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Research

Formulation And Evaluation Of Nanoparticles Of Tulasi (Anti-Microbial Activity)

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Abstract

Controlled drug release system is one of the most favorable technique of novel drug delivery system owing to its reproducibility and ease of formulation. Nanotechnology is very useful for controlling the drug release and thus improving the pharmacokinetic and pharmacodynamic properties of the drug. The technique improves patient compliance by reducing both dose and the frequency of administration and thus minimizing the local as well as systemic toxic effects. The aim of the present research work was to formulate and evaluate nanoparticles of *Tulasi* by using the Emulsion solvent evaporation method. Sustained release nanoparticles of *Tulasi* were prepared to increase the drug residence time in gastrointestinal tract and thus improving the bioavailability of drug. The nanoparticles were prepared by using Chitosan and Carbopol940 as polymers. Different formulations were prepared with varying concentrations of Chitosan and Carbopol940in order to achieve the optimum particle size and maximum encapsulation efficiency. The particle size of nanoparticles was found to be in the range of 0.181±0.051 nm to 0.390±0.101 nm. Drug encapsulation efficiency ranged between 58.1±0.651 percent to 82.9±1.216% with controlled drug release up to 99.29% in phosphate buffer pH 6.8, 12 hrs. FT-IR studies showed that the drug and polymers were compatible. The results ofNanoparticles indicated that optimized formulation exhibited excellent properties.Nanoparticles in the doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg were used in Wistar rats of either sex. Were measured by standard biochemical methods. Silymarin (500 mg/kg) was used as a standard drug for assessment of Anti-Microbial status.When compared with the standard Anti-Microbial agent Ciprofloxacin, The antibacterial activity of these prepared nanoparticles against pathogenic bacterium has shown. The observed results

	suggest that *Tulasi* loaded Nanoparticles could be a potential source of Anti-Microbial Activity. However, further studies are required to explore this therapeutic property of *Tulasi*.
	Keywords: Nanoparticles, Chitosan,Carbopol 940, *Tulasi* and Emulsion solvent evaporation method and Anti-Microbial Activity.

INTRODUCTION

Nanotechnology has gained huge attention over time. The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometres in size and are made up of carbon, metal, metal oxides or organic matter. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, increased reactivity or stability in a chemical process, enhanced mechanical strength, etc. . These properties of nanoparticles has led to its use various applications. The nanoparticles differs from various dimensions, to shapes and sizes apart from their material . A nanoparticle can be either a zero dimensional where the length, breadth and height is fixed at a single point for example nano dots, one dimensional where it can possess only one parameter for example graphene, two dimensional where it has length and breadth for example carbon nanotubes or three dimensional where it has all the parameters such as length, breadth and height for example gold nanoparticles.

The nanoparticles are of different shape, size and structure. It be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular and differ from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated. Numerous synthesis methods are either being developed or improved to enhance the properties and reduce the production costs. Some methods are modified to achieve process specific nanoparticles to increase their optical, mechanical, physical and chemical properties. A vast development in the instrumentation has led to an improved nanoparticle characterisation and subsequent application. The nanoparticles are now used in every objects like from cooking vessel, electronics to renewable energy and aerospace industry. Nanotechnology is the key for a clean and sustainable future.

Advantages of nanoparticles

Nanoparticles offer numerous advantages in drug delivery system. These advantages include, but are not limited:

- Nanoparticles have many significant advantage over conventional and traditional drug delivery system.
- Nanoparticles are control and sustain release form at the site of localization, they alter organ distribution of drug compound. They enhance drug circulation in blood, bioavailability, therapeutic efficacy and reduce
- Nanoparticles can be administer by various routes including oral, nasal, parenteral, intra-ocular etc.
- In the tiny areas of body nanoparticles shows better drug delivery as compare to other dosage form and target to a particular cell type or receptor.
- Due to small particle size nanoparticles overcome resistance by physiological barriers in the body and easily penetrates to cell walls, blood vessels, stomach epithelium and blood–brain barrier.
- Nanoparticle enhance the aqueous solubility of poorly soluble drug, which improves bioavailability of drug.
- As a targeted drug carrier nanoparticles reduce drug toxicity and enhance efficient drug distribution.
- By using polymers drug release form nanoparticles can be modified which makes polymeric nanoparticle an ideal drug delivery system for cancer therapy, vaccines, contraceptives and antibiotics.
- Useful to diagnose various diseases
- Enhanced stability of ingredients
- Prolonged shelf life
- Used in dental surgery also as filling the tiny holes in teeth.

Change the method of drug delivery to improve customer acceptance or reduce manufacturing costs.

MATERIALS AND METHODS

Plant collection and identification

Tulasi is an aromatic much branched erect herb with 4 angled stems, bearded nodes leaves. It is a common weed of open lands. For the present study fresh plants were collected from locality and brought to laboratory in air tight polythene bags for further processing.

