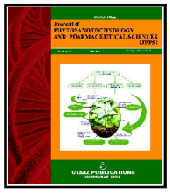
Journal of Phytonanotechnology and Pharmaceutical Sciences 1(1): 24-31, 2021

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.21276/jpps.2021.1.1.5

Journal of Phytonanotechnology and Pharmaceutical Sciences



Original article: Open access

Synthesis, characterization and anticancer activity of rutin loaded chitosan nanoparticles

M.D. Imad Uddin◆, K. Saivani, K.V. Akhil, K. Nandini, K. Sandhya and Faizan Sayeed*

Department of Pharmacology, Pulla Reddy Institute of Pharmacy, Hyderabad-502313, T.S., India

**Department of Pharmaceutics, Mesco College of Pharmacy, Hyderabad-500006, T.S., India*

Article Info

Article history

Received 1 February 2021
Revised 17 March 2021
Accepted 19 March 2021
Published online 30 March 2021

Keywords

Rutin
Nanoparticles
Chitosan
Anti cancer activity
Ovarian cancer

Abstract

Rutin, a potent antioxidant has been reported with anticancer activity. Our study aims to prepare rutin loaded chitosan nanoparticles (RCNPs) by ionic gelation method. These nanoparticles are characterized by fourier transform infrared spectroscopy (FTIR), X-ray diffraction spectroscopy (XRD), scanning electron microscopy (SEM), and zeta potential. FTIR spectra of RCNPs showed the presence of different functional groups of chitosan and also the peaks corresponding to functional group of rutin indicating successful encapsulation of rutin in chitosan nanoparticles. Debye-Scherrer equation was applied for XRD data and RCNPs size was found to be 32.15 nm. SEM image revealed the porous and rough morphology of the surface of RCNPs. Zeta potential was found to be +0.68 mV indicating stability of nanoparticles. Entrapment efficiencies (EE %) was found to be 68.7%, Loading efficiencies (LE %) was found to be 50.8%, % yield of RCNPs was found to be 31.08 %. Based on the above test values, RCNPs were further subjected to anti-proliferative activity by MTT assay on different cancer cell lines, *viz.*, HeLa (Cervical cancer cells), MDAMB231 (Breast cancer cells), Panc-1 (Pancreatic cancer cells), PC3 (Prostate cancer cells), SK0V3 (Ovarian cancer cells). The results showed that rutin and RCNPs have anti-proliferative effect on SK0V3 cell line only and effects of RCNPs were greater when compared to that of pure rutin. On this basis, we can suggest that rutin loaded chitosan nanoparticles have promising therapeutic role in cancer treatment.

1. Introduction

Cancer is a second leading cause of death and includes 277 various types (Hassanpour and Dehghani, 2017). Cancer cells are characterized by abnormal growth and uncontrolled division (Balachandran and Govindarajan, 2005). Main treatment option is chemotherapy, surgery, radiotherapy, and hormone therapy (Shihabul *et al*., 2018). Alopecia, anemia, exhaustion, and nausea are the major side effects. Moreover, therapeutic application of above said options was also decreased due to high toxicity, high cost, and low efficacy (Shaûei *et al*., 2015). In this context, scientists all over the world are focusing on plant based therapies. Considering the significance of all phytoconstituents, in particular flavonoids play a vital role in management of life threatening diseases. A flavonoid 7-methyl gallic acid has a role in treatment of neurodegenerative related disorders (Pugazhendhi *et al*., 2018). Other flavonoids such as rutin, vinca alkaloids, etoposides, *etc*., are used in the treatment and management of cancer, as compounds derived from plants are more tolerant, non-toxic to normal human cells (Mariyappan *et al*., 2018). Majority of flavonoids are polar compounds and water soluble in nature which limits their absorption. Two are the important factors which decrease their bioavailability, first factor is molecular size which blocks their passive absorption and second factor is weak lipid solubility which restricts their entry through phospholipid layer. Hence, there is a challenge to increase bioavailability of these flavonoids, in order to increase their therapeutic efficacy (Khan *et al*., 2013; Li *et al*., 2015; Sepahvand *et al*., 2014).

Rutin is also known as quercetin-3-rutinoside (Mauludin *et al*., 2009) which is commonly found in tea, passion flower, apple, and buckwheat (Harborne, 1986). It is reported with many pharmacological activities, *viz*., cardioprotective (Annapurna *et al*., 2009), neuroprotective (Pu *et al*., 2007), anticonvulsant activity (Nieoczym *et al*., 2014), antidepressant effects (Machado *et al*., 2008), analgesic (Rylski *et al*., 1979), antinociceptive effects (Selvaraj *et al*., 2014), and anticancer effects (Alonso-Castro *et al*., 2013). It is reported to possess anticancer potential against HL-60 human leukemia cells (Lin *et al*., 2012), LAN-5 human neuroblastoma cells (Chen *et al*., 2013), colorectal cell (Araujo *et al*., 2011), OVCA 433 ovarian cancer cells, (Scambia *et al*., 1990) and pancreatic cancer cells (Mouria *et al*., 2002).

