List of ten best papers highlighting the important discoveries and contributions

1. Jagwani S, Jalalpure S, Dhamecha D, Jadhav K, Bohara R. Pharmacokinetic and pharmacodynamic evaluation of resveratrol loaded cationic liposomes for targeting hepatocellular carcinoma. ACS Biomaterials Science & Engineering. 2020 Jul 27;6(9):4969-84.

Important Discoveries:

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. Resveratrol (RS) is a model natural nonflavonoid drug known for its anti-cancer activity. However, its clinical application is limited due to its poor bioavailability. This research work aims to formulate, optimize, and characterize RS loaded cationic liposomes (RLs) for specific delivery in HCC. The optimized liposomes formulation (RL5) was spherical with a vesicle size (VS) of 145.78 ± 9.9 nm, ζ potential (ZP) of 38.03 ± 9.12 mV, and encapsulation efficiency (EE) of 78.14 ± 8.04%. In vitro cytotoxicity studies in HepG2 cells demonstrated an improved anticancer activity of RL5 in comparison with free RS. These outcomes were supported by a cell uptake study in HepG2 cells, in which RL5 exhibited a higher uptake than free RS. Furthermore, confocal images of HepG2 cells after 3 and 5 h of incubation showed higher internalization of coumarin 6 (C6) loaded liposomes (CL) as compared to those of the free C6. Pharmacokinetic and pharmacodynamic (prophylactic and therapeutic treatment modalities) studies were performed in N-nitrosodiethylamine (NDEAcarcinogen) induced HCC in rats. Pharmacokinetic evaluation of RL5 demonstrated increased localization of RS in cancerous liver tissues by 3.2- and 2.2-fold increase in AUC and Cmax, respectively, when compared to those of the free RS group. These results implied that delivery of RS loaded cationic liposomes substantially controlled the severity of HCC and that they can be considered as a promising nanocarrier in the management of HCC.

2. Kurangi B, **Jalalpure S**, Jagwani S. A validated stability-indicating HPLC method for simultaneous estimation of resveratrol and piperine in cubosome and human plasma. **Journal of Chromatography B**. 2019, Vol.1122-1123, 39-48.

Important Discoveries:

Resveratrol and piperine are proven for their therapeutic benefits to treat various diseases. Due to their synergistic actions and combined drug delivery application, a rapid and specific RP-HPLC method was developed and validated as per ICH guidelines, by using an isosbestic point. The chromatographic separation was performed with Luna 5 µ 100 A C-18(2) HPLC column by using acetonitrile (ACN): phosphate buffer (0.01% orthophosphoric acid) (55:45) as mobile phase, at 1 mL/min of flow rate and 330 nm. The developed method was found to be linear over the concentration range of 0.25–8 µg/mL with correlation coefficient value>0.999. The developed method was accurate (percent recovery 98.06–101.74%), precise (percent relative standard deviation< 2.0%), and robust. The limit of detection and limit of quantification for resveratrol were found to be 0.02 and 0.08 µg/mL, respectively and 0.04 and 0.11 µg/mL, for piperine, respectively. The developed method was also validated in human plasma as per ICH guidelines. Moreover, stress degradation studies of both phytoconstituents were studied and the relevancy of the developed method was analyzed on cubosome nanoformulation. A good separation of drug peaks was observed in the presence of the degradation products. This method could thus be used for regular *in vitro* and *in vivo* estimation of piperine and resveratrol.

3. Rajeshwari HR, Dhamecha D, Jagwani S, Rao M, Jadhav K, Shaikh S, Puzhankara L, **Jalalpure S.** Local drug delivery systems in the management of periodontitis: A scientific review. **Journal of Controlled Release**. 2019 Jun 27.