Preparation of leaf extract

For the preparation of leaf extract, fresh leaves were collected in a beaker and washed several times with water to remove the dust and finally with double distilled water. 10 g washed leaves were cut into fine pieces and crushed with the help of mortar and pestle in 100 ml double distilled water. After grinding the aqueous extract was taken in 250 ml beaker and boiled for 10 min at 80 °C temperature. The plant extract was allowed to cool at room temperature and then filtered with whatman filter paper. The filtrate was centrifuged for 20–25 min at 10000 rpm, the supernatant was collected and stored at 4 °C. This filtrate was used as a stabilizing and reducing agents.

Preparation of 0.1M NaOH Solution

Dissolved 4 g Sodium Hydroxide pellets in 1000mL of Water and mixed well.

Preparation of pH 7.4 Phosphate buffer medium

Weighed and dissolved about 6.8 g of Potassium dihydrogen phosphate in 1000mL of Purified water and mixed well. Then adjusted the pH of this solution to 7.4 with 0.1 M NaOH Solution.

Analytical Method Development

Determination of absorption maxima: Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

Procedure: For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of Methanol(1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate buffer (7.4pH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 400-800 nm using double beam UV spectrophotometer. The absorption maxima were obtained at 420 nm with a characteristic peak.

Preparation of calibration curve: It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Saxagliptin was prepared in Methanoland subsequent working standards (2, 4, 6, 8 and 10 µg/mL) were prepared by dilution with phosphate buffer of pH-5.5. These solutions were used for the estimation Saxagliptin by UV method. The whole procedure was repeated three times and average peak area was calculated. Calibration plot was drawn between concentrations and peak area. Calibration equation and R^2 value are reported.

Preparation of nanoparticles

Preparation of Tulasiloaded nanoparticles

`Polymeric Nanoparticles were prepared by Emulsion solvent evaporation method. Required quantity of polymer and drug were weighed & dissolved in 12ml of dichloromethane. Quantity of Tween 80 and Span 60 was mixed with of water & this solution was kept in another beaker.Both the phases were kept for sonication for 15 min. until it become clear. Solution containing drug and polymer were added drop wise to aqueous phase under continues stirring. The formed nanoparticles suspension were homogenized at 18000 rpm for 15min then followed by magnetic stirring for 3hr. The suspension was centrifuged at 9,000 rpm for 45 min. The samples were added to glass vials & freeze-dried with mannitol 2% (w/v) as cryprotectant in a lyophilizer.

Table 1: Composition of nanoparticles formulations (F1 to F8)

Excipients	F1	F2	F3	F4	F5	F6	F7	F8
Tulasi	100	100	100	100	100	100	100	100
Chitosan	100	200	300	400	-	-	-	-
Carbopol940 %	-	-	-	-	100	200	300	400
Tween 80 (mL)	5	5	5	5	-	-	-	-
Span 60 (mL)	-	-	-	-	10	10	10	10
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Dichloromethane (ml)	10	10	10	10	10	10	10	10
Methanol	10	10	10	10	10	10	10	10

RESULTS AND DISCUSSION

Preparation of Standard Graph
Determination of absorption maxima
The standard curve is based on the spectrophotometry. The maximum absorption was observed at 215nm.

Calibration curve
Graphs of Tulasi was taken in 7.4 Phosphate buffer

Table 2: Calibration curve data for Tulasi at 215 nm

Concentrations [µg/mL]	Absorbance
0	0
2	0.128
4	0.265
6	0.381
8	0.487
10	0.619

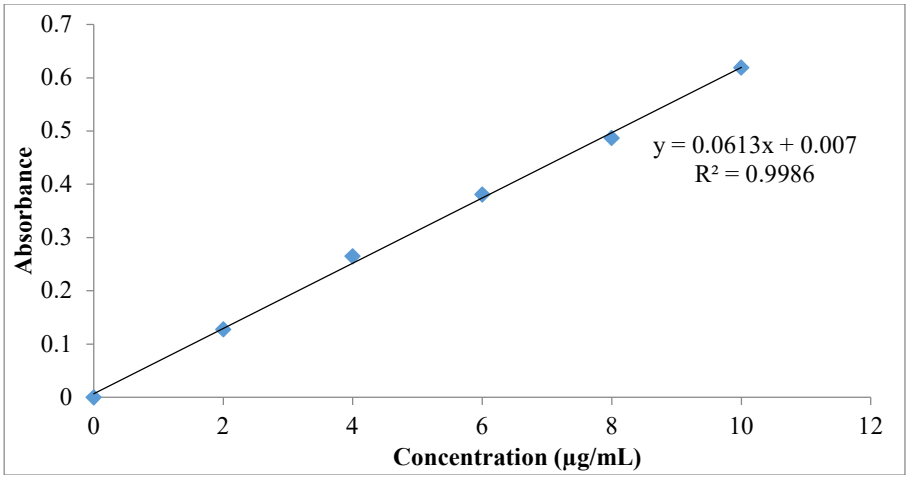

Fig 1: Standard graph of Tulasi in 7.4 Phosphate buffer

Standard graph of Tulasi was plotted as per the procedure in experimental method and its linearity is shown in Table 8.1 and Fig 8.1. The standard graph of Tulasi showed good linearity with R^2 of 0.998, which indicates that it obeys "Beer- Lamberts" law.