Taking in to consideration of different pharmacological activities of rutin and its action profile against cancer cells many studies are conducted to increase its clinical use by increasing its bioavailability (Imad Uddin *et al*., 2020). Rutin loaded nanophytosomes was prepared with better antioxidant potential and bioavailability (Hooresfand *et al*., 2015). Targeted delivery to brain for treating

Corresponding author: Mr. M.D. Imad Uddin
Associate Professor, Department of Pharmacology, Pulla Reddy Institute of Pharmacy, Hyderabad-502313, T.S., India
E-mail: imadpharmal11@gmail.com
Tel.: +91-8374175556

cerebral ischemia was reported with rutin loaded chitosan nanoparticles (Ahmad *et al.*, 2016). Chitosan nano formulations reported a key role in increasing bioavailability of many plant derived drugs. This is a polymer obtained by the de-acetylation (Ravi Kumar, 2001) of chitin which is a fibrous substance present in crab's exoskeleton, scales of fish, and cell walls of fungi (Shu and Zhu, 2002). Majority of biomedical uses of this polymer is due to its non-toxic, biodegradable, mucoadhesive, and biocompatible applications (Shanmuganathan *et al.*, 2019). Moreover, it has the ability to attach to mucosal layer and fleetingly opens epithelial tight junctions (Qian *et al.*, 2006). Based on above discussion, present work was designed to encapsulate rutin in chitosan to increase its therapeutic efficacy by improving its bioavailability.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and cell lines

Acetic acid, sodium hydroxide, DMSO (S.D. Fine Chemical Limited), sodium tripolyphosphate (STTP) (Qualikems Fine Chemical Limited), ethanol, rutin (NR Chemicals). Chitosan with MW= 68 kDa and Deacetylaton degree = 90% from Chemsworth Suppliers Pvt Ltd. HeLa, MDAMB231, PC3, SKOV3, Panc-1 cell lines, Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI), Fetal Bovine Serum (FBS), 3-(4,5-dimethyl-2-yl)-2,5-diphynyltetrazoliumbromide (MTT) are obtained from American Type Culture Collection, United States. Sodium pyruvate, Bovine insulin is procured from Sigma Aldrich.

2.1.2 Equipments and Instruments

Sensitive weighing balance (Citizen scale-CY220; India), pH Meter (Digisun Electronics-India), Sonicator (Citizen scale-CUD25; India), Magnetic stirrer (REMI-1MLH; India), Rapid cooling centrifuge (Remilabworld-CM12; India), Refrigerator (Whirlpool-DC20045; India), Hot air oven (Lab care-TC344; India) UV spectrophotometer (LabIndia-T60; India), FTIR (BRUKER-ALPHA; USA), XRD (SCHIMADZU-XRD7000; Japan), Zeta potential (MALVERN-MAL1004428; United Kingdom), SEM (Hitachi-3000N; Japan), Multimode plate reader (Perkin Elmer- HH35000500; USA).

2.2 Methods

2.2.1 Synthesis of RCNP's

1mg/ml chitosan solution of pH 5.0 (adjusted with NaOH (0.1M conc)) was prepared by using 1% CH_3COOH. 1mg/ml STTP solution of pH 5.0 was prepared by using double distilled water (DDW). 17.5 ml of chitosan solution containing rutin (4 mg/ml) was kept in a volumetric flask on a magnetic stirrer with a speed of 200 rpm at room temp. To this solution, 9 ml of above made STTP solution was added drop by drop. Above solution is kept on overnight stirring for the formation of nanoparticles. After 24 h, solution is subjected for centrifugation at 8000 rpm at 4°C for 20 min. RCNPs are deposited at the base of centrifugation tube as a pellet which was washed thrice with 10% aqueous ethanol. Finally, pellet was stored at 4^0C after resuspending in 10% aqueous ethanol (Patil *et al.*, 2010).

2.2.2 EE %, LE % and % yield of RCNP's

3 mg of synthesized RCNP's was dissolved in 10% DDW and sonicated for 10 min at amplitude of 40%. Above solution is centrifuged for 15 min at a speed of 3000 rpm and at a Temp. of 4°C. Amount of rutin released from RCNPs in supernatant was estimated by measuring absorption at 354 nm by using UV visible spectroscope. Standard curve of pure rutin was obtained by taking absorbance of different concentrations of rutin, *viz.*, 100, 200, 300, 400 and 500 µg/ml at 354 nm by using UV visible spectroscope (Anitha *et al.*, 2011).

$$EE\% = \left(\frac{\text{Total amount of rutin with in the pellet}}{\text{Intial amount of rutin for loading studies}}\right) \times 100$$

$$LE\% = \left(\frac{\text{Total amount of rutin trapped with in the pellet}}{\text{Yield of RCNP's}}\right) \times 100$$

%Yield = (W1/W2) 100

where W1= Dried wt. of RCNPs; W2= Wt. of Rutin + Wt. of STTP + Wt. of chitosan. (Sharma and Garg, 2010)

2.2.3 Fourier transform infrared spectroscopy mediated assessment of RCNP's

This was recorded using FTIR operated in the range from 4000 to 500 cm^{-1}. For FTIR mediated assessment, obtained RCNP's are diluted in DDW, centrifuged at 5000 rpm for 15 min to remove the unbound moieties. Supernatant obtained after centrifugation is discarded, pellet is suspended in DDW and procedure is repeated for 3 times, final pellet obtained is dried in hot air oven at 60°C to obtain dried and pure form of RCNP's which are then used for characterization (Kiran *et al.*, 2010)

2.2.4 X-Ray diffraction mediated assessment of RCNPs

Crystallographic structural pattern of RCNPs was determined by using XRD operated at 30 mA and a voltage of 40 kV. 2-theta scale was set from 2.0000° to 50.0014°, step=0.0053°, step time = 13.93 with anode Cu, wavelength - 1.5406 (Thomas *et al.*, 2009). Average size of RCNP's was assessed by using the Debye-Scherrer equation (Wang, 2000.).

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where, θ = Bragg's angle in degrees, λ = x-ray wavelength (1.5406), β = Full width at half the maximum (FWHM), k = constant (0.94), D = Average size of CCNP's.