Important Discoveries:

Periodontitis (PD) is a microbial disease of tooth supporting tissues that results in progressive destruction of surrounding soft and hard tissues with eventual tooth mobility and exfoliation. Perioceutics, which includes the delivery of therapeutic agents via systemic and local means as an adjunct to mechanical therapy has revolutionized the arena of periodontal therapy. Selection of a right antimicrobial agent with appropriate route of drug administration is the key to successful periodontal therapy. Irrigating systems, fibers, gels, strips, films, microparticles, nanoparticles and low dose antimicrobial agents are some of the local drug delivery systems (LDDS) available in the field, which aims to deliver antimicrobial agents to sub-gingival diseased sites with minimal or no side-effects on other body sites. The present review aim to summarize the current state-of-the-art technology on LDDS in periodontal therapy ensuring the the practitioners are able to choose LDD agents which are custom made for a specific clinical condition.

4. Peram MR, **Jalalpure SS,** Kumbar VM, Patil SR, Joshi SA, Bhat KG, Diwan PV. Factorial design based curcumin ethosomal nanocarriers for the skin cancer delivery: In vitro evaluation. **Journal of Liposome Research**. 2018:1-64.

Important Discoveries:

Melanoma is the most deadly and life-threatening form of skin cancer with progressively higher rates of incidence worldwide. The objective of the present investigation is to develop and to statistically optimize and characterize curcumin (CUR) loaded ethosomes for treatment of melanoma. A two factor, three level (3^2) factorial design approach was employed for the optimization of ethosomes. The prepared ethosomes were evaluated for size, zeta potential, entrapment efficiency, *in vitro* skin permeation and deposition ability. The optimized ethosomal formulation was evaluated for *in vitro* cytotoxicity and cellular uptake studies using A375 human melanoma cells. The optimized formulation has imperfect round shaped unilamellar structures with a mean vesicle size of 247 ± 5.25 nm and an entrapment efficiency of $92.24 \pm 0.20\%$. The *in vitro* skin permeation studies proved the superiority of ethosomes over the traditional liposomes in terms of the amount of drug permeated and deposited in skin layers. Fluorescence microscopy showed the enhanced penetration of ethosomes into the deeper layers of the skin. *In vitro* cytotoxicity and cellular uptake studies revealed that curcumin ethosomes have significantly improved cytotoxicity and cellular uptake in A375 human melanoma cell lines. The colony formation assay results showed that curcumin ethosomes have a superior antiproliferative effect as they effectively inhibit the clonogenic ability of A375 cells. The flow cytometry results indicate that curcumin ethosomes induce cell death in A375 cells by apoptosis mechanism. The present study provides a strong rationale and motivation for further investigation of newly developed curcumin ethosomes as a potential therapeutic strategy for melanoma treatment.

5. Jadhav K, Deore S, Dhamecha D, Jagwani S, **Jalalpure S**, Bohara R. Phytosynthesis of Silver Nanoparticles: Characterization, Biocompatibility Studies, and Anticancer Activity. **ACS Biomaterials Science & Engineering**. 2018 Jan 28;4(3):892-9.

Important Discoveries:

Silver nanoparticles (SNPs), owing to their wide range of biomedical applications, have recently attracted remarkable interest for use in cancer nanomedicine. The present research work investigated the anticancer activity of phytosynthesized SNPs against human cancer cell lines. Phytosynthesis of SNPs was achieved by using an aqueous extract of Salacia chinensis (SC) bark as a green source to reduce silver nitrate to silver nanoparticles. Characterization of synthesized nanoparticles demonstrated a UV−visible peak at 443 nm, ζ-potential (zetasizer) of −25.6 ± 0.34 and particle size (transmission electron microscopy analysis) in the range of 40−80 nm, which validates formation of stable silver nanoparticles. The absence of cytotoxicity against normal human fibroblasts and blood erythrocytes confirms the biocompatible nature of green synthesized SNPs. In vitro anticancer assay demonstrated IC50 values of 6.31, 4.002, 5.228, 8.452, 14.37, 7.46, and 6.55 μg/mL against liver (Hep G2), lungs (L-132), pancreas (MIA-Pa-Ca-2), breast (MDA-MB-231), oral (KB cells), prostate (PC-3), and cervical (HeLa) cancer cell lines respectively, which confirms its potent anticancer action. The results of the present study give an experimental proof that the SC mediated green synthesized SNPs could serve as a promising anticancer agent to overcome limitations of existing conventional cancer chemotherapeutics.

