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	formulation	

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 outstanding flow property.

Flow property of powdered extract

The powdered material of poly-herbal capsule formulation was filled in size 1 capsules. Prior to filling of powder in capsules, its flow property was properly checked. The flow property of polyherbal formulation was found to be best and under acceptable limit as per Indian Pharmacopoeia. The results are shown below in Table 5. 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. The optimized poly-herbal capsule formulation showed excellent flow characters and was taken for capsule filling. All parameters were within the specified limits.

Table 6. Preformulation parameters.

Parameters	**Poly-herbal formulation**
Bulk density	0.823g/ml
Tapped density	0.702 g/ml
Carrs index	14.7023
Hausners ratio	1.14 ± 0.04
Angle of repose	19.04

Standardization of formulation

Capsule evaluation

Description "light brown" coloured granules packed in "0" size blue capsules. The poly-herbal capsules were evaluated for their description, microbial load, uniformity of dosage units, weight variation, disintegration time, and moisture content, and compared with Indian pharmacopoeial standards.

Organoleptic characters like brown colour, characteristic odour and taste was found. The Physiochemical parameters results were found such as pH 7.7, moisture content 1.01%, average weight 550 mg and weight variation 2.83%. The pharmacognostic powder characters results are loss on drying 2.55%, total ash 5.66%, acid-insoluble ash 1.28%, and water-soluble ash 3.51%, water-soluble extractive value 16.72%, ethanol-soluble extractive value 13.38%, arsenic not more than 5 ppm, microbial load analysis, presence of *E. coli* (should be absent), presence of salmonella (should be absent), presence of streptococcus (should be absent), presence of pseudomonas (should be absent) and total microbial count of yeast and molds are under limit (Table 7).

Tablet 7. Standardization of poly-herbal capsule.

Name of the test	Observations
Organoleptic characters	
Description	Brown colour powder
Colour	Brown
Odour	Characteristic
Taste	Bitter
Physiochemical parameters	

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pH	7.7
Moisture content	0.0101
Average weight	563 mg
Weight variation	0.0283
Disintegration time (Mean ± SEM)	3 min 25 seconds ± 0.21
Loss on drying	0.0255
Total ash	0.0566
Acid-insoluble ash	0.0128
Water-soluble ash	0.0351
Water-soluble extractive value	0.1672
Ethanol-soluble extractive value	0.1338
Limits for heavy metals	
Arsenic not more than 5ppm	Complies
Lead not more than 10ppm	Complies
Microbial load analysis	
Total microbial count NMT 1000 cfu/g	113 cfu/g
Yeast and molds	Nil
Presence of *E. coli* (should be absent)	Absent
Presence of *Salmonella* (should be absent)	Absent
Presence of *Streptococcus* (should be absent)	Absent
Presence of *Pseudomonas* (should be absent)	Absent

Disintegration

Disintegration test was performed (550mg) by using six capsules. The minimum time of disintegration was 6 minutes and the maximum time observed was 13 minutes. All the six capsules fulfill the criteria of dissolution according to the Indian Pharmacopoeia (Table 8).

Table 8. Disintegration and dissolution pattern of poly-herbal capsule.

No of capsules	Disintegration time (Min)
1st Capsule	6 Min
2nd Capsule	8 Min
3rd Capsule	9 Min
4th Capsule	11 Min
5th Capsule	12 Min
6th Capsule	13 Min
Mean X	9.83
± S.D.	2.64
%CV	26.856

Dissolution

The optimized poly-herbal formulation of dissolution was studied by using *in-vitro* method. The % of cumulative drug release of bioactive drug molecule is maximum 98.6734 % up to 30 minutes (Table 9 and Figure 5).

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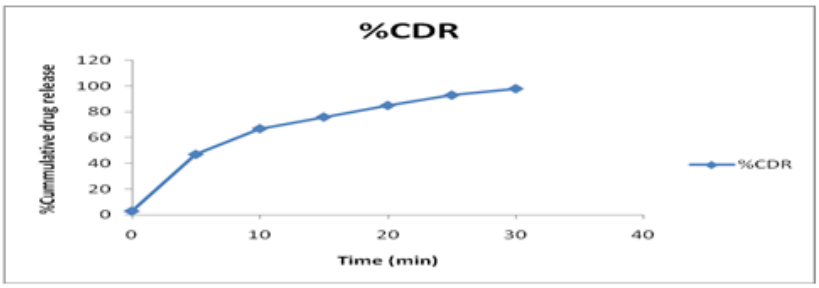
Table 9. *In-Vitro* Dissolution Studies.