2.2.5 Scanning electron microscope mediated assessment of RCNP's

Morphology, shape and size of synthesized RCNP's were determined by SEM. For this small quantity of RCNP's was dropped on carbon coated copper grid, blotting paper was used to remove extra solution and final grid was used for SEM analysis (Imad Uddin *et al.*, 2018).

2.2.6 Zeta potential analysis of RCNP's

Light scattering method was employed to detect stability of RCNP's particles at room temperature in suspension from by using zeta potential analyzer (Dodane and Vilivalam, 1998).

2.2.7 Anti-proliferative activity of RCNPs by MTT Assay

DMEM containing 10% FBS at 37°C and 5% CO_2 was used to grow four different cell lines, *viz.*, MDAMB231, SKOV3, Panc-1 and HeLa. RPMI medium containing non-essential amino acids, 10% FBS, 10

mg/ml bovine insulin and 1mM sodium pyruvate was used to grow PC3 cells. 100 µl aliquots of trypsinized cells were seeded into 96-well microtiter plates. These plates were incubated for 24 h in atmospheric conditions like 100% relative humidity, 5% CO_2, Temp. of 37°C and 95% of air. After 24 h, rutin and RCNPs was added to each cell line at a conc. of 50 µM and was incubated for more 48 h. To stop the reaction 5% MTT (10 µl) was added to all wells and incubated for 1 h at 37°C. Air dried plates are eluted with DMSO (100 µl) and used to record absorbance at 560 nm on a multimode plate reader (Imad Uddin *et al.*, 2019).

3. Results

3.1 Characterization of RCNPs

Standard curve of rutin was obtained and R^2 value was found to be 0.999 (Figure 1). For screening, nanocarrier property of chitosan EE% and LE % are estimated which were found to be 68.7%, and 50.8%, respectively. FTIR analysis of chitosan nanoparticles are shown in Figure 2. Peak at 3273.96 cm^{-1} indicates –OH stretching vibration, 1635.50 cm^{-1} indicates interaction between STTP and chitosan, 1539 cm^{-1} indicates –NH bending vibration of primary amine, 1152.65 cm^{-1} indicates the presence of C-O stretching vibration, 1070.51cm^{-1} and 1023.50 cm^{-1} indicates -CO vibrations. In case of RCNPs (Figure 3) 3341cm^{-1} and 1650.50 cm^{-1} indicates –OH stretching vibration and C=O stretching vibrations, respectively. 1454 cm^{-1}, 1203 cm^{-1}, and 1060 cm^{-1} indicates C=C vibrations, P=O vibrations and C-O-C vibrations of rutin, respectively.

Physical nature of RCNPs was assessed by XRD technique and XRD-diffractogram is presented in Figure 4. 2θ peaks of RCNPs were found to be from 5.210 to 46.150 with intensity (cps) from 44.9 to 8.7. SEM image (Figure 5) revealed rough and porous surface of RCNPs. Stability of nano formulations is estimated by measuring potential on either surface or interface of particles. Irrespective of the charge particles with higher zeta potential are stable but the particles with lower value are unstable and are prone to flocculate. Zeta potential of RCNP (Figure 6) was found to be +0.68 mV which indicates low stability.

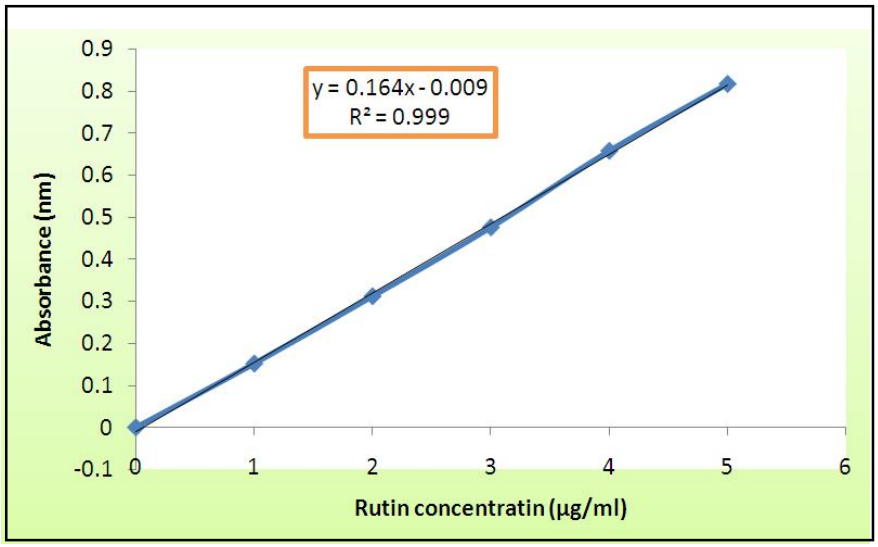


Figure 1: Standard curve of pure rutin.

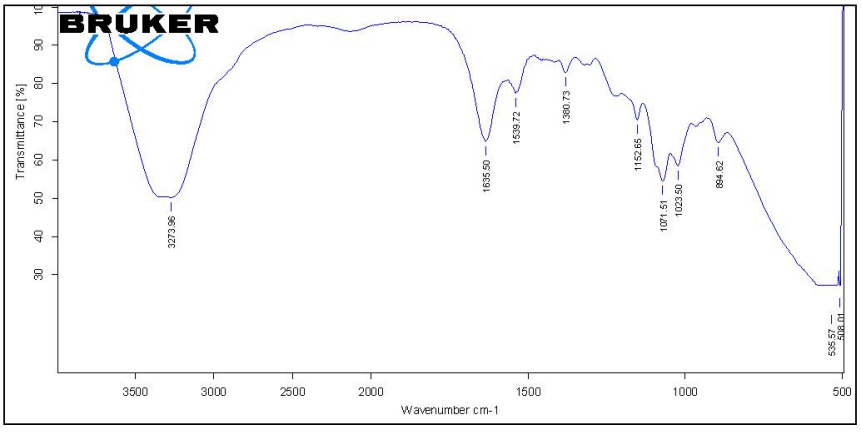


Figure 2: FTIR analysis of chitosan nanoparticle.