6. Dhamecha D, **Jalalpure S**, Jadhav K, Jagwani S, Chavan R. Doxorubicin loaded gold nanoparticles: Implication of passive targeting on anticancer efficacy. **Pharmacological research**. 2016 Nov 1;113:547-56.

Important Discoveries:

The present work aims to investigate targeting potential of doxorubicin (Dox) functionalized gold nanoparticles (D-GNPs) for treatment of chemically induced fibrosarcoma in mice. Carrier GNPs were synthesized by green chemistry method and loaded with doxorubicin by incubation method. D-GNPs were studied for its biocompatibility using normal mouse fibroblasts (L929) and found to be cell compatible and non-toxic. D-GNPs (at a dose of 2.5, 2 and 1.5 mg/kg equivalent to Dox) demonstrated passive targeting measured as function of antitumor efficacy against chemical induced fibrosarcoma which showed higher latency to the tumour growth as compared to free Dox (2.5 mg/kg). D-GNPs exhibited significantly higher therapeutic anticancer efficacy (~81% tumour suppression at dose of 2.5 mg/kg equivalent to Dox) in the same model as compared to that of free doxorubicin (~48% tumour suppression at dose of 2.5 mg/kg). Safety profile and targeting efficiency of developed formulation was established by assessing cardiac and blood markers.

7. Nannapaneni NK, **Jalalpure SS**, Muppavarapu R, Sirigiri SK. A sensitive and rapid UFLC-APCI-MS/MS bioanalytical method for quantification of endogenous and exogenous Vitamin K1 isomers in human plasma: Development, validation and first application to a pharmacokinetic study. **Talanta**. 2017 Mar 1;164:233-43.

Important Discoveries:

Due to lack of suitable bioanalytical methods in previous literature, for simultaneous estimation of Vitamin K1 isomers, in compliance with the current regulatory expectation, we aimed to develop a sensitive and rapid method with UFLC-APCI-MS/MS (ultrafast liquid chromatography - tandem mass spectrometry) using human plasma. A simple and cost effective procedure was implemented with the combination of protein precipitation and liquid extraction, to isolate the targets from plasma sample, while achieving an insignificant matrix effects and high recovery (≥88.2%). A short 9.0 min run time per sample was accomplished by using water in methanol (1.0% v/v) and acetonitrile, which pumped at 0.8 mL/min, on to the COSMOSIL® packed column, for separating the trans and cis isomers of Vitamin K1 along with the corresponding stable labeled D7 internal standards (ISs). The analytes and ISs were quantified, at their parent to product ion mass transitions of 451.3 →187.1 m/z and 458.1→194.3 m/z respectively, using an APCI (atmospheric pressure chemical ionization) source of the tandem mass, in MRM (multiple reaction monitoring) mode. Performance of the method over the calibration range: 0.1–150.0 ng/mL, while using a low sample volume (0.3 mL), was successfully evaluated through full method validation in compliance with the latest regulations. Fully validated method with significant results was applied to human pharmacokinetic study, and had a potential to further advance the clinical research programs and generic drug development of Vitamin K1, intended for the regulatory submission.

8. Nagaraj Kumar Nannapaneni, **Sunil S. Jalalpure**, Rajendra prasad Muppavarapu, Sunil Kumar Sirigiri, An ultra-high performance liquid chromatography-tandem mass spectrometry method for the quantification of linagliptin in human plasma. **RSC Advances**, 2016, 6, 66756 – 66766.

