Original Article

Characterization of Liquid Oral Containing Lycopene Phytosomes for Improved Absorption

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ABSTRACT

Objective: Owing to life style modifications and use of Nutraceuticals present study was aimed to develop stable and controlled release oral liquid containing lycopene phytosomes for better therapeutic activity. Tomatoes are consumed raw or in the processed forms like Ketchup, Sauce, Soup and salad. The global lycopene market size was valued at $107.2 million in 2020, and is projected reach $187.3 million in 2030, registering a CAGR of 5.2% from 2021 to 2030. It has several medicinal properties, which has increased its demand in the Nutraceutical market. **Method:** Extracted lycopene was complexed with phospholipid (1:1) to form phytosomes and subjected to pre and post evaluations of liquid oral nanosuspension (LPS1 to LPS4) containing different concentration of Sodium Carboxymethylcellulose, and Hydroxy Propyl Methyl Cellulose K4M **Results:** Acceptable results were seen for pre evaluation for FTIR, DSC compatible studies and post evaluation like pH(4.4 to 5.1), viscosity (15.2 to 42.2 cp), Drug content (96.5 to 98.8%), specific gravity (1.1042 to 1.1062), Particle size(450.5 to 594.5nm) and zeta potential (-21.2 to -23.2) respectively. **Conclusion:** Overall LPS3 was found to be better and stable formulation containing 10ml of 1% HPMC K4M as suspending agent. Anti-Oxidant property was found comparatively same for plain and complexes of lycopene, difference was found for duration of antioxidant activity which can be correlated with *in-vitro* dissolution studies.

Key words: Lycopene, Liquid Oral, Anti-Oxidant, Nutraceutical, Phytosomes.

Recent studies have an impact on how people feel about using dietary supplements, and consumer interest in self-care is rising [1]. The potential of complementary and alternative medicine—these so-called "community-based lifestyle interventions"—to prevent diseases has been the subject of a growing body of scientific research over the past ten years. One of the active components derived from a natural source is lycopene [2]. Lycopene, a chemical with a high lipophilicity, has a variety of pharmacological effects. Its bioavailability is however constrained by its poor water solubility and significant pre-systemic metabolism. Due to their poor oral bioavailability, many barriers prevent the medical use of medications derived from herbs, especially those that include polyene chains with 35–40. Carbon atoms in their chemical structure. The use of phytosomal technology is one approach to address these more recent issues. Lycopene and the consumption of foods containing lycopene may impact the risk of cancer or cardiovascular disease, according to encouraging results from epidemiological, cell culture, and animal research, but more clinical trial data is required to support this idea [3].

A unique herbal formulation called a phytosome, which resembles a tiny cell, combines the bioactive phytoconstituents of an herb extract complexed with phospholipids echnique is a ground-breaking approach for dramatically ito create lipid-compatible molecular complexes. This phytosome tmproving bioavailability, substantially increasing clinical benefit, assuring delivery to the tissues, and maintaining nutrient safety. Over traditional botanical extracts, phytosomes offer superior pharmacokinetic and pharmacodynamic behaviour. This method made use of the complex formation that can occur when phospholipid molecules interact with herbal extracts or their constituents to create a lipid-compatible molecular complex that is soluble in both a lipid environment and water [4]. Delivering an effective level of the active components is necessary for any herbal product to be effective. This problem is solved by the phytosome technology, which significantly increases the bioavailability of phytomedicines [5].

MATERIALS & METHODS

Tomatoes were collected from local market at Mangalore of Karnataka in the month of January 2018 and authenticated by Pilikula Nisarga Dhama (Botanical garden) Mangalore. Natural or synthetic phospholipids, such as phosphatidylcholine, were obtained from Hi media, aprotic

solvent, such as dioxane or acetone, n-hexane, ethanol were obtained by Merck chemicals.

Preparation of oral Nanosuspension containing Lycopene Phytosomes: This preparation is majorly includes two steps. Extraction and Preparation of Lycopene Phytosomes was done as per Aghel N *et al*., 2011 and Jain S *et al.*, 2019 respectively [6, 7] and composition of Suspension containing Lycopene Phytosomes was prepared as per Table 1.