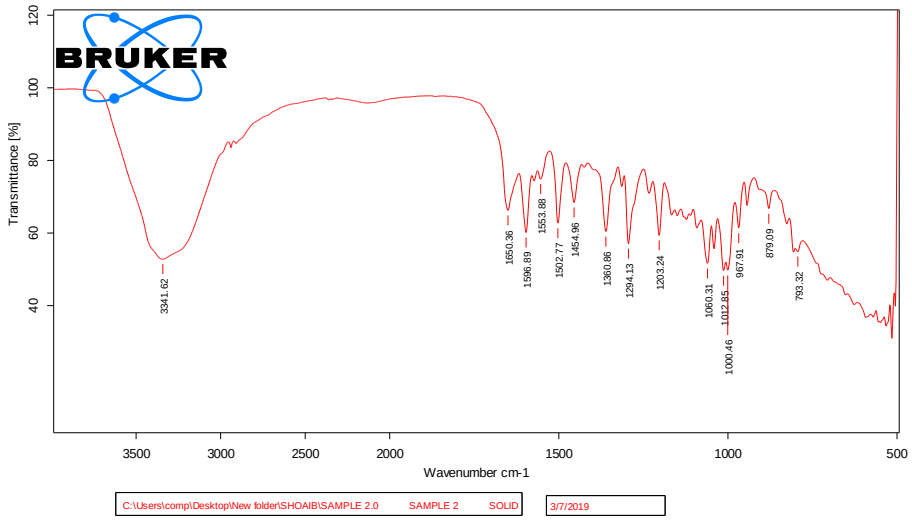


Figure 3: FTIR analysis of RCNP's.

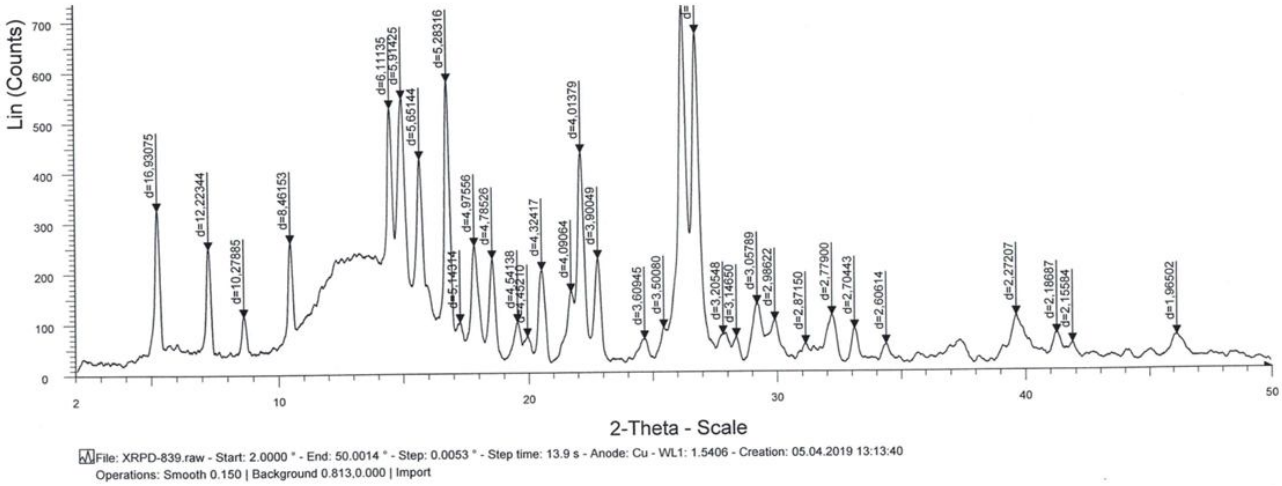


Figure 4: XRD pattern of RCNPs.

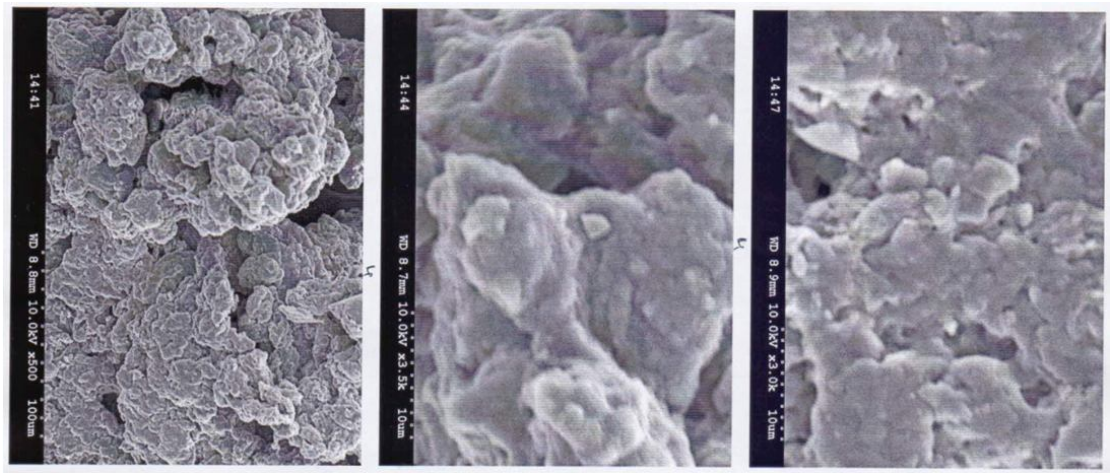


Figure 5: SEM image of RCNPs.