Evaluation oftulasi loaded nanoparticles:

Table 3: Evaluation of Nanoparticles

Batch No	Mean Particle size(nm)	%Yield	Drug Content	Drug encapsulation efficiency	PDI	Zeta Potential(mV)
F1	286.12 ± 18	68.14	93.51	63.92	0.668	-26.12 ± 1.8
F2	292.22 ± 19	71.54	95.81	72.29	1.268	-28.22 ± 1.9
F3	305.19 ± 16	75.92	97.65	80.41	1.153	-30.19 ± 1.6
F4	267.22 ± 20	75.20	91.54	76.91	0.868	-27.22 ± 2.0
F5	278.56± 18	79.81	94.82	82.83	0.577	-28.56± 1.8
F6	281.72± 23	86.34	98.84	87.92	0.309	-32.61± 2.3
F7	351.72± 23	73.92	95.14	62.79	0.498	-25.72± 2.3
F8	368.32± 42	77.69	97.14	70.30	0.385	-26.32± 2.2
F9	371.52± 32	83.44	97.82	76.98	0.325	-27.52± 2.4

Percentage yield of formulations F1 to F9 by varying drug was determined and is presented in Table. Highest drug content, Highest Entrapment efficiency observed for F6 formulation.
PDI observed in the F6 formulation i.e., 0.309 respectively. The Zeta potential range from -25.72 mV to -32.61 mV to all the formulations.

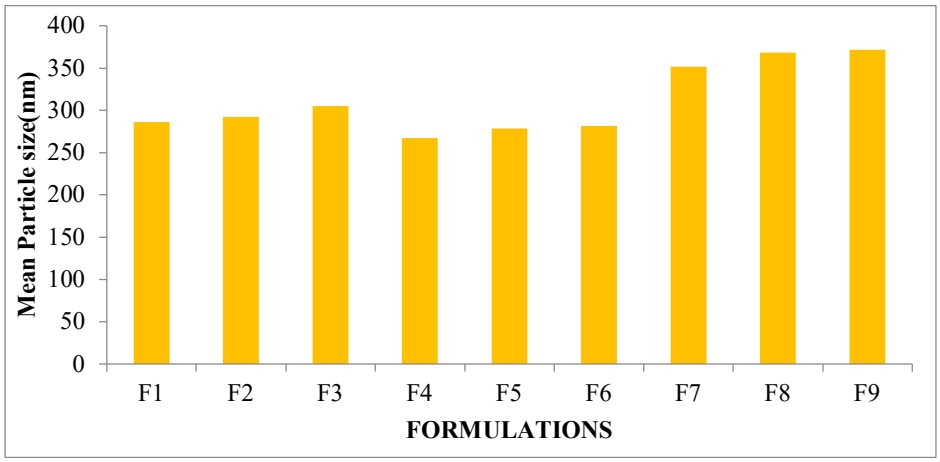

Fig 2: Mean Particle size(nm)

