemic disorders. Many of the traditional/indigenous systems of medicine are effective but they suffer from lack of complete standardization which is one of the important challenges posed by the traditional systems of medicine. The concept of polyhedral formulation is well documented in the ancient literature. Compared to the single herb, the polyherbal formulation has better and extended therapeutic potential. Hence, the present study was planned to formulate and standardize a polyherbal formulation using plants having known anti tubercular potential.

In poly-herbal preparations it will be very difficult if we want to estimate each and every ingredient in term of their chemical constituent. But if few major constituents having particular therapeutic action indicated in the labelled can be pinpointed then these constituents should be estimated quantitatively along with the other parameters through which presence of all ingredients can be confirmed [9].

2. Materials and Methods
2.1 Selection of plant material
All the four plants were selected on the basis of their antitubercular activity previously studied using cup and bore method on *Mycobacterium smegmatis*.

Plant material used for polyherbal formulation: following four plants were used for the preparation of polyherbal antituberculosis formulation.

Table 1: Herbal Drugs Used in Polyherbal Formulation

Plant name	Botanical source	Family	Part used
Tulsi [10]	*Oscimum sanctum*	*Liliaceae*	Leaves
Rasna [11]	*Alpinia galanga*	*Zingiberaceae*	Rhizomes
Vasaka[12]	*Adhatoda vasica*	*Acanthaceae*	Leaves
Long pepper[13]	*Piper longum*	*Piperaceae*	Fruits

2.2 Formula for Polyherbal formulation

The polyherbal formulation (capsules) contained the aqueous extracts of *Oscimum sanctum* (leaves), *Alpinia galanga* (Rhizomes), *Adhatoda vasica* (leaves) and *Long pepper* (Fruits) in the ratio of 1:1:1:1.

2.3 Preformulation studies

Preformulation parameters such as bulk density, tap density, Compressibility index, Hausner's ratio, and angle of repose were determined for the prepared polyherbal granules and the best trial batch were taken for capsule filling and further studies [14, 15].

Preformulation parameters

2.3.1 Bulk density, tap density and Carr's index [16, 17]

A weighed quantity (15g) of powdered material was taken in a 50ml measuring cylinder and recorded the initial volume (vo). tapped the contents and recorded the powdered volumes after 50 taps(v50).

Fluff density = w/vo g/cc

Tapped density = w/v50 g/cc

Carr's index = Tapped density- Fluff density/ Tapped density * 100

Value for Carr's index below 15 indicate excellent flowing material and value over 20-30 suggested poor flowing material.

2.3.2 Angle of repose [18]

A funnel was fixed at a particular height (1.5, 2.5, 3.5 cm) on a burette stand. A white paper was placed below the funnel on the table. The powdered drug passed slowly through the funnel until it forms a pile. The radius of the pile was noted down.

Angle of repose of the powder material was calculated by using the formula:

$\tan\theta = h/r$

$\theta = \tan (h/r)$

Where, h = height of the pile, r = radius.

Values for angle of repose 30^o usually indicate a free flowing material and angle 40^o suggest a poor flowing material.

2.3.3 Hausner's ratio [18]

The basic procedure is to measure the unsettled apparent volume, V0 and the final tap volume Vf of the powder tapping the material until no further volume changes occur. The Hausner's ratio was calculated as follows:

Hausner's ratio = V0 / Vf

Hausner's ratio between 1.00 to 1.11 shows excellent flow and value more than 1.60 shows very, very poor flow.

2.4 Preparation of polyherbal formulation by wet granulation method

The formulation preparation began with trials by adding a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. *Oscimum sanctum* (leaves), *Alpinia galanga* (Rhizomes), *Adhatoda vasica* (leaves) and *Long pepper* (Fruits) extracts were powdered (sieve 40), and mixed in the ratio of 1:1:1:1 and taken for the preparation of capsules by wet granulation technique using 5% starch paste as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray [19].

2.5 Standardization of polyherbal formulation (hard gelatin capsule)

2.5.1 Capsule evaluation

The polyherbal capsules were evaluated for their description, average weight, weight variation, moisture content, disintegration time, pH and microbial load and compared with Indian pharmacopoeial standards [20].

2.5.1.1 Average weight: Twenty capsules were individually weighed and the average weight of the capsule was calculated.

2.5.1.2 Weight variation: The individual weights of the each capsule should be within the limits of 90% and 110% of the average weight.

2.5.1.3 Moisture content: Moisture content was determined by using automatic Karl Fischer titration apparatus.

2.5.1.4 Disintegration time: Disintegration test was performed using the digital microprocessor based disintegration test apparatus. One capsule was introduced into each tube and a disc was added to each tube. The assembly was suspended in water in a 1000 ml beaker. The volume of water at its highest point was at least 25 mm below the surface of the water and at its lowest point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37 ± 2ºC.

pH value: pH of 1% solution was determined by using a digital pH meter.

