

Pharmacognostic quantification of flavonoids by high performance thin layer chromatography and *in vitro* cell line study on developed herbal formulation from *Cestrum nocturnum* plant extract

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Abstract

Background: Herbal therapy is eventually based on the knowledge and practitioner skills. In India, local plants, as well as shrubs, can be procured easily due to influence of ancient Ayurveda. Taking into due considerations, we investigated *Cestrum nocturnum* (CN) Linn. commonly known as Raat rani. The shrub has been reported to have a wide arena of ethnopharmacological relevance. **Objective:** In the current research studies, our aim was to develop controlled release herbal tablets from the CN extract followed by *in vitro* screening on colon cancer cell line and correlate the quantified phytoconstituents such as rutin and quercetin by high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC). **Materials and Methods:** The leaves were macerated in methanol. The extract after preliminary phytochemical screening was subjected to sophisticated methods such as HPTLC and HPLC to elucidate rutin and quercetin which were found to positive by thin layer chromatography. To develop herbal tablets, the extract was subjected to excipient profile compatibility which was confirmed by Fourier transmission infrared (FTIR) spectrometry. Wet granulation method was used to develop the formulation. The developed herbal tablets were screened for colon cancer targeted cell line (HCT 15) using sulforhodamine B assay method with four dose levels of different concentrations. The results were analyzed as a percentage of growth inhibition 50%. **Results:** HPTLC confirmed the presence of spots of rutin (4.15% w/w) and quercetin (10.61% w/w) in comparison to the standard at R_f value 0.47 and 0.29, respectively. HPLC chromatogram showed the presence of rutin and quercetin with retention time 5.391 min and 6.698 min, respectively, in comparison to standard at 360 nm. The FTIR revealed no interaction of drug and excipients since the peaks of both are observed to be identical. Unsatisfactory results were obtained for the formulation on the selected cell line. **Conclusion:** In future, the world needs to explore, screen and develop herbal formulations which can be found to be compatible for cancer chemotherapy.

Key words: *Cestrum nocturnum*, colon cancer, fourier transmission infrared spectrometry, HCT 15, high performance liquid chromatography, high performance thin layer chromatography, sulforhodamine B assay

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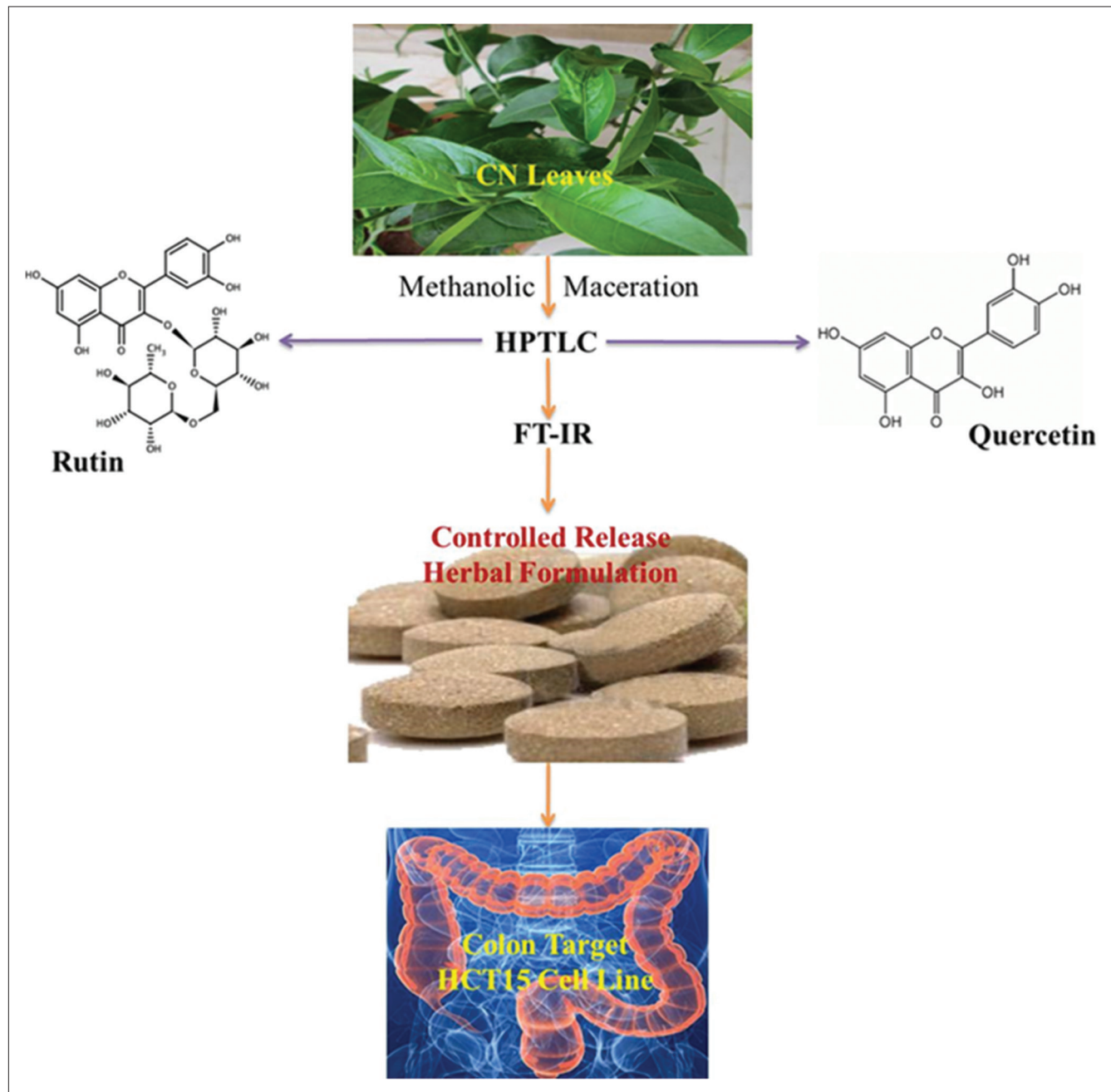
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Graphical Abstract