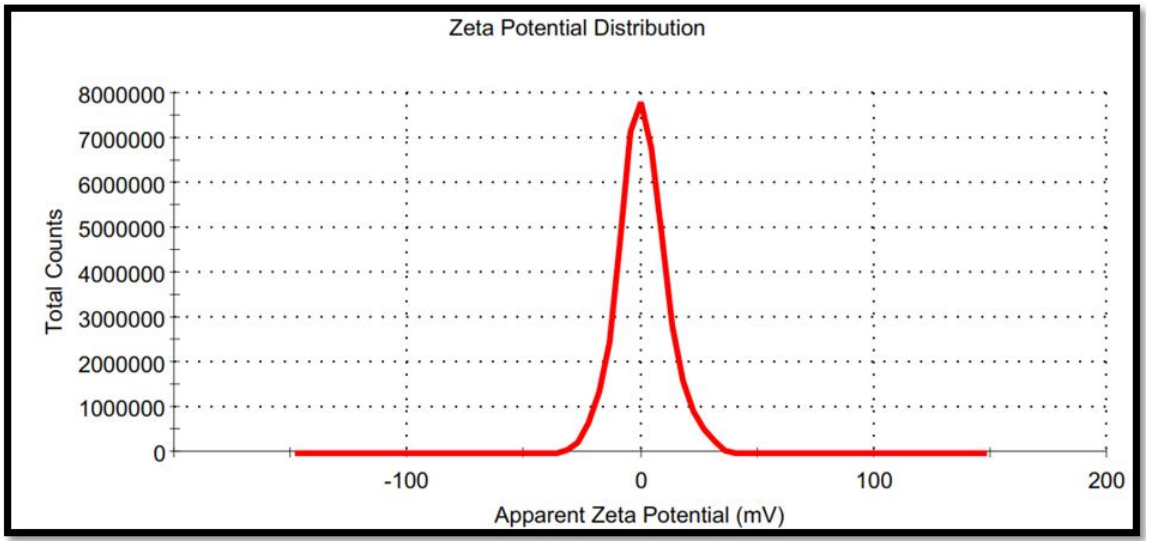


Figure 6: Zeta potential of RCNPs.

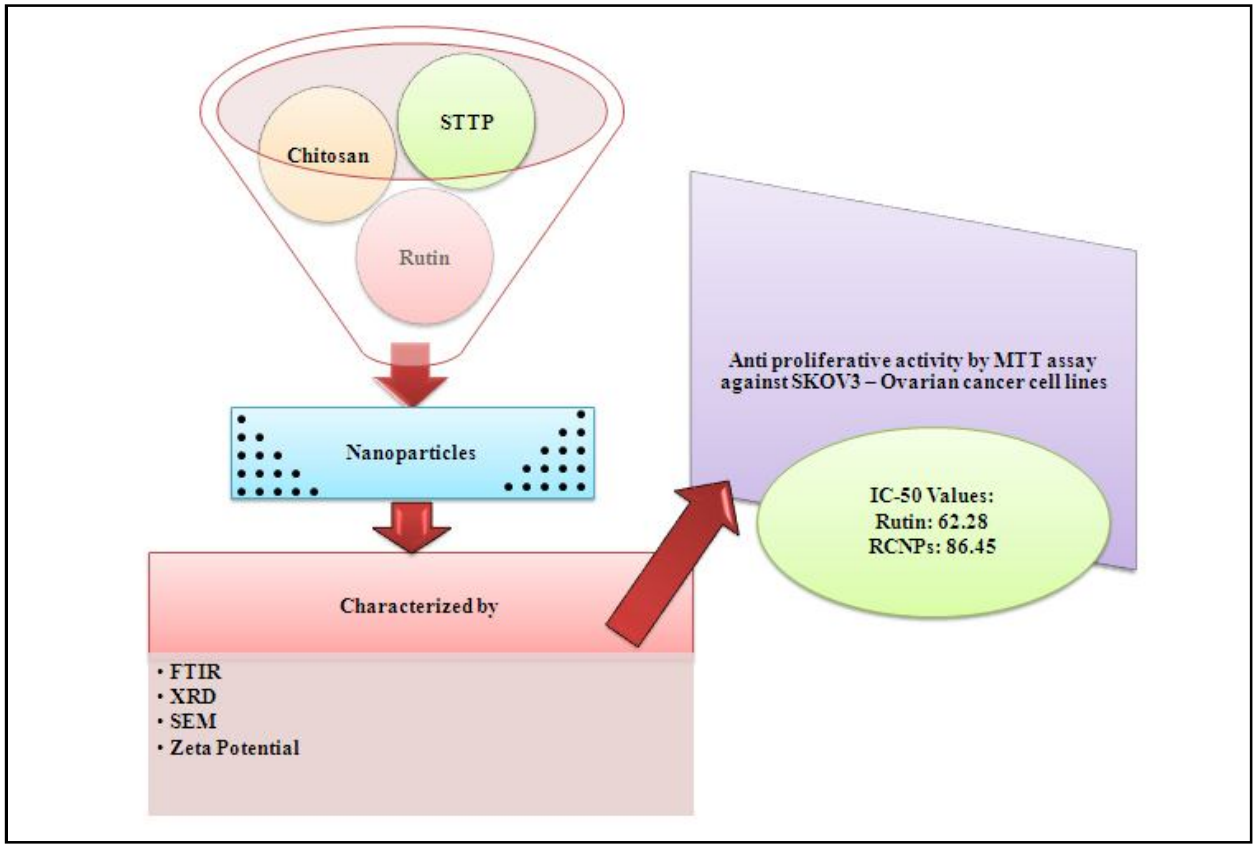


Figure 7: Graphical abstract explaining synthesis, characterization and antiproliferative activity of rutin and RCNPs.