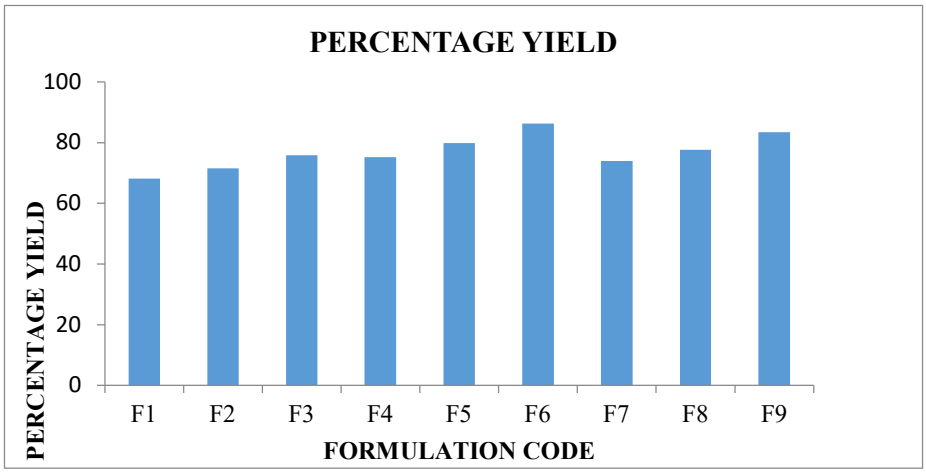

Fig 3: %Yield

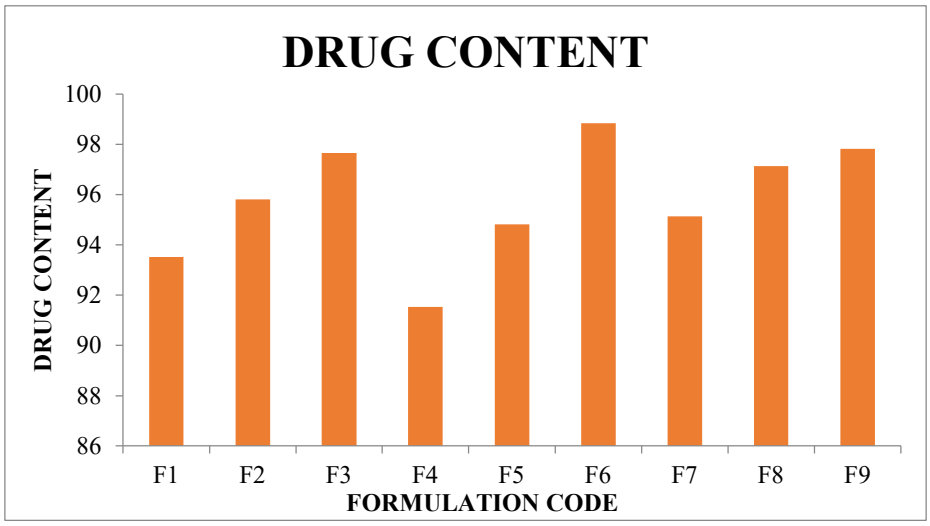

Fig 4:Drug content

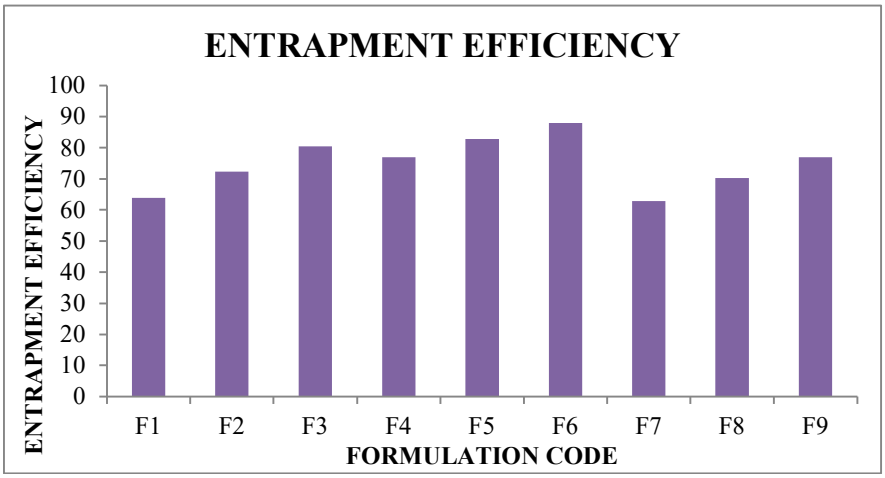

Fig 5: Drug encapsulation efficiency

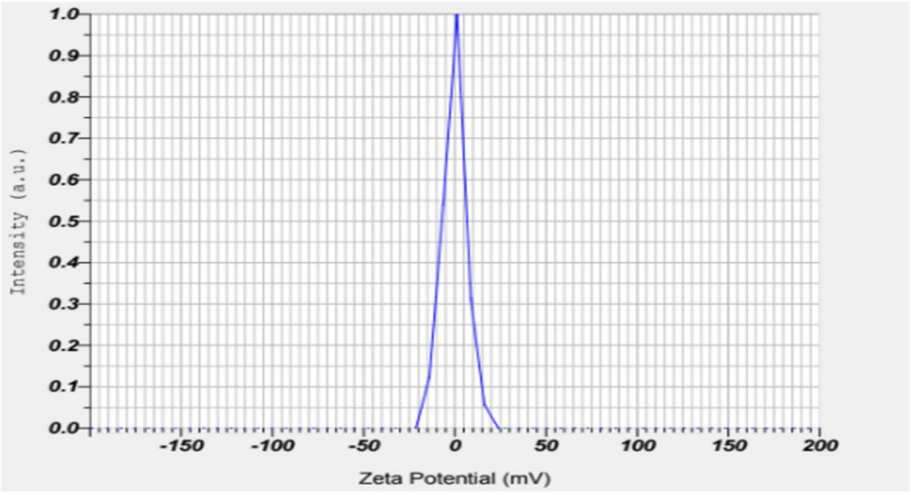

Fig 6: Zeta Potential of F6 Formulation

***In vitro* Drug release studies**

Table 4: *In vitro* Drug release studies of Tulasi

TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	27.42	29.69	32.41	27.93	16.85	22.26	20.92	17.92	14.01
2	34.39	40.09	47.69	41.62	22.76	28.78	34.36	22.65	20.08
3	47.60	46.16	58.34	48.02	30.50	35.36	42.61	33.89	31.51
4	56.51	57.65	64.61	60.47	49.11	57.23	54.53	44.32	43.98
5	67.62	65.19	70.08	66.85	61.78	66.98	61.88	52.87	50.31
6	78.37	78.67	78.39	78.68	76.89	77.46	72.46	65.90	62.57
7	85.26	81.76	84.56	87.39	83.43	85.68	81.87	73.36	67.04
8	96.78	89.54	87.98	98.77	97.14	93.14	89.29	79.77	75.91
10	99.82	95.34	93.18			98.13	98.14	90.53	83.09
12		97.54	97.14			99.37		96.91	94.91

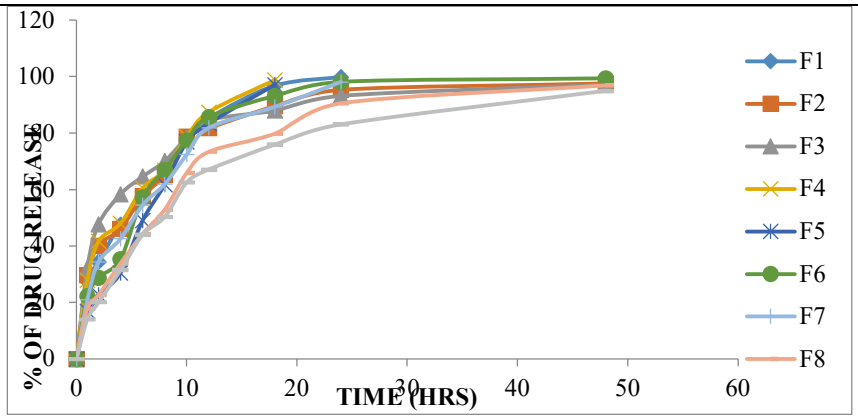

Fig 7: Dissolution study of Tulasi Nanoparticles

Hence based on dissolution data of 9 formulations, F6**Carbopol p934 (1:3)(**300**mg)**formulation showed better release (99.37%) up to 12 hours. So F6 formulation is optimised formulation.