INTRODUCTION

Medicinal plants have been used as important sources of raw materials in the development of herbal formulations. Herbal medicines from local plants are often used to provide basic health services and the first line treatment for various ailments. Although herbal medicines used by traditional systems of medicine have been tested through long historical practice, scientific research on herbal medicines will provide additional evidence of their safety and efficacy.^[1] The local plant extracts have proved its

elucidative roles with probable ethnopharmacology potential in major illness, viz., cancer, malaria, diabetes, etc.^[2] The local plant selected for study is *Cestrum nocturnum* (CN) Linn. (Family Solanaceae) commonly known as Raat rani, lady of night, or night jessamine.^[3] It has been reported for anti-inflammatory, antidiabetic, antibacterial, and astringent potential.^[4] Herbal shrubs are useful as dietary supplements as well as in a plethora of remedies.^[5]

In recent years, analytical identification of phytoconstituents has gained a large importance. Nowadays, the isolated

constituents are purified and used in enormous treatment strategies.^[6] The general reason for the use of high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) methods was to optimize the separation and identification of phytoconstituents from the extract. Accuracy, preciseness, specificity, sensitivity, and reproducibility are some of the advantages of these powerful visualization techniques.^[7]

In recent decades, herbal products have gained high popularity.^[8] India being an agricultural hub, use of medicinal and local plants forms a unique component of Traditional Ayurvedic Healthcare System. The herbal formulation comprises complex organic chemicals including raw or processed parts of plants such as roots, seeds, stems, and leaves.^[9] Ethical issues have aroused due to synthetic nature of drugs which has enforced pharmaceutical industries to face challenges during the course of research and development. Herbal medicines play an important role in treatment of chronic diseases in turn causing health promotion. However, usage of herbal medicine increases when conventional medicine is ineffective in the treatment of disease such as advanced cancer and new infectious diseases. Furthermore, these medicines are widely perceived as natural and non-toxic.^[10,11] Thus, taking into account the current situation, the world relies heavily on the development of traditional medicine which could improve the setbacks. Herbal formulations are available in different forms such as tablets, capsules, gels, ointments, creams, suspensions, and tinctures.

The oral route is the most convenient and preferred route. Coated tablets are selected for variety of reasons such as taste masking, aesthetic appearance, control release, sustained or prolong release, and compatibility.^[12] In addition, tablets offers advantage of being economic, safe, requires no nursing, more tamperproof than other dosages like capsules, most stable and convenient in packaging, shipping, and transportation. Nowadays, as most of the industries focus on the good manufacturing practices, it is utmost important to identify the compatibility of excipients to develop the herbal formulation.

Oral control release formulations for small intestine and colon have received considerable attention in the past few years for a variety of reasons including pharmaceutical superiority and clinical benefits derived from the drug release pattern that is not achieved with traditional immediate or sustained release products.^[13] Prevention and early detection are key factors in controlling and curing colorectal cancer which is the second most preventable cancer. Early treatment can often lead to an excellent outcome. Colorectal cancer is cancer that occurs in the large intestine and rectum. Today, the average chance of developing colorectal cancer during person's life is 1 in 20. The allopathic treatments available in market have many side effects such as loss of appetite, anemia, low blood count, taste changes, mouth sores, nausea, and vomiting.^[14]

Although many allopathic anticancer agents such as 5-fluorouracil, irinotecan, capecitabine, and eloxatine are available in the market, there is still a need to develop new drugs that have activity with minimal or no side effects. As there is the minimum availability of herbal formulations in the market, newer herbal drugs mentioned in the literature can be formulated so as to have minimum side effects.^[15] Thus, considering the literature views, the article aims for the development of controlled release tablets which will be screened for *in vitro* anticancer potential on selected colon cancer cell line (HCT 15) at different concentrations using sulforhodamine B (SRB) assay method with four dose levels of different concentrations. The results will be analyzed in terms of growth inhibition 50% (GI50).