Table 1: Antiproliferative activity of pure rutin and RCNPs

Compounds	HeLa	MDAMB231	Panc^{-1}	PC3	SKOV3
Pure rutin (50 µM)	NA	NA	NA	NA	62.28 ± 19.42
RCNP (50 µM)	NA	NA	NA	NA	86.45 ± 19.46

Antiproliferative activity of pure rutin and RCNPs by MTT assay. All values are expressed as Mean ± SEM, NA=Not active

3.2 Antiproliferative activity of RCNPs by MTT assay

The IC_{50} values against SK0V3 cell lines for rutin and RCNPs was found to be 62.28 ± 19.42 and 86.45 ± 19.46, respectively (Table 1). Thus, efficacy of rutin and RCNPs against ovarian cancer cell line is reported for the first time. However, in our study, both rutin and RCNPs have not showed any activity against HeLa, MDA – MB-231, Panc^{-1} and PC3. Graphical abstract explaining synthesis, characterization and antiproliferative activity of rutin and RCNPs is presented in Figure 7.

4. Discussion

RCNPs were prepared by adding rutin to the chitosan solution. Later on STPP is added drop wise to the above solution. After overnight stirring, the above solution is subjected to centrifugation at 8000 rpm then the nanoparticles are separated by removing the supernatant and then washed with ethanol and stored for further studies. EE% can be increased by optimizing the quantities of chitosan, drug and also by optimizing temperature, rotating speed, and pH. LE% is the actual amount of drug loaded in RCNPs. % yield of RCNPs was found to be 31.08 %. Our results are in accordance with other studies, *viz*., Patil and Jobanputra (2015) got 36.72% of % yield and 32.24% of EE% when they prepared rutin loaded chitosan nanoparticles. % yield was less but EE% was far better in our study. Moreover, both efficiencies and % yield can be increased by optimizing the ratio between chitosan and STTP.

In FTIR peaks of chitosan nanoparticles 1635.50 cm^{-1} indicates interaction between STTP and chitosan, 1539cm^{-1} indicates –NH bending vibration of primary amine. In case of RCNPs (Figure 3) 3341cm^{-1} and 1650.50 cm^{-1} indicates –OH stretching vibration and C=O stretching vibrations, respectively. 1454 cm^{-1}, 1203 cm^{-1}, and 1060cm^{-1} indicates C=C vibrations, P=O vibrations and C-O-C vibrations of rutin, respectively. This confirms loading of rutin in chitosan nanoparticles. 1596 cm^{-1} indicates –NH bending vibration of primary amine of chitosan. Our results are in conformity with the study conducted by Patil and Jobanputra (2015) where they reported –OH stretching vibration is due to a broad peak at 3498 cm^{-1}, interaction between STTP and chitosan is due to a peak at 1637 cm^{-1}, confirmation of loading rutin is indicated by C-O-C vibrations of rutin which showed a peak at 1064 cm^{-1} (Ashwini *et al.,* 2015). Diffractogram showed many XRD peaks below 30^{0} which indicates semi crystalline nature of chitosan. Size of RCNPs was found to be 22.31nm to 42.32 nm with an average size of 32.15 nm by using Debye-Scherrer's equation. Similar results were obtained by Khan *et al.* (2016).

According to SEM study, porous morphology of RCNPs is reported. This facilitates in swelling and rapid release of drug. Surface morphology depicted in our study is in accordance with SEM results of Cahyono *et al.* (2017). Low cross linking densities of nanoparticles is credited due to porous, rough, loose and open surface morphology (Bhumkar and Pokharkar, 2006). Results of zeta potential measurement showed reduced stability of rutin loaded chitosan nanoparticles. This is due to various factors such as types of biopolymers and their quantity, ionic strength, and pH of the solution. Value near to zero may be due to –vely charged groups of rutin (Ashwini *et al.,* 2015). RCNPs are effective against SKOV3 cells. Rutin is a citrus flavonoid found in many plants. Long term and high intake of flavonoids will decrease the incidence of ovarian cancer (Evans, 2011). Results indicated that RCNPs are more effective than rutin. This increased activity of rutin loaded chitosan nanoparticles is due to targeted delivery of nanoparticles. Moreover, both pure rutin and RCNPs are not active against other cell lines, *viz*., HeLa, MDAMB231, Panc^{-1} and PC3.

5. Conclusion

Current investigation portrays easy method for incorporation of insoluble phytoconstituent rutin in chitosan nanoparticles. Synthesized RCNPs are stable and water soluble. Moreover, successful encapsulation of rutin in nanoparticles improves bioavailability of rutin and their by increasing its therapeutic effect. Obtained results more prominently authenticate the effectiveness of rutin loaded chitosan nanoparticles; this may be attributed to increased antiproliferative action, intracellular accumulation and cellular uptake. Thus, chitosan loaded rutin nanoparticles provide a proficient tool for encapsulation and delivery of rutin by increasing its stability and water solubility for making it an efficient treatment option for cancer. Further studies should be carried out to screen anticancer potential by using higher conc. of RCNPs against other cell lines (MDAMB231, Panc-1, HeLa and PC3). Study also directs the conduct of *in vivo* studies of RCNPs against ovarian cancer.

Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

References

Ahmad, N.; Ahmad, R.; Naqvi, A.A.; Alam, A.; Ashafaq, MD.; Samim, MD.; Iqbal, Z. and Ahmad, F.J. (2016). Rutin-encapsulated chitosan nanoparticles targeted to the brain in the treatment of Cerebral Ischemia. Int. J. Biol. Macromol., **91**:640-655.