Application of Release Rate Kinetics to Dissolution Data

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of drug release from Nanoparticles. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyer peppas mechanisms and the results were shown in below table it follows the zero order kinetics.

Table 5: Release kinetics data for optimized formulation (F6)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
22.26	1	1.000	1.348	0.000	1.891	22.260	0.0449	-0.652	77.74	4.642	4.268	0.374
28.78	2	1.414	1.459	0.301	1.853	14.390	0.0347	-0.541	71.22	4.642	4.145	0.496
35.36	4	2.000	1.549	0.602	1.811	8.840	0.0283	-0.451	64.64	4.642	4.013	0.628
57.23	6	2.449	1.758	0.778	1.631	9.538	0.0175	-0.242	42.77	4.642	3.497	1.144
66.98	8	2.828	1.826	0.903	1.519	8.373	0.0149	-0.174	33.02	4.642	3.208	1.433

77.46	10	3.162	1.889	1.000	1.353	7.746	0.0129	-0.111	22.54	4.642	2.825	1.817
85.68	12	3.464	1.933	1.079	1.156	7.140	0.0117	-0.067	14.32	4.642	2.428	2.213
93.14	18	4.243	1.969	1.255	0.836	5.174	0.0107	-0.031	6.86	4.642	1.900	2.741
98.13	24	4.899	1.992	1.380	0.272	4.089	0.0102	-0.008	1.87	4.642	1.232	3.410
99.37	48	6.928	1.997	1.681	-0.201	2.070	0.0101	-0.003	0.63	4.642	0.857	3.784