Table 1: Composition of Liquid Orals

Ingredients	LPS1	LPS2	LPS3	LPS4
Lycopene Phytosomes equivalent to	10mg / 5ml.	10mg / 5ml.	10mg / 5ml.	10mg / 5ml.
Simple Syrup	10 ml	10 ml	10 ml	10 ml
Glycerin	10 ml	10 ml	10 ml	10 ml
Aqueous Solution of Na-CMC (1%)	5 ml	10 ml	-	-
Aqueous solution of HPMC K4M (1%)	-	-	5 ml	10 ml
Methyl Paraben (%)	0.09	0.09	0.09	0.09
Propyl Paraben (%)	0.01	0.01	0.01	0.01
Purified Water	50 ml	50 ml	50 ml	50 ml

Preparation of Lycopene Phytosomal Suspension dosage form: To lower the interfacial tension between liquid and air, the powdered form of the medication complex was completely moistened with syrup and glycerin solution. The wetted material was then slowly added to, with continuous triturating, the suspending agents such as sodium carboxy methyl cellulose (Na-CMC) or hydroxy propyl methyl cellulose K4M in the aqueous medium containing specified preservatives. In order to create four different suspension formulations, LPS1, LPS2, LPS3, and LPS4, 5 and 10 millilitres of 1% aqueous Na-CMC and HPMC K4M solution, respectively, were used. Lastly, a continuous trituration process was used to raise the suspension to the final volume using filtered water in order to produce a uniform product. The production quality of all four suspension formulations was then assessed in accordance with established guidelines. [8].

Formulation of Lycopene Phytosome Liquid Orals

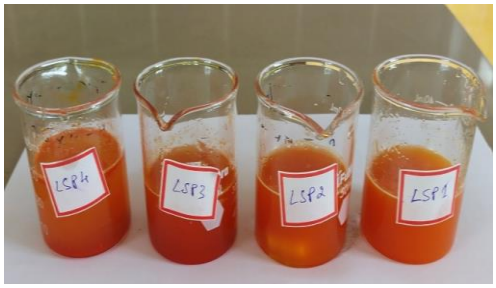

Fig 1: Lycopene Phytosomal Liquid Orals.

Evaluation of Lycopene Phytosomes Suspensions: Evaluation of Suspension: All the four Suspension dosage form (LPS1, LPS2, LPS3 and LPS4) were evaluated for pH, viscosity, Drug content, Drug Release studies and stability study of the final suspension was carried out.

pH: Using common buffer solutions, such as pH 4 and 7, the pH metre was calibrated. The pH of the suspension was tested after it had been dissolved in 50.0 ml of distilled water in an amount of about 5 ml. With the use of a Systronic Digital pH metre, the pH of the samples was determined.

Viscosity: Using a Brookfield Viscometer, the sample's viscosity was measured (DV - E Model). A tiny volume holder was used to hold the necessary amount of suspension, and the LV4-27 spindle with a 100 rpm rotational speed was employed. Centipoises (cp) and the associated percent torque value were recorded.

Specific Gravity: Relative density, or specific gravity, is the ratio of the density (mass of a unit volume) of a substance to the density of a given reference material. Specific gravity usually means relative density with respect to water [9].

Determination of drug content: Accurately 5mL of formulation from different batches was measured and transferred to 100 mL volumetric flask. To this 50-70mL of 0.1 N HCl was added and sonicated for 30 min. Volume was adjusted to 100mL. Complete dispersion of contents was ensured visually and the dispersion was filtered using Whattman Filter Paper. From this solution, 1 mL of sample was withdrawn and diluted to 10mL with 0.1 N HCl. Contents of lycopene was measured at maximum absorbance at 471nm using Jasco UV Spectrophotometer [10].

Particle Size: Particle size and zeta potential of the optimized lycopene phytosomes were done using Malvern Particle Size and Zeta Analyzer (Malvern Panalytical version 7.13) [11].

In-Vitro Release Studies: The drug release study was carried out using USP type II paddle type apparatus at 37 ± 0.5ºC and at 50 rpm using 900 ml of 0.1 N HCl (pH 1.2). Lycopene Suspension equivalent to 10 mg of lycopene was used for the test. Sample solution (5 ml) was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted and suitably analyzed by UV spectrophotometric JASCO at 471 nm. Fresh dissolution medium was replaced immediately after withdrawal of the test sample to maintain sink condition. The dissolution studies were carried out for a period of 2 h. and further dissolution medium pH was raised to 6.8 and dissolution studies was carried out for 10 h [12].

Anti-Oxidant Activity by DPPH Method: DPPH solution, 1mmol/L, was prepared by dissolving 31.54 mg of DPPH in 95% v/v buffered methanol (40mL of 0.1 mol/L acetate buffer

pH 5.5 with 60 mL of methanol) and made up to 50 ml with buffered methanol. The different concentrations of Lycopene and lycopene phytosomes such as 0.5 mg, 1 mg, 2mg, 4mg and 8 mg. were made up to 4 ml with distilled water. 1 ml of DPPH (1mmol, $3.953x10^{-10}$ µg/ml) was added to each test tube, shaken and the mixture was kept at 30^0C for 30 min. The 471 nm is a measuring absorbance of the resulting solution. The effect of ascorbic acid (Vitamin C) on DPPH was also assessed for comparison with that of lycopene phytosomes. A buffered methanolic dilution (0.2, 0.4, 0.6, 0.8, 1.0 ml) of 1 mg/ml ascorbic acid was made to 4 ml with distilled water. 1 ml DPPH radical (1nmom/L) was added to each test tube and same procedure as in DPPH scavenging experiment was followed. The absorbance measured for the control solution (Buffered methanol with DPPH) was in the range 0.500 ± 0.040. Antiradical activity was expressed as inhibition percentage (1%) and calculated using the following equation: Inhibition percentage = [(Abs control – Abs sample)/ Abs control] x 100 [13].