Time (min)	Abs	%CDR
0	0.0571	3.6864
5	0.3612	47.852
10	0.446	67.1841
15	0.5833	76.2171
20	0.6712	85.0103
25	0.7371	93.8643
30	0.769	98.6734

Figure 5. *In-vitro* % cumulative drug release of poly-herbal capsule.



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 10,11 and 12 for three stability parameters.

Table 10. Effect of different intensities of lights on poly-herbal capsules.

Light source	Sun light				Fluorescence				Tube light				UV Light				Infra-Red (IR)				Lamp light			
Time of exposure (hours)	01-Feb	1	3	6	01-Feb	1	3	6	½	1	3	6	01-Feb	1	3	6	01-Feb	1	3	6	01-Feb	1	3	6
600 mg polyherbal capsule	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Table 11. Stability test of poly-herbal capsule at different temperature.

Storage condition	Testing condition	Time duration (hours)				Result
		44593	1	3	6	
Ambient	30°C	-	-	-	-	No change during 6 hours after
Warm (30-40 °C)	35°C	-	-	-	-	No change during 6 hours after
Accelerated	50°C	-	-	-	-	No change during 6 hours after
Accelerated	55°C	-	-	-	+	Degradation start after 4 hours

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Accelerated	65°C	-	-	+	+	Degradation start after 2 hours

Table 12. Stability of polyherbal capsule at different humidity and temperature.

Temperature	30% Humidity	50% Humidity	70% Humidity	90% Humidity
0.3	-	-	-	-
0.35	-	-	-	-
0.55	-	-	+	++
0.65	-	-	++	+++

In-Vitro anti-cancer activity of prepared polyherbal formulation

Polyherbal mixture shows significant cytotoxicity on the A549 cell line. The percentage cytotoxicity on A549 cell line at different concentration is as shown in Table 13 (Figure 6-8).

Table 13. Table showing the IC50 concentrations of the test compounds, S1 against A549 cell lines after the incubation period of 24 hrs.

S. NO	Sample code	IC_{50} (µG/ml) A549 Cell
1	S1	17.68

Figure 6. Overlaid Bar graph showing the IC50 values of Test compounds against A549cell lines by MTT study.

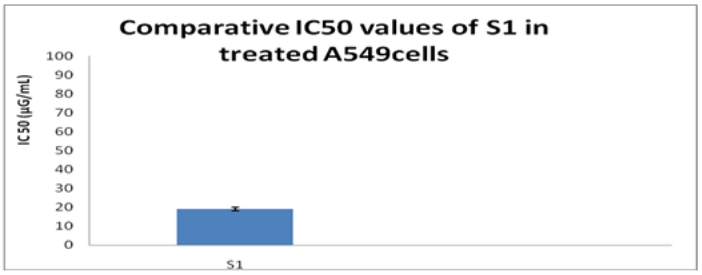

Figure 7. Graph showing the % cell viability of Test compounds against A549cell lines by MTT study.

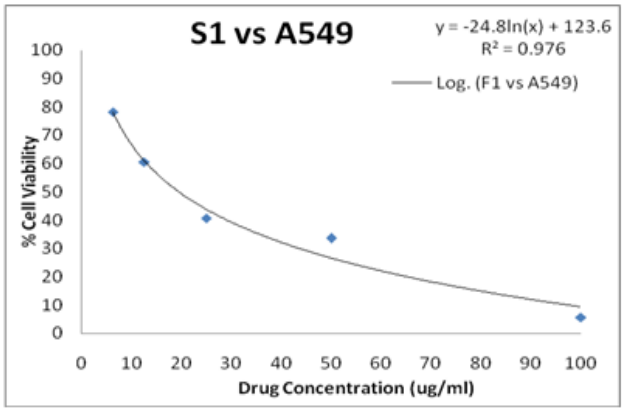

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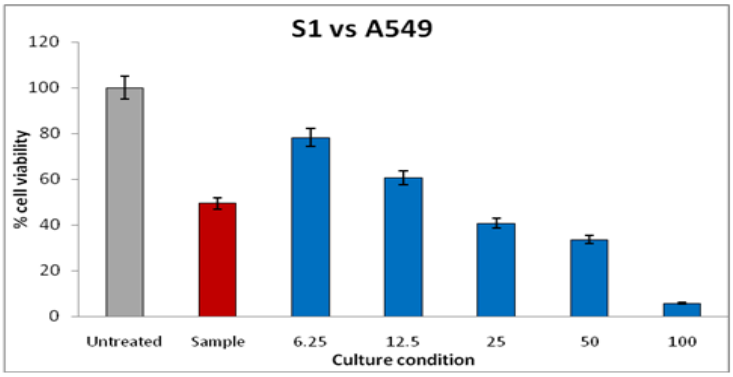
Figure 8. Bar graph showing the % cell viability of Test compounds against A549cell lines by MTT study.