MATERIALS AND METHODS

Part A: Collection, Authentication Extraction and Preliminary Phytochemical Screening

The plant was collected from the local market in Mumbai and authenticated at Agharkar Research Institute, Pune, India, with voucher number L-075. The leaves were air dried at room temperature. The dried leaves were grinded to powder and macerated in methanol for 10 days in an extraction bottle. The bottle was occasionally stirred and finally the material was filtered through Whatman filter paper No. 1. The residue obtained was evaporated on rotary evaporator at 35°C. The extract obtained was further dried, converted to freely flowing powder by using a suction pump and subjected to preliminary phytochemical screening.^[16,17] The extract was used in developing oral controlled release tablets which were further screened for anticancer activity.

Part B: Analytical Studies

Analytical section of the research article covers details of methods studied such as thin layer chromatography (TLC), high HPTLC, and HPLC for determining the percentage of the flavonol constituent such as rutin and quercetin.

TLC^[18]

- Rutin: Mobile phase used was methanol:glacial acetic acid:formic acid:water (3:0.9:0.9:0.5)
- Quercetin: Mobile phase used was methanol:glacial acetic acid:formic acid:water (3:0.9:0.9:0.5).

The standards used were dissolved in methanol. The sample was dissolved in methanol and filtered before spotting the sample (10 mg/ml). The chamber was saturated for 30 min.

HPTLC

HPTLC was performed at Anchrom Test Lab Pvt. Ltd., Mumbai, India. The analysis was performed by application of

the standards and sample dissolved in methanol on HPTLC plate silica gel 60F254 (20 cm × 10 cm). The details of the instrument are as CAMAG Linomat 5 with 5 application parameters. Spray gas was used as inert gas; sample solvent type was methanol with dosage speed 150 nl/s and predosage volume 0.2 µl. The syringe size was 100 µl; application position Y was 8.0 mm and band length was 6 mm. Calibration parameters used were multilevel calibration mode, CV statistics mode and peak area as the evaluation mode. Formula used will be:

$$\% \text{ of constituent A or B} = \frac{\text{Area of sample} \times \text{standard dilution} \times \text{purity of gel} \times 100}{\text{Area of standard} \times \text{sample dilution}}$$

Spots of extract (20 µg/ml) and standard rutin (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 µg/ml) and standard quercetin (0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 µg/ml) were applied on the plates. The scanning of developed plates was carried out, and the images were observed under ultraviolet (UV) visible wavelength of 254 nm but well-resolved spots were obtained only after derivatization at 540 nm.

HPLC

HPLC analysis was performed at Indian Institute of Technology, Mumbai, India. The instrument used was Varian GC/MS with software as Chromtech N 2000 data with a detector type as 335, having a flow rate of 1.5 ml/min and run time of 10 min. The injection volume was 20 µl and column dimensions were RP C-18, 250 mm × 4.6 mm, 5 µ. Acetonitrile and water (50:50) was used as mobile phase. The sample was prepared by taking 100 µg of standard and sample dissolved in 1 ml solvent. From this stock solution, 20 µl was injected.

Part C: Fourier Transmission Infrared (FTIR) Spectroscopy

Physical mixtures of herbal extract and various excipients were mixed in specific ratio. The ratios were I (CN

extract:diluent [1:1] such as lactose and microcrystalline cellulose [MCC] 101); II (CN extract:superdisintegrant [1:0.5] like crosspovidone); III (CN extract:binder [1:0.5] corresponding to polyvinylpyrrolidone K 30 [PVP K30]); IV (CN extract:lubricant [1:0.5] such as aerosil, MCC 102, talc and Mg stearate) and kept under specified storage conditions such as dry and wet closed vials at 25°C/60% RH and 40°C/75% RH for 1 month. They were evaluated for color change, flowability, and moisture uptake. To check the compatibility, FTIR scanning was performed at Vivekanand Education Society's College of Pharmacy, Mumbai, India. Shimadzu instrument of model IR infinity and software of IR was used. IR spectrum of the extract and excipients was done using FTIR spectrophotometer. The spectra were scanned over the wavelength region of 4000-550/cm at resolution of 4/cm.