Alonso-Castro, A.J.; Dominguez, F. and Garcia-Carranca, A. (2013). Rutin exerts antitumor effects on nude mice bearing SW480 tumor. Arch. Med. Res., **44(5)**:346-351.

Anitha, A.; Deepagan, V.G.; Divya Rani, V.V.; Menon, D.; Nair, S.V. and Jayakumar, R. (2011). Preparation, characterization, *in vitro* drug release and biological studies of curcumin loaded dextran sulphate–chitosan nanoparticles. Carbohyd. Polym., **84(3):**1158-1164.

Annapurna, A.; Reddy, C.S.; Akondi, R.B. and Rao, S.R. (2009). Cardioprotective actions of two bioûavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. J. Pharm. Pharmacol., **61(10):**1365-1374.

Araujo, J.R.; Goncalves, P. and Martel F. (2011). Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutr. Res., **31(2):**77-87.

Asad Khan, M.D.; Zafaryab, M.D.; Mehdi, S.H.; Ahmad, I. and Rizvi, M.A.M. (2016). Characterization and anti-proliferative activity of curcumin loaded chitosan nanoparticles in cervical cancer. Int. J. Biol. Macromol., **93**:242-253.

Ashwini, G.P.; Harshada, R.B.; Mahesh, P.M.; Prashant, KD. and Pravin, O.P. (2015). Rutin-chitosan nanoparticles: Fabrication, characterization and application in dental disorders. Polymer-Plastics Technology and Engineering, **54(2):**202-208.

Balachandran, P. and Govindarajan, R. (2005). Cancer: An ayurvedic perspective. Pharmacol. Res., **51**:19-30.

Bhumkar, D.R. and Pokharkar, V.B. (2006). Studies on effect of pH on Cross-linking of chitosan with sodium tripolyphosphate: A technical note, AAPS Pharm. Sci. Tech., **72:**2-7.

Cahyono, B.; Sestri Bela Pratiwi, S.B.; Hadiyanto. and Suzery, M. (2017). Encapsulation rutin with chitosan-NATPP using coaservation Method. Reaktor., **17(4):**215-220.

Chen, C.Y.; Li, H.; Yuan, Y.N.; Dai, H.Q. and Yang, B. (2013). Antioxidant activity and components of a traditional Chinese medicine formula consisting of *Crataegus pinnatifida* and *Salvia miltiorrhiza*. BMC Complement. Altern. Med., **523:**99-102.

Dodane, V. and Vilivalam, D. (1998). Pharmaceutical applications of chitosan. Pharm. Sci.Technol. Today, **1(6):**246-253.

Evans, K. (2011). Flavonoids in fruits, vegetables and nuts dramatically lower cancer rates. Tags: cancer, flavonoids, health news, September.

Harborne, J.B. (1986). Nature, distribution and function of plant ûavonoids. Prog. Clin. Biol. Res., **213**:15-24.

Hassanpour, S.H. and Dehghani, M. (2017). Review of cancer from perspective of molecular. Journal of Cancer Research and Practice, **4:**127-129.

Hooresfand, Z.; Ghanbarzadeh, S. and Hamishehkar, H. (2015). Preparation and characterization of rutin-loaded nanophytosomes. Pharmaceutical Sciences, **21(1):**145-151.

Imad Uddin, M.D. and Veeresh, B. (2020). Systematic review on screening the role of chemosensitizer or synergistic drug and doxorubicin as dual drug loaded nanoparticle in overcoming multidrug resistant breast cancer. Ann. Phytomed., **9(2):**1-12.

Imad Uddin, M.D.; Kavya, G.; Soundarya, G.; Ropa, G. and Sridhar, C.H. (2018). Antidiabetic activity of silver nanoparticles synthesized from ashwagandha root aqueous extract. Asian Journal of Pharmaceutical Education and Research, **7(2):**45-49.

Imad Uddin, M.D.; Rachana, N.; Suraj, N.; Naveena, N. and Mounica P. (2019). Screening anticancer activity of colchicine loaded chitosan nanoparticles. Pharmacophore, **10(2):**37-42.

Khan, J.; Alexander, A.; Ajazuddin.; Saraf, S. and Saraf, S. (2013). Recent advances and future prospects of phytophospholipid complexation technique for improving pharmacokinetic profile of plant actives. J. Control Release, **168:**50-60.

Kiran, D.; Khan, M.A.; Sharma, Y.D.; Moinuddin. and Alam, K. (2010). Physicochemical studies on peroxynitrite-modiûed H3 histone. Intl. J. Biol. Macromol., **46(1):**20-26.

Li, Z.; Jiang, H.; Xu, C. and Gu, L. (2015). A review: Using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. Food Hydrocoll., **43:**153-164.

Lin, J.P.; Yang, J.S.; Lin, J.J.; Lai, K.C.; Lu, H.F.; Ma, C.Y.; Sai-Chuen, Wu.R.; Wu, K.C.; Chueh, F.S.; Gibson Wood, W. and Chung, J.G. (2012). Rutin inhibits human leukemia tumor growth in a murine xenograft model *in vivo*. Environ. Toxicol., **27(8):**480-484.

Machado, D.G.; Bettio, L.E.; Cunha, M.P.; Santos, A.R.; Pizzolatti, M.G.; Brighente, I.M. and Rodrigues, A.L. (2008). Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: Evidence for the involvement of the serotonergic and noradrenergic systems. Eur. J. Pharmacol., **587(1-3):**163-168

Mariyappan, S.; Pichaiyan, V.; Ramalingam, S. and Saravanan, R. (2018). A review on the role of phytoconstituents in cancer cells. Journal of Global Pharma. Technology, **10(2):**1-10.