The Observations in Statistical data of MTT cytotoxicity Study suggesting us that against A549cell lines test compounds namely S1 showing significant cytotoxic potential properties with the IC50 concentrations at 19.12 uG/ml used in the study. The compound, S1 showing effective cytotoxicity on A549 cells and may be considered as potent anti-lung cancer agent due to their low IC50 values on A549 cells. Overall, S1 compound showing effective anti-cancer potency on Human lung cancer cells. Further studies like cell cycle study by PI staining, Apoptosis study by Annexin V/PI staining, Apoptotic Protein expressions like Caspase 3,7,9, Bcl2,p53 and ROS study to evaluate the mechanism of action of test compounds *viz.*, S1 behind the anticancer potential in *in-vitro* conditions.

DISCUSSION

Different types of poly-herbal dosage forms have been used as therapeutic agents for the treatment of different disease. These bioactive derived molecules are obtained from traditional herbs. It may have potential therapeutic relevance in the treatment of cancer or many diseases. Therefore need do more research work for development of best combination of herbal formulation. In the present research work *Ocimum sanctum* 50 mg, *Curcuma longa* 50 mg, *Emblica officinalis* 50 mg, *Teriminalia bellerica* 50 mg, *Teriminalia chebula* 50 mg, *Piper longum* 50 mg, *Piper nigrum* 25 mg and *Zingiber officinale*25 mg were used for the poly-herbal 550 mg capsule. Poly-herbal combination of capsule formulation was developed with wet granulation method and then evaluated for quality poly-herbal product. It is very significant irrespective of their medicinal content and therapeutic states therefore the pre-formulation and formulation studies of the formulated polyherbal capsule solid dosage form were evaluated. Preformulation parameters are with 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 inter-particulate friction). These are useful tools in the development of new poly-herbal formulation. A value of <30° indicates 'excellent' flow whereas >56° indicates 'very poor' flow. Based on this, the flow was rated as 'excellent'. The CI and HR were found to be 14.7023and 1.14 ± 0.04. Lower CI or lower Hausner ratios of a material indicates better flow properties than higher ones. 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 eight herbal drugs were approved as quality drug when undergone by phytopharmaceutical evaluation according to the pharmacopoeial standards. Each poly-herbal capsule 550 mg disintegrated in meantime 13.14 ± 15 minutes and in vitro condition. The dissolution of drug determined where the release of a drug from solid dosage format which the substance dissolved in the fluid of gastrointestinal tract. Dissolution results represented that all of six capsules dissolved equal to 90% in 30 minutes. Drug releasing pattern of drug from capsule shell during *in-vitro* study is predicting the releasing sequence. The correlations of *in-vitro* and *in-vivo* results are developing a tool for bioavailability of drug, and to determine bioequivalence. In light of the phyto pharmaceutical studies and stability studies of the poly-herbal capsule was found almost stable.

Poly-herbal mixtures of selected plants were screened for their anti-cancer activity. Poly-herbal dosage form of plant shows significant cytotoxicity and thus anti-cancer activity on A549 cell line. Further studies using further precise methods are necessary to explore the constituents responsible for the activity and the mechanism of this activity which might confirm important and better therapies for the treatment and management of lung cancer [20,21].

CONCLUSION

Thus our study findings demonstrate that the poly-herbal capsule formulation evaluation of oral dosage form (550 mg capsule according to the Indian Pharmacopeia was successfully done. In the present research work, poly-herbal formulation was evaluated for its ability to reduce oxidative stress well. The oxidative stress is a vital factor for the pathogenesis of cancer disease, and decreases it; antioxidant may have been seen shown to be beneficial in the management of lung cancer disease.

Therefore, it can be concluded that the formulated poly-herbal solid dosage form, containing eight good anticancer bioactive molecule. It can be used for the treatment and management of cancer diseases.

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