Part D: Development of Controlled Release Herbal Formulation

The controlled release formulation comprises of CN (150 mg) extract and excipients like crosspovidone, sodium starch glycolate (superdisintegrants); lactose, MCC 101 (diluent); talc and magnesium stearate (lubricant) and aerosil, MCC 102 (glidant). Table 1 shows the composition of various batches of formulations. All the batches contain lactose and MCC 101 in the ratio 70:30. The steps followed for the preparation of the formulation are as follows:

1. Sieving: All ingredients were first sieved through #40 mesh followed by sieving of extract through #60 mesh
2. Dry mixing: All the ingredients were mixed in increasing order of weight and blended for 5 min
3. Granulation: Hydro-alcoholic wet granulation method was used for granulation. Isopropyl alcohol was used as alcoholic solvent and PVP K30 was used as binding agent. Isopropyl alcohol was added in solution till the granules of required consistency were formed. Granulation was done manually
4. Drying: The wet granules were dried in tray dryer at temperature of 50°C ± 5°C until completely dry

Table 1: Composition of batches of controlled release formulation of CN extract

Ingredients	Quantity per tablet (mg)							
	F1	F2	F3	F4	F5	F6	F7	F8
Active ingredient	150	150	150	150	150	150	150	150
Lactose	331	352	352	373	390	381	360	351
MCC 101	142	151	151	160	167	163	155	152
Crosspovidone	18.75	18.75	3.7	3.75	1.5	7.5	1.5	7.5
PVP K30	52.5	22.5	52.5	22.5	30	30	45	45
MCC 102	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Aerosil	20	20	20	20	20	20	20	20
Talc	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Magnesium stearate	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Total	750	750	750	750	750	750	750	750

PVP K30: Polyvinylpyrrolidone K 30, MCC: Microcrystalline cellulose, CN: *Cestrum nocturnum*

5. Milling and sifting: The dried granules were sifted through #22 mesh and retained on #40 mesh
6. Lubrication: MCC 102, aerosil, talc, and magnesium stearate were sifted through #60 mesh. The sifted ingredients were mixed with prelubricated blend for 5-10 min using polythene bag
7. Compression: The blend was compressed on single punch compression machine using punch size 16 mm caplet shape.

The developed herbal formulation was optimized using design expert 8.0 software for batch optimization. The strategy followed was 2² factorial design. PVP K30 (binder) and CP (superdisintegrant) were the two excipients considered for the design. The design was applied to disintegration (range: 4-10 min) and dissolution (95-105%) studies as per IP.

Part E: Evaluation of the Developed Herbal Formulation

Tablet evaluation parameters such as pre- and post-compression as well as dissolution profile were carried out as per guidelines described in Indian Pharmacopoeia.^[19]

Pre-compression parameters

Various parameters of lubricated granules such as angle of repose, bulk and tapped density, compressibility index, Hausner ratio, and loss on drying were checked. Granules were evaluated for following parameters:

- a. Angle of repose – The angle of repose gives an indication of the flowability of the substance. The funnel was adjusted such that the stem of the funnel lies 2 cm above the horizontal surface. The drug powder was allowed to flow from the funnel under the gravitational force till the apex of the pile to just touch the stem of the funnel. Height of the pile was taken as 2 cm drawing boundary along the circumference of the pile and taking the average of six diameters determined the diameter of the pile. These values of height and diameter were then substituted in the following equation:

$$\text{Angle of repose} = \tan^{-1} \frac{h}{r}$$

Where, h - height of pile and r - radius of pile

- b. Untapped bulk density – Powder weighing 10 g was placed into 50 ml measuring cylinder. Volume occupied by the powder was noted without disturbing the cylinder, and bulk density was calculated by the following equation.

$$\text{Untapped bulk density} = \frac{\text{Mass of the bulk granules}}{\text{Volume of the bulk granules}}$$

- c. Tapped density – Powder weighing 10 g was placed into 50 ml measuring cylinder. The cylinder was then subjected to a fixed number of taps (50 in this research)

until the powder bed volume had reached the maximum level. The final volume was recorded, and the tap density was calculated by the following equation.

$$\text{Tapped bulk density} = \frac{\text{Mass of tapped granules}}{\text{Volume of tapped granules}}$$

- d. Compressibility index – Compressibility index was determined by using following equation.

$$\% \text{ Compressibility} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

- e. Hausner ratio – Hausner ratio was determined by using following equation:

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

- f. Loss on drying – Loss on drying was determined by IR moisture balance using following equation:

$$P' = \frac{100P}{100 - P}$$

Where, P = % of moisture lost by the sample, P' = % of moisture on dry basis.