2.5.2 Dissolution

Dissolution is considered as a tool for predicting rate of absorption and bioavailability in some cases, replacing clinical studies to determine bioequivalence of drug. We were added six capsules in the basket type dissolution apparatus containing distilled water as a dissolution media. The speed was set on 50 rpm for 1 hour and the sample was drawn at every 10 minutes and the amount of dissolved active ingredient in the solution was calculated as percentage dissolved in 1 hour.

2.5.3 Stability

Pharmaceutical products are generally studied for stability profile at accelerated temperature, humidity and also at different intensities of light. The studies were performed to determine the physical, chemical, and therapeutic changes occurring in the monoherbal capsule by extrinsic factors [21, 22].

a) **Light:** Sample was stored in different intensities of light i.e. sunrays, fluorescent (tube) light, UV and infrared light for detection of degradation of powder material.
b) **Temperature:** The effect of temperature on the stability of polyherbal capsule was checked by keeping all the capsule at different temperatures i.e. ambient, 35^0C, 50^0C, 55^0C, 65^0C for 30 minutes, 1, 3, and 6 hours.

c) **Humidity**: The effect of humidity on the stability of capsule was checked by keeping the entire capsule at four different humidity percentage i.e. 30%, 50%, 70% and 90%.

Composition of capsule

Each 500mg capsule contains:
Oscimum sanctum 125mg
Alpinia galanga 125mg
Adhatoda vasica 125mg
Piper longum 125mg
Excipients q.s.

2.6 In-Vitro Anti-Tuberculosis activity of prepared polyherbal formulation:

The Agar Diffusion Cup Method [23]

This method is used to screen the anti-tuberculosis activity of selected ethnomedicinal plants. Agar plates were seeded with 0.5 McFarland standards bacterial culture of *M. smegmatis*. Agar plates were then bore holed using 6 mm diameter cork bore.100, 500, 1000 µg/ml concentration of granules were prepared to perform this assay. 0.3 ml each of the concentration was introduced into the hole and allowed to diffuse for 5-10 minutes before incubation. The Petri dishes used for antitubercular screening were incubated at 37 ^{0}C for 48 hours. All the concentration was done in triplicate to minimize the error. The inhibition zone diameters (IZD) were determined and recorded for further analysis. Isoniazid and Rifampicin were used as a standard.

3. Results

The most important part of any formulation is standardization which ensures the quality, safety and reproducibility. It involves the complete process of bioprospection right from the collection of raw materials to development of finished product. In the present study, standardized polyherbal mixture was formulated in hard gelatin capsule.

Polyherbal formulation composed of four ingredients, belonging to different families, different morphological plant parts and different phytoconstituents.

3.1 Preformulation studies

Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were obtained for the laboratory granules. The granules showed excellent flow property.

Table 2: Preformulation parameters

S. No.	Parameters	Results
1	Bulk density	0.6
2	Tap density	0.7
3	Carr's index	18.4
4	Hausner's ratio	1.19
5	Angle of repose	13.95

As per the standards, the flow property of the blend to be filled in the capsule should be in good range and was confirmed by the above parameters. Trail batch IV showed excellent flow characters and batch IV was taken for capsule filling.

The trial IV flow properties were Excellent and all parameters were within the Specified limits. So, fourth trial was chosen for further studies.

Table 3: Evaluation of in process Parameters

Parameter	Trial I	Trial II	Trial III	Trial IV
Flow property	Poor flow	Poor flow	Fair	Good
Uniformity of Filling	-	-	Uniform	Uniform
Uniformity of Weight	-	-	Less weight	Uniform

3.2 Standardization of formulation

3.2.1 Capsule evaluation

Description "light brown" coloured granules packed in "0" size blue capsules. The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature.

Table 4: Organoleptic Characters of Capsules

Parameters	Observation
Description	Light brown granule in blue cap and body "0" size capsule
Colour	Light brown granule
Odour	Characteristic odour
Taste	Bitter taste

Table 5: Evaluation of capsules

Parameter	Observation
Average weight	Within limits
Weight variation	Within limits
Moister content(LOD)	3.56%
Disintegration time	8 mins 15 secs
pH(1% aqueous solution	5.52± 0.68

Result (n=3) are reported as Mean ± Standard deviation

Table 6: *In Vitro* Dissolution Studies

Time (min)	Abs	Conc. (µg/ml)	Amt (mg/5ml)	Amt (mg/ml)	Amt (mg/900ml)	CDR	%CDR
0	0.047	9.62963	0.04815	0.00963	8.66667	8.6666	3.46664
5	0.358	124.815	0.62407	0.12482	112.333	112.382	44.9526
10	0.493	174.815	0.87407	0.17482	157.333	157.957	63.183
15	0.575	205.185	1.02593	0.20519	184.667	185.541	74.2163
20	0.648	232.222	1.16111	0.23222	209	210.026	84.0104
25	0.714	256.667	1.28333	0.25667	231	232.161	92.8644
30	0.756	272.222	1.36111	0.27222	245	246.283	98.5133