Stability Studies: In order to determine the change in evaluation parameters like physical appearance, drug content, *in vitro* drug release profile on storage, stability studies of optimized batch was carried out at accelerated storage conditions at temperature 40±2˚C and 75±5% RH in a humidity chamber (ROTEK) as per ICH Q1C guidelines for 6 months. Sample were withdrawn after each month and evaluated for changes in physical appearance, drug content and *in vitro* drug release profile [11].

Fig 2: Viscosity of Liquid Orals.

RESULTS AND DISCUSSION

Satisfactory attempt to formulate phytosomal Oral suspension dosage form for controlled delivery of Lycopene using polymer like sodium CMC and HPMC K4M showed reproducible results of the executed experiments, it can be concluded that: The lycopene phytosomal Liquid Oral Suspension dosage form can be prepared by best use of suspending agents like sodium CMC and HPMC K4M. The prepared Liquid Orals were evaluated and showed to be uniform and stable in terms of pH of the liquid oral formulations was found to be in acceptable range for gastric absorption (pH 4.5); best to be absorbed by oral route, Viscosity of Liquid oral dosage forms were found to be satisfactory and shows the ease of pour ability from the container. Different concentration of suspending agents showed noticeable change in viscosity. Sodium CMC showed better suspending property than HPMC K4M. Specific gravity found to be near to one which is essential for a stable suspension and uniformity in the drug content as given in Table 2.

Particle size and Zeta potential: Particle Size (450.5nm to 467.1nm), Zeta Potential (- 21.7 to – 23.2) and Poly dispersity index (0.515 to 0.591) of all the respective suspension dosage form were up to the acceptable values for the stable suspension. Table 3 and Fig 3.

Table 2: pH, Viscosity and Specific Gravity of Lycopene Suspension

Code	pH	Viscosity 100 rpm		Specific Gravity	Drug Content %
		cp	% Torque		
LPS1	4.4	29.5	12.6	1.1058	98.8
LPS2	4.5	42.2	18.0	1.1062	97.8
LPS3	4.9	15.2	10.2	1.1042	96.5
LPS4	5.1	22.3	11.6	1.1052	97.2

Table 3: Particle Size and Zeta potential of Lycopene Suspension.

Code	Avg. Particle Size (nm)	Zeta Potential	Poly Dispersity Index (PDI)	Product Quality
LPS1	464.8	- 21.7	0.515	Good
LPS2	450.5	- 21.9	0.578	Good
LPS3	467.1	- 23.2	0.521	Good
LPS4	594.4	-	0.591	Good

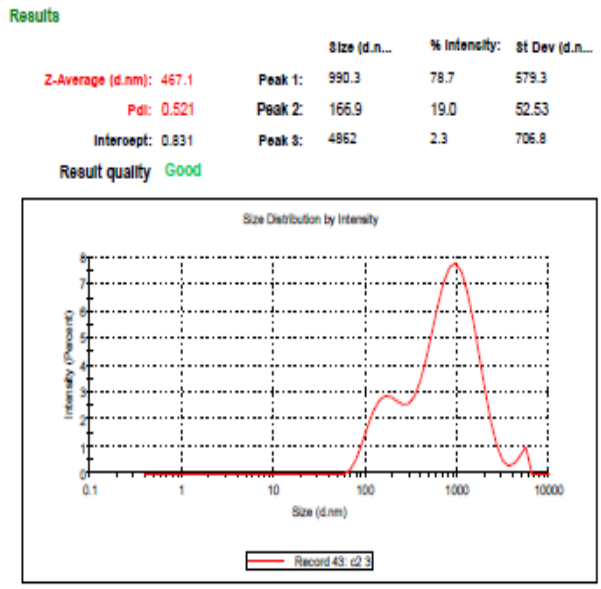

Fig 3: Particle Size distribution of Lycopene Phytosome LPS3

Anti- Oxidant Activity: The antioxidant activity of the lycopene and phytosomal complex was compared taking ascorbic acid as standard; the results are given in Fig 4. Anti-Oxidant activity of the extracted lycopene and Lycopene Phytosomal complex was performed and it found to be for lycopene 8 to 60 % antioxidant activity with increase in concentration to that of Lycopene phytosomal complex between 9 to 61 % compared to the standard antioxidant ascorbic acid. The anti-oxidant activity was found comparatively same for plain and complexes of lycopene, difference was found for duration of antioxidant activity which can be correlated with *in-vitro* dissolution studies.