Post-compression parameters

- a. Hardness – It was measured using Monsanto hardness tester
- b. Thickness – It was measured using Vernier caliper
- c. Weight variation – The average weight of tablets was calculated, and then, each tablet was weighed individually. These weights were compared with the average weight
- d. Friability – Friability testing was carried out using Roche friability tester. Since the weight of a tablet was 750 mg, a sample of 7.5 g of tablets was taken

$$\% \text{ Friability} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

- e. Disintegration time – Distilled water at 37°C ± 2°C was used as disintegration medium for uncoated tablets. For coated tablets phosphate buffer of pH 1.2, pH 6.8 and pH 7.4 were used for 2 h, 3 h, etc. as disintegration medium. The time taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured. The test was performed using disintegration test apparatus IP Type I Paddle for uncoated tablets and IP Type II Basket for coated tablets. Basket speed was 100 rpm, tablet strength used was 150 mg active ingredient, volume of dissolution was 900 ml, and the detection was done at 267 nm. Each time volume of sample removed was 5 ml with a sampling profile of 5-60 min for uncoated tablets (5 min interval) and 30-360 min (30 min interval) for coated tablets. The volume of sample withdrawn was replaced by the same volume of fresh dissolution medium maintained at the

same temperature. The sample removed was analyzed by UV spectrometry.

Part F: Coating of Developed Herbal Formulation

Eudragit S100 pH dependent polymer was used for the controlled release targeted drug delivery to colon. The following procedure was followed:

Selection of solvent mixture

Solvent mixture for Eudragit S-100 was selected on the basis of drop test. A drop of water was placed on the tablet and time taken for tablet to absorb was recorded. Therefore, organic coating for gastrointestinal targeting was used.

Preparation of coating solution

The standard high sheer overhead stirrer was used for suspension of Eudragit S100. Eudragit powder was added slowly into 50% of the diluent mixture and stirred until the polymer completely dissolved. Talc and ethyl citrate was added in the remaining diluent mixture and stirred for 10 min in high shear mixture. While stirring, the excipient suspension was also poured slowly into the Eudragit solution. Finally, spray suspension was passed through a 0.5 mm sieve.

Procedure for coating

Coating of the tablet was carried out in conventional coating pan (Newtech) with the pan rpm set at 12. The temperature of the inlet air was set at 55°C. Tablets were loaded along with the placebo tablets for pre-heating for 10 min and the average weight of 20 tablets were noted before coating. During the coating process, the spray gun nozzle was frequently checked for proper spraying. The appearance of tablets was checked for smoothness and intactness of edges. After completion of coating procedure, the tablets were checked for final weight gain. The coated tablets were dried for 10 min at the inlet air temperature and then gradually allowed to cool down to the room temperature. The dried coated tablets were unloaded and stored in controlled conditions.

Part G: In Vitro Screening of the Developed Herbal Formulation for Colon Cancer Cell Line HCT 15^[20,21]

All the cell cultures and media used in studies comprised human colon cancer cell Line HCT 15, human breast cancer cell line MCF7, and human leukemia cell line MOLT4. Standard drug adriamycin (doxorubicin) was procured and maintained at ACTREC, Kharghar, Mumbai, during the studies. All the chemicals were procured from local suppliers throughout the experimentation with the help of ACTREC, Kharghar, Navi Mumbai.

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present

screening, cells were inoculated into 96-well microtiter plates in 100 μ l at plating densities depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. CN extract was initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen before use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400, and 800 μ g/ml with complete medium containing test extracts. Aliquots of 10 μ l of these different drug dilutions were added to the appropriate microtiter wells already containing 90 μ l of the medium, resulting in the required final drug concentrations, i.e. 10, 20, 40, and 80 μ g/ml. A positive control used in experimentation was adriamycin (doxorubicin), and each experiment was repeated thrice.

After addition of the extract and the standard drug, plates were incubated at standard conditions for 48 h and the assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by gentle addition of 50 μ l of cold 30% w/v TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. SRB solution (50 μ l) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and the plates were incubated for 20 min at room temperature. After staining, the unbound dye was recovered and the residual dye was removed by washing 5 times with 1% acetic acid. The plates were air-dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells. Using the six absorbance measurements (time zero [Tz], control growth [C] and test growth in the presence of drug at the four concentration levels [Ti]), the percentage growth was calculated at each of the extracts and standard drug concentration levels. Percentage GI was calculated as:

$$[\text{Ti}/\text{C}] \times 100\%.$$

RESULTS AND DISCUSSION

Part A: Extraction Yield

The extraction yield of methanolic extract of CN leaves was found to be 14.44% w/w. The extract was found to be present positive for alkaloids, glycosides, carbohydrates, steroids, flavonoids, tannins, and saponins as depicted in Table 2.