Drug – Excipient compatibility studies

Fourier Transform-Infrared Spectroscopy

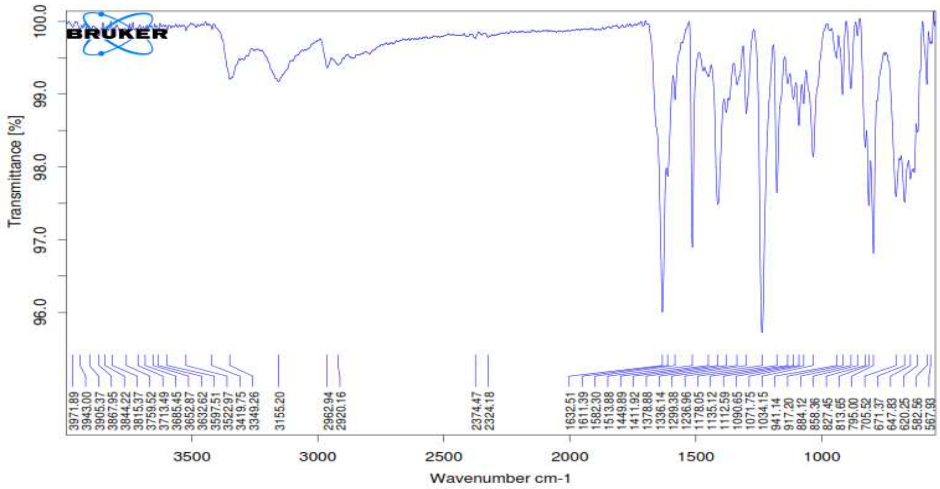

Fig 8: FT-TR Spectrum of Tulasi pure drug

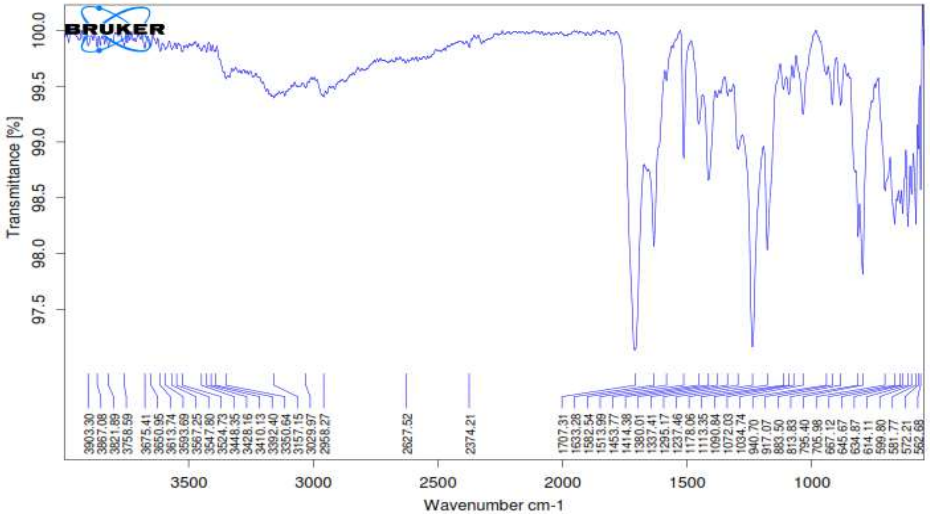

Fig 9: FT-IR Spectrum of Optimised Formulation

There is no incompatibility of pure drug and excipients. There is no disappearance of peaks of pure drug and in optimised formulation.

SEM

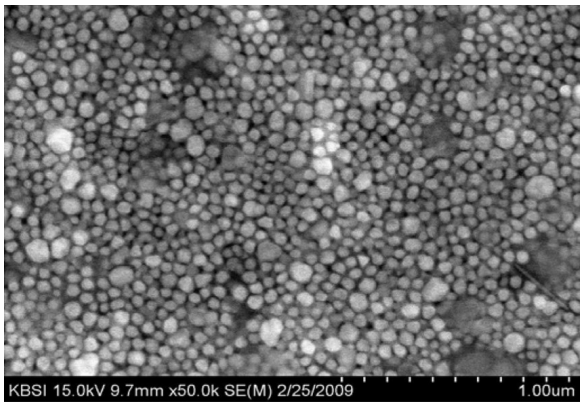

Fig 10: SEM graph of optimized formulation

SEM studies showed that the Tulasi - loaded nanoparticles had a spherical shape with a smooth surface as shown in Figure.

XRD

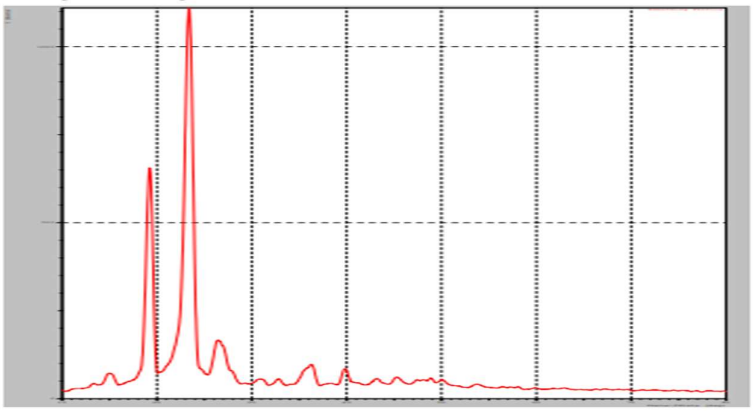

Fig 11: Tulasi F6 optimised formulation

Anti-microbialb activity

Table 6: Zone of inhibition of solvent extract of Tulasi against*E.Coli*

Compounds	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)
MEBM	6	10	12
BME	10	12	16
BMC	11	15	18
BMH	8	10	14
BMQ	**12**	**18**	**20**
CPN	12	16	20
MXN	14	18	24