3.2.2 Stability

The stability parameters were analyzed for 30 minutes, 1, 3 and 6 hours of storage at accelerated conditions of temperature, light and humidity were found to be comparable. It was indicating that there gross physical characteristics does not produce any significant change, observation have been tabulated in table 4, 5 and 6 for three Stability parameters

Table 7: Effect of different intensities of lights on polyherbal capsules (500 mg)

Light Source	Sun light				Fluorescence				Tube light				UV Light				Infra-Red (IR)				Lamp Light			
Time of Exposure (hours)	1/2	1	3	6	1/2	1	3	6	1/2	1	3	6	1/2	1	3	6	1/2	1	3	6	1/2	1	3	6
500mg polyherbal capsule	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

(-) No change, (+) Degradation

Table 8: Stability test of polyherbal Capsule (500mg) at different Temperature

Storage condition	Testing condition	Time Duration (hours)				Result
		1/2	1	3	6	
Ambient	30ºC	-	-	-	-	No change during 6 hours
Warm (30-40 ºC)	35 ºC	-	-	-	-	No change during 6 hours
Accelerated	50 ºC	-	-	-	-	No change during 6 hours
Accelerated	55 ºC	-	-	-	+	Degradation start after 4 hours
Accelerated	65 ºC	-	-	+	+	Degradation start after 2 hours

(-) No change, (+) Degradation starts

Table 9: Stability of monoherbal Capsule (250 mg) at different Humidity with respect to different Temperature

Temperature	30% Humidity	50% Humidity	70% Humidity	90% Humidity
30%	-	-	-	-
35%	-	-	-	-
55%	-	-	+	++
65%	-	-	++	+++

(+) Degradation (-) No Change

3.3 *In-Vitro* Anti-Tuberculosis activity of prepared polyherbal formulation:

Polyherbal mixture shows significant zone of inhibition on *Mycobacterium smegmatis* at different concentration is shown in Table 10.

Table 10: Zone of inhibition of polyherbal mixture

S. No.	Test substance	Concentration		
		100 µg/ml	500 µg/ml	1000 µg/ml
1	Polyherbal mixture	14mm	17mm	20mm
2	Isoniazid+Rifampicin (100µg/ml)	21mm	22mm	22mm

4. Discussion

Various types of herbal medicines have been used as curative agents in different parts of the world [24]. Drugs derived from traditional herbs may have possible therapeutic relevance in the treatment of illness [25].

In the present research work *Oscimum sanctum* (leaves), *Alpinia galanga* (Rhizomes), *Adhatoda vasica* (leaves) and *Long pepper* (Fruits) were used for the polyherbal 500 mg capsule. First it was formulated and then evaluated for quality herbal product which is very important irrespective of their medicinal content and therapeutic states therefore the pre-formulation and formulation studies of the formulated polyherbal capsule were evaluated.

Preformulation parameters including angle of repose (a traditional characterization method for pharmaceutical powder flow), porosity (packing geometry), Carr's index and Hausner's ratio (a measure of the interparticulate friction) are useful tools in the development of new formulation. A value of <30° indicates 'excellent' flow whereas >56° indicates 'very poor' flow. Based on this, the flow was rated as 'excellent' (Table-2). The CI and HR were found to be 18.4 and 1.19. Lower CI or lower Hausner ratios of a material indicates better flow properties than higher ones. A Carr's index of <10 or HR of <1.11 is considered 'excellent' flow whereas CI>38 or HR>1.60 is considered 'very very poor' flow [26, 27]. Based on the results obtained (Table-2) flow of selected plant powder was rated as 'good'. Good flow of powder help to avoid the extensive costs and time involved in unloading powders that will not flow out of storage containers. As well as help to achieve the best formulation and improve the quality and consistency of the product.

All the four drugs were approved as quality drug when undergone by phytopharmaceutical evaluation according to the pharmacopoeial standards. 500 mg polyherbal capsules

disintegrated in meantime 8.14±15 minutes and in vitro condition and we determined the release of a drug from solid dosage format which the substance dissolved in the fluid of gastrointestinal tract. Results indicates that all of six capsules dissolved equal to 90% in 30 minutes and this releasing pattern of drug from their capsule shell in-vitro help in predicting the releasing sequence in-vivo that developing a tool for bioavailability of drug, as well as in some cases, replacing clinical studies to determine bioequivalence. In light of the phytopharmaceutical studies of the polyherbal capsule was found almost stable.

Polyherbal mixtures of selected plants were screened for their anti-tuberculosis activity. Polyherbal mixure of plant shows maximum anti-tuberculosis activity on *M. smegmetis*. Further studies using more specific methods are required to explore the constituents responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and prevention of tuberculosis.

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