Table 2: Optimization of formulation by design of Expert Software 8

ANOVA for selected factorial model						
ANOVA table (Partial sum of squares – Type III)						
Sources	Sum of squares	Difference	Mean square	F value	P value	P>F
Model	28.39	3	9.46	3262.83	<0.0001	Significant
A-PVP	1.54	1	1.54	531.32	<0.0001	Significant
B-CP	19.10	1	19.10	6586.77	<0.0001	Significant
AB	7.74	1	7.74	2670.39	<0.0001	Significant
Pure error	0.023	8	2.900E-003			
Corrected total	28.41	11				

PVP K30: Polyvinylpyrrolidone K 30

Part B: Analytical Studies

TLC

By comparing with the standard, the extract was found to be showed the presence of rutin and quercetin at R_f values 0.78 and 0.60, respectively.

HPTLC

The extract showed the presence of the rutin at tracks 9 and 10 in comparison to standard tracks 1 to 8 and quercetin at tracks 1 to 6 in comparison to standard at tracks 7 and 8. Rutin and quercetin showed R_f values at 0.47 and 0.29 with area of 7776.5 and 15218.2, respectively. According to the formula mentioned by Doshi *et al.*, rutin (4.15% w/w) and quercetin (10.61% w/w) were found to be present in the extract. The details are depicted in Figure 1.

HPLC

CN extract showed well resolved peak at 360 nm. Rutin and quercetin showed retention time of 5.391 and 6.698 in comparison with the standard as 5.401 and 6.700, respectively. The chromatogram depicts the details as Figure 2 (A - Rutin, B - Quercetin and C - CN extract).

Part C: FTIR Spectroscopy

No color change was observed and all the excipients mentioned in the specific ratio in the experimental design passed the drug excipient compatibility and were taken further for formulation. FTIR of herbal extract and excipients exhibited peaks at similar spaces. This shows that there was no interaction between drug and excipients.

Part D: Development of Controlled Release Herbal Formulation

As mentioned in Table 2 and Figure 3 using design expert software 8 the results were interpreted. The results obtained imply that the model $F = 3262.83$ was found to be significant. " $P > F$ " values < 0.0500 indicate that model terms were significant. In

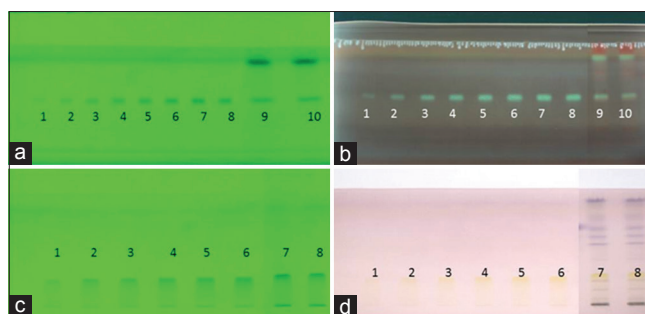


Figure 1: High performance thin layer chromatography fingerprint of rutin (a) Before derivation, (b) after derivation, and quercetin (c) before derivation, (d) after derivation

this case, A and B are significant model terms. Values >0.1000 indicate that the model terms were not significant.

Part E: Evaluation of the Developed Herbal Formulation

Pre-compression parameters

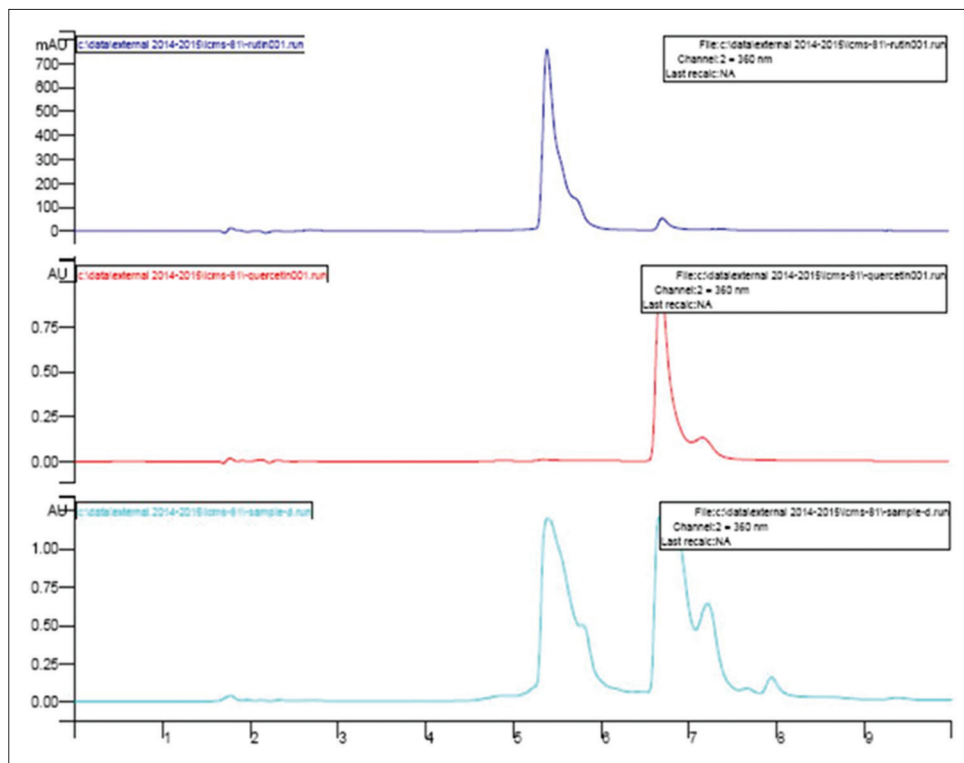
The results of pre-compression parameters such as angle of repose, bulk density, tapped density, compressibility index, Hausner ratio, and limit of detection of batches are shown in Table 3. The bulk density of all batches was found to be in the range of 0.337-0.372 g/ml. Tapped density of all batches was found to be in the range of 0.402-0.454 g/ml. Angle of repose showed good flow property for the developed granules. Compressibility index result obtained was found that batches F1, F2, F3, F6 showed fair flow while batches F4, F5 showed good flow property. In the case of Hausner ratio, batch F5 showed good flow while other batches showed fair flow. Loss on drying of all the batches was in the range of 2.39-2.87.