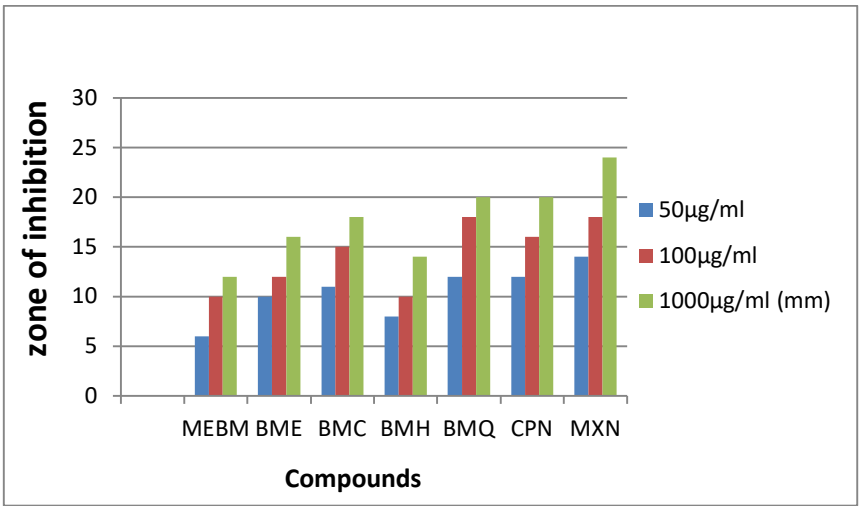

Fig 12: Zone of inhibition of solvent extract of Tulasi against*E.Coli*

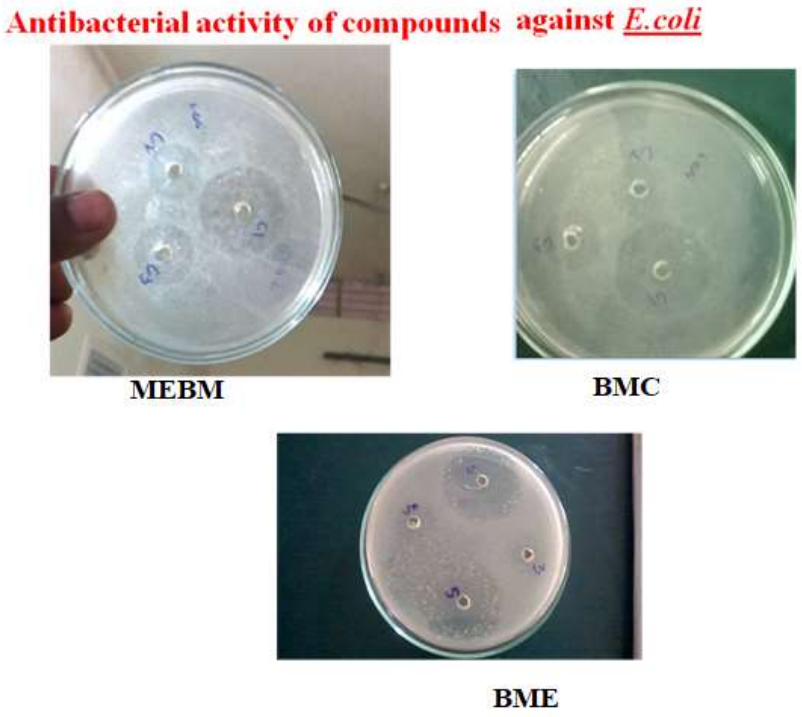

Fig 13: Zone of inhibition of solvent extract of Tulasi against*E.Coli*

Table 7: Zone of inhibition of Tulasi against on *Streptococcus aureus*

Compounds	50µg/ml	100µg/ml	1000µg/ml
MEBM	5 mm	9 mm	14 mm
BME	10 mm	12 mm	18 mm
BMC	6 mm	10 mm	15 mm
BMH	**10 mm**	**12 mm**	**14 mm**
BMQ	6 mm	10 mm	16 mm
CPN	14 mm	18 mm	22 mm
MXN	16 mm	20 mm	24 mm