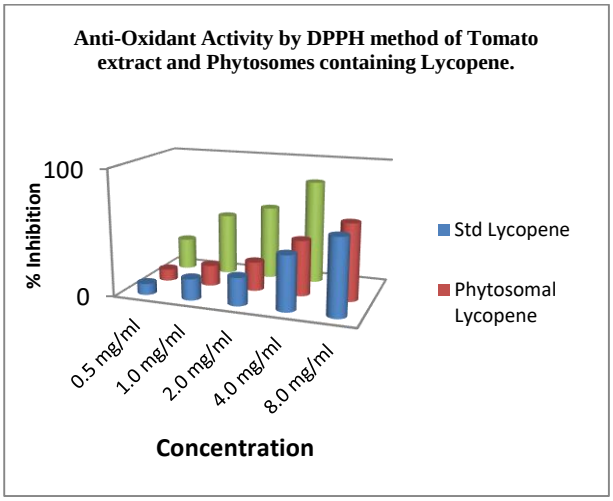

Fig 4: Anti-Oxidant Activity by DPPH method of Tomato extract and Phytosomes containing Lycopene.

***In-vitro* Drug Release Studies:** The percentage release of the drug during dissolution studies showed less than 14% release in first 2 hours in the acidic pH and more than 95% of release within 10 hours of all the formulations in pH 6.8 for LPS2 containing 10 ml of 1% sodium CMC for 12 hours compared to HPMC K4M which sustained up to 10 hours.Fig 5.

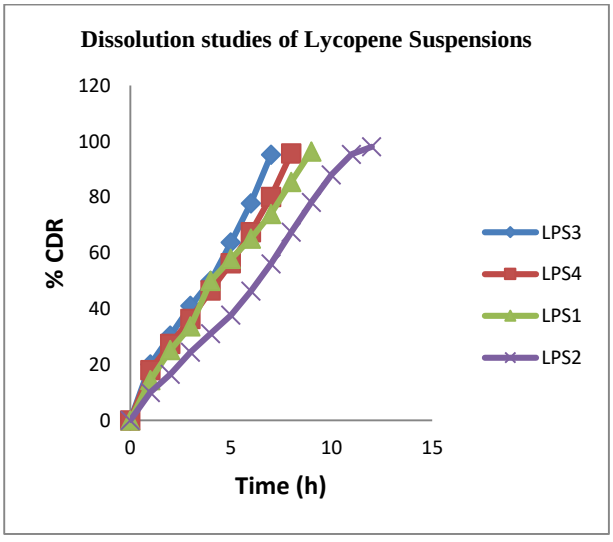

Fig 5: Dissolution studies of Lycopene Suspensions

Stability Studies: Stability studies conducted for Suspension was found to be stable throughout the period stating that formulations are stable. By enhancing its therapeutic usefulness, phytosomes demonstrated potential technology for maintaining the stability of the components that are light-sensitive. Alternative dosage forms are advised because liquid dosage forms choose to shorten their shelf life.

Table 4: Stability Studies of best formulations LPS2

Code LPS2	At 25±2º C / 65±5% RH* and At 40±2º C / 75±5% RH** for 6 months			
Month	**pH**	**Viscosity**	**%CDR at the end of 12 h**	**Remark**
1	4.50*	42.23*	98.06*	Stable
	4.60**	41.23**	98.01**	Stable
2	4.50*	42.20*	98.00*	Stable
	4.65**	41.00**	98.00**	Stable
3	4.50*	42.23*	98.00*	Stable
	4.60**	41.20**	98.00**	Stable
4	4.60*	42.40*	96.58*	Stable
	4.60**	41.21**	96.40**	Stable
5	4.60*	42.31*	96.21*	Stable
	4.65**	42.00**	96.11**	Stable
6	4.65*	42.24*	95.86*	Stable
	4.60*8	41.23**	95.12**	Stable

CONCLUSION

For the reproducible outcomes of the carried out studies, a satisfactory attempt has been made to manufacture phytosomal Oral suspension dosage forms for controlled delivery of lycopene utilising 10 mL of 1% polymers like sodium CMC and HPMC K4M. In order to include stable goods into oral liquid dosage forms, lycopene complexes with phospholipids were successfully created into phytosomes. This was a good innovation in the field of nanotechnology. The pH, specific gravity, particle size, and zeta potential of an elegant suspension, among other characteristics, were all found to be within acceptable limits. Compared to the commercially available traditional dosage form, the therapeutic concentration of the food component could be sustained for a period of 12 hours (Syrup).

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