Post-compression parameters

The results of post-compression parameters such as hardness, thickness, weight variation, and friability for all the batches are given in Table 4. All the batches showed hardness in the range of 4-6 which were acceptable. All the developed herbal formulations from the extract showed average thickness of 5.4 mm. The maximum weight variation was found to

Table 3: Results of pre-compression parameters of batches (F1-F6)

Batch No.	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (°)	Compressibility index (%)	Hausner ratio	Loss on drying (%)
F1	0.372	0.454	32.54	18.06	1.22	2.39
F2	0.349	0.420	34.21	16.90	1.21	2.59
F3	0.348	0.432	33.28	19.44	1.24	2.74
F4	0.337	0.406	31.87	15.99	1.20	2.83
F5	0.341	0.402	34.72	15.17	1.17	2.67
F6	0.362	0.438	31.53	17.35	1.20	2.87

**Figure 2:** High performance liquid chromatography chromatogram of *Cestrum nocturnum* extract

be within the prescribed limits of I.P. Friability of all the formulations was found to be <1%. All batches showed disintegration time within the limit, out of which batch F5 showed least disintegration time. The result of disintegration time in minutes and % drug content are shown in Table 5. All the batches showed satisfactory results of the formulation. *In vitro* release profile of F5 was 97.7% [Figure 4] and disintegration time was less than the other batches. Hence, F5 was selected as optimized batch.

Part F: Coating of Developed Herbal Formulation

The result for coating done on the developed herbal CN tablet is shown in Table 6. *In vitro* drug release of the coated table is depicted in Figure 5. Results show that there is no significant

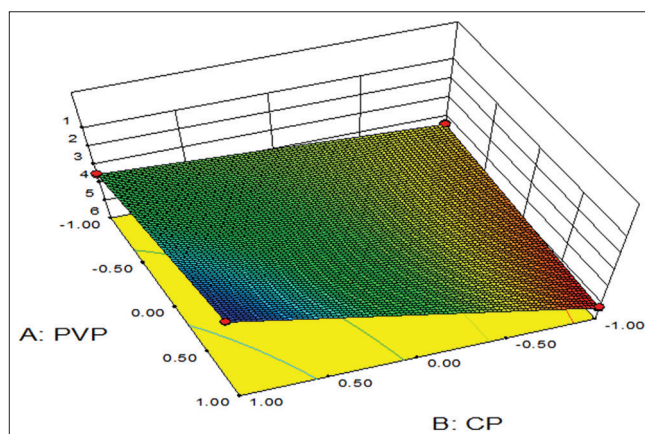
**Figure 3:** Optimization of formulation by design of Expert Software 8

Table 4: Results of post-compression parameters of batches (F1-F6)

Batch No.	Hardness (kg/cm ²)	Thickness (mm)	Diameter (mm)	Weight variation (mg)	Friability (%)
F1	5.5	5.4±0.02	19	750±4	0.64
F2	5.5	5.4±0.02	19	750±4	0.59
F3	5.5	5.4±0.02	19	750±3	0.67
F4	5.5	5.4±0.02	19	750±4	0.54
F5	5.5	5.4±0.02	19	750±3	0.66
F6	5.5	5.4±0.02	19	750±4	0.62

Table 5: Disintegration time in minutes and % drug content of all the batches

Batch No.	Disintegration (min)	Drug content %
F1	7:10	92.1
F2	6:28	96.3
F3	5:50	95.3
F4	6:28	91.8
F5	5:40	96.4
F6	7:45	94.7