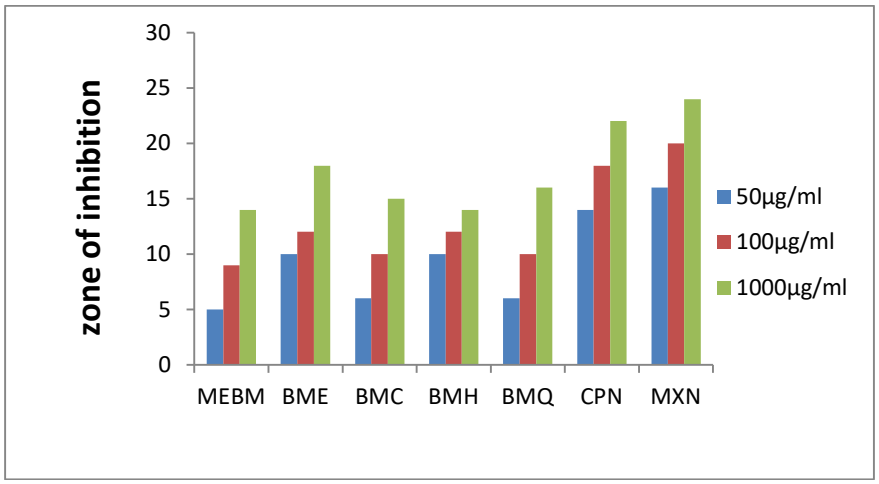

Fig 14: Zone of inhibition of Tulasi against on *Streptococcus aureus*

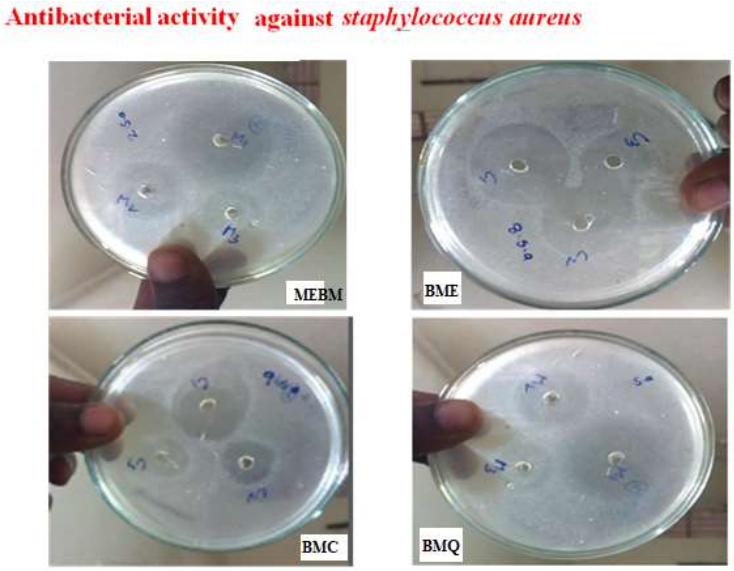

Fig 15: Zone of inhibition of Tulasi against on *Streptococcus aureus*

Antimicrobial Activity

The Extracted compounds **MEBM, BME, BMC, BMH, BMQ** has been evaluated for their antimicrobial activity against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent Ciprofloxacin as standard drug.

- All the Extracted compounds (BMC &BMQ), were found to be effective antibacterial agents.
- The compounds MEBM showed minimum activity against *E.coli*with zone of inhibition 6mm,10mm, 12mm.
- The compounds BMC showed maximum activity against Gram positive bacteria *E. Coli* at 100µg/ml with zone of inhibition 15mm. Among all those compounds, MEBM, BMC and BMQ showed Poor activity against Gram-positive (*Staphylococcus aureus)* with zone of inhibition of 5mm, 6mm and 5mm.

CONCLUSION

The method used for preparation of nanoparticles of *Tulasi* was found to be simple and reproducible. The slow and constant release of *Tulasi* from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. The developed formulation overcome and alleviates the drawbacks and limitations

of *Tulasi* sustained release formulations. The development of effective nano delivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators. On the basis of different parameters i.e. physicochemical and *in-vitro* release study, nanoparticles of batch F6 are concluded as optimum formulations. Further, it can be concluded that the nanoparticulate formulation can be an innovative and promising approach for the delivery of Rutin. The nanoparticles demonstrated significant Anti-Microbial properties in experimental animals in this study. These activities may be attributed to the various Phytoconstituents of nanoparticles such as flavonoids, tannins, saponins, alkaloids, anthocyanins, glycosides, polyphenols, and steroids. However, further experimental studies are required to explore the exact mechanism of actions and next level of clinical trials to generate novel drugs. This might prove helpful to use its immense therapeutic efficacy as a potent Anti-Microbial phytomedicine.

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