Mauludin, R.; Muller, R.H. and Keck, C.M. (2009). Development of an oral rutin nanocrystal formulation. Int. J. Pharmaceut., **370:**202-209.

Mouria, M.; Gukovskaya, A.S.; Jung, Y.; Buechler, P.; Hines, O.J.; Reber, H.A. and Pandol, S.J. (2002). Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis. Int. J. Cancer, **98(5):**761-769.

Nieoczym, D.; Socala, K.; Raszewski, G. and Wlaz, P. (2014). Effect of quercetin and rutin in some acute seizure models in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry, **54:**50-58.

Patil, A.G. and Jobanputra, A.H. (2015). Rutin chitosan nanoparticles: fabrication, characterization and application in dental disorders. Polymer-Plastics Technology and Engineering, **54(2):**202-208, doi:10.1080/03602559.2014.935425

Patil, J.S.; Kamalapur, M.V.; Marapur, S.C. and Kadam, D.V. (2010). Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: A review. Digest Journal of Nanomaterials and Biostructures, **5(1):**241-248.

Pu, F.; Mishima, K.; Irie, K.; Motohashi, K.; Tanaka, Y.; Orito, K.; Egawa, T.; Kitamura, Y.; Egashira, N.; Iwasaki, K. and Fujiwara, M. (2007). Neuroprotective effects of quercetin and rutin on spatial memory impairment in an 8-arm radial maze task and neuronal death induced by repeated cerebral ischemia in rats. J. Pharmacol. Sci., **104:**329-334.

Pugazhendhi, A.; Shafreen, R.B.; Devi, K.P. and Suganthy, N. (2018). Assessment of antioxidant, anticholinesterase and antiamyloidogenic effect of *Terminalia chebula*, *Terminalia arjuna* and its bioactive constituent 7-Methyl gallic acid: An *in vitro* and *in silico* studies. J. Mol. Liq., **257:**69-81.

Qian, F.; Cui, F.; Ding, J.; Tang, C. and Yin, C. (2006). Chitosan graft copolymer nanoparticles for oral protein drug delivery: Preparation and characterization. Biomacromolecules, **7(10):**2722-2727.

Ravi Kumar, M.N.V. (2001). A review of chitin and chitosan applications. React. Funct. Polym., **46**:1-27.

Rylski, M.; Duriasz-Rowinska, H. and Rewerski, W. (1979). The analgesic action of some flavonoids in the hot plate test. Acta Physiol. Pol., **30(3):**385-388

Scambia, G.; Ranelletti, F.O.; Benedetti, Panici, P.; Bonanno, G.; De Vincenzo, R.; Piantelli, M. and Mancuso, S. (1990). Synergistic antiproliferative activity of quercetin and cisplatin on ovarian cancer cell growth. Anticancer Drugs, **1(1):**45-48.

Selvaraj, G.; Kaliamurthi, S.; Thirungnasambandam, R.; Vivekanandan, L. and Balasubramanian, T. (2014). Antinociceptive effect in mice of thillai flavonoid rutin. Biomed. Environ. Sci., **27(4):**295-299.

Sepahvand, R.; Delfan, B.; Ghanbarzadeh, S.; Rashidipour, M.; Veiskarami, G.H. and GhasemianYadegari, J. (2014). Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*. Asian Pac. J. Trop. Med., **7:**S491-S496.

Shaûei, S.S.; Solati-Hashjin, M.; Samadikuchaksaraei, A.; Kalantarinejad, R.; Asadi-Eydivand, M. and Abu Osman, N.A. (2015). Epigallocatechin gallate/layered double hydroxide nanohybrids: Preparation, characterization, and *in vitro* antitumor study. PLoS ONE, **10(8):** 1-18.

Shanmuganathan, R.; Edison, T.N.J.I.; LewisOscar, F.; Ponnuchamy, K.; Shanmugam, S. and Pugazhendhi, A. (2019). Chitosan nanopolymers: An overview of drug delivery against cancer, Int. J. Biol. Macromol., **130:**727-736.

Sharma P and Garg S. (2010). Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti-HIV drugs. Adv. Drug Delivery Rev., **62:**491-502

Shihabul Islam, M.D.; Sifat Rahi, M.D.; Arif Jahangir, C.; Habibur Rahman, M.D.; Jerin, I.; Amin, R.; Kazi, M.D.; Hoque, F. and Abu Reza, M.D. (2018). *In Vivo* anticancer activity of *Basella alba* leaf and seed extracts against Ehrlich's Ascites Carcinoma (EAC) cell line. Evid-Based Compl. Alt., **2018:**1-11.

Shu, X.Z. and Zhu, K.J. (2002). Controlled drug release properties of ionically cross-linked Chitosan beads: The influence of anion structure. Int. J. Pharm., **233(1-2):**217-225.

Thomas, V.Y.; Mohan, M.B. and Sreedhar, S.K.B. (2009). Fabrication, characterization of chitosan/nanosilver film and its potential antibacterial application. J. Biomater. Sci. A Polym. Ed, **20(14):**2129-2144.

Wang, Z.L (2000), A textbook of characterization of nanophase materials. Wiley-VCH, Germany, First Edition.

Citation **M.D. Imad Uddin, K. Saivani, K.V. Akhil, K. Nandini, K. Sandhya and Faizan Sayeed (2021). Synthesis, characterization and anticancer activity of rutin loaded chitosan nanoparticles. J. Phytonanotech. Pharmaceut. Sci., 1(1):24-31. http://dx.doi.org/10.21276/jpps.2021.1.1.5**