Table 6: Results of the coated batch F5

Evaluation parameters	Uncoated tablet (F5)	Coated tablet (F5)
Weight (mg)	750±4	779±3
Thickness (mm)	5.4±0.1	5.65±0.1
Hardness (kg/cm ²)	5.5	6
Friability (%)	0.66	0.2
% Drug content	96.4	95.7
% Drug release	97.7	96.2

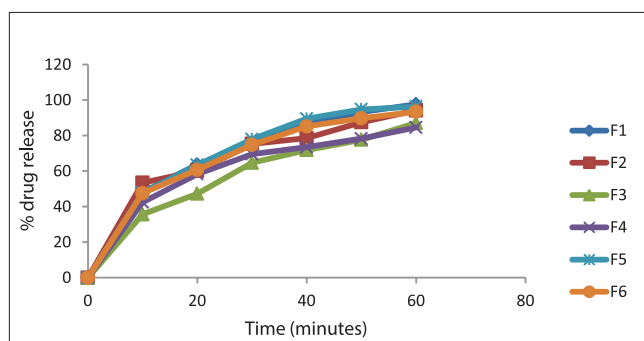
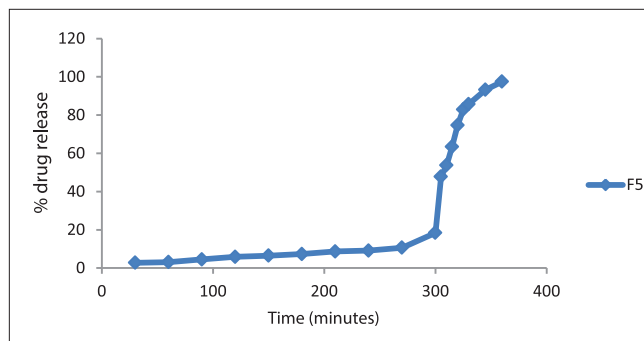
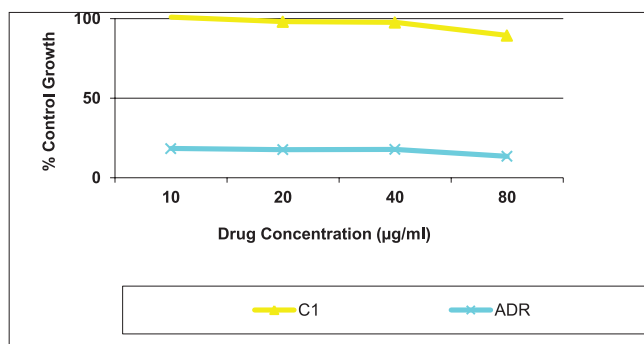
difference between coated and uncoated tablets. Optimized coated batch F5 was kept for stability studies as per ICH guidelines.

Part F: *In vitro* Screening of the Developed Herbal Formulation for Colon Cancer Cell Line HCT 15

Unsatisfactory results were obtained for the selected concentrations of the formulation when subjected to *in-vitro* screening on human colon cancer cell line HCT 15 in comparison to the standard adriamycin (doxorubicin). The results are depicted in Tables 7 and 8 and Figure 6.

CONCLUSION AND FUTURE PROSPECTS

From these research studies, we have concluded that there were no significant interactions among CN extract and excipients and found to be compatible with each other. The developed controlled release formulation from extract passed the evaluations parameters satisfactorily and gave

**Figure 4:** *In vitro* release profile of batches**Figure 5:** *In vitro* release profile of coated tablets**Figure 6:** *In vitro* screening of the developed herbal formulation for colon cancer cell line HCT 15

good results. The studies target for colon cancer for the specific cell line was found to give negative results but can be tried for another organ as well as different cell lines. Stability studies are needed to be planned out as per ICH guidelines for designing safe, stable and effective herbal dosage form.

Table 7: Human colon cancer cell line HCT 15, % control growth and drug concentrations ($\mu\text{g/ml}$)

Details	Experiment 1				Experiment 2				Experiment 3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
C1	100.0	98.1	99.0	88.9	100.0	95.4	95.7	90.3	102.6	100.8	98.0	89.0	100.9	98.1	97.6	89.4
ADR	18.1	18.2	18.6	10.9	18.3	17.4	17.9	13.2	18.8	17.5	17.0	16.3	18.4	17.7	17.9	13.5

Table 8: Human colon cancer cell line HCT 15, % control growth and drug concentrations ($\mu\text{g/ml}$)

Details	Drug concentrations ($\mu\text{g/ml}$) calculated from graph		
	LC ₅₀	TGI	GI ₅₀
HCT 15 cell line of colon cancer			
CN (C1)	>80	>80	>80
ADR - Adriamycin (doxorubicin)	>80	>80	<10

LC₅₀: Lethal dose 50%, TGI: Total growth inhibition, GI₅₀: Growth inhibition 50%

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