Important Discoveries:

A simple, rapid, sensitive, reliable and selective ultra high performance liquid chromatography (UHPLC)-tandem mass spectrometry (MS/MS) method was developed for the quantification of linagliptin (LGN) in human plasma. LGN and its deuterated internal standard (IS) LGN-d4 were extracted from a low-plasma sample (volume: 300 μL) by a simple liquid–liquid extraction protocol. Efficient estimation of the analyte and IS at a mean retention time (RT) of 1.75 and 1.74 min respectively, with a rapid 3.5 min run time per sample was chromatographically established on a Gemini C18 (100 mm × 4.6 mm, 3 μ) column under simple isocratic elution conditions, using a mixture of 10 mM ammonium formate : methanol [20 : 80 (v/v)] delivered at a flow rate of 0.5 mL min^{-1}. Following the separation of the compounds, protonated precursor → product ion transitions were monitored for LGN (*m*/*z*: 473.3 → 420.1) and IS (*m*/*z*: 477.5 → 424.3) on a triple quadrupole mass spectrometer, operating in a multiple reaction monitoring (MRM) mode. The most recent regulatory guidelines were adopted during the method validation. The method demonstrated very good analyte and IS recovery (not less than 71.0%), precision (≤8.6% CV), accuracy (range: 86.7% to 95.6%) and linearity ($r > 0.99$) across a clinically relevant LGN plasma concentration range: 50.3 to 12 115.5 pg mL^{-1}. The validated method was successfully applied to pharmacokinetic study samples for measuring linagliptin plasma levels.

9. Dinesh Dhamecha, **Sunil Jalalpure**, Kiran Jadhav, Nepenthes khasiana mediated synthesis of stabilized gold nanoparticles: characterization and biocompatibility studies, **Journal of Photochemistry and Photobiology B: Biology**, 154, 2016, 108-117.

Important Discoveries:

The current study summarizes a unique green process for the synthesis of gold nanoparticles by simple treatment of gold salts with aqueous extract of *Nepenthes khasiana* (NK)—a red listed medicinal plant and its characterization. Study on the effect of different process parameters like temperature, pH and stirring on surface and stability characteristics has been demonstrated. Formation of GNPs was visually observed by change in color from colorless to wine red and characterized by UV-Visible spectroscopy, FT-IR spectroscopy, Zetasizer, X-RD, ICP-AES, SEM-EDAX, AFM and TEM. *In vitro* stability studies of gold colloidal dispersion in various blood components suggest that, NK mediated GNPs exhibit remarkable *in vitro* stability in 2% bovine serum albumin, 2% human serum albumin (HSA), 0.2 M histidine, and 0.2 M cysteine but unstable in 5% NaCl solution and acidic pH. Biocompatibility of NK stabilized GNPs against normal mouse fibroblasts (L929) cell lines revealed nontoxic nature of GNPs and thus provides exceptional opportunities for their uses as nanomedicine for diagnosis and drug therapy. The role of antioxidant phytochemicals (flavonoids and polyphenols) of NK extract in synthesis of biocompatible and stabilized GNPs was demonstrated by estimating total flavonoid content, total phenolic content and total antioxidant capacity of extract before and after formation of GNPs. Fast and easy synthesis of biocompatible GNPs possesses unique physical and chemical features which serve as an advantage for its use in various biomedical applications. The overall approach designated in the present research investigation for the synthesis of GNPs is based on all 12 principles of green chemistry, in which no man-made chemical other than the gold chloride was used.

10. Dinesh Dhamecha, **Sunil Jalalpure**, Kiran Jadhav. Doxorubicin functionalized gold nanoparticles: characterization and activity against human cancer cell lines, **Process Biochemistry**, 50 (2015) 2298-2306.

Important Discoveries:

The aim of the study was to synthesize doxorubicin (DOX)-functionalized gold nanoparticles (GNPs) by a green method and to evaluate their anticancer potential against human cancer cell lines. These GNPs were synthesized with a green chemistry method and characterized by ultraviolet (UV) spectrophotometry, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Zetasizer measurements. Surface plasmon resonance studies showed a clear UV-Visible peak at 532 nm, suggesting the formation of GNPs. FT-IR and XRD were used to determine the surface characteristics (presence of phytoconstituents) and crystalline nature of GNPs, respectively. The TEM and Zetasizer studies revealed a particle size of 74.7 ± 2.47 nm with a zeta potential of -19.13 ± 0.2. The synthesized GNPs were loaded with DOX by simple incubation method and evaluated for particle size, zeta potential, FT-IR and XRD to confirm drug loading. An in vitro anticancer assay of DOX-loaded GNPs (D-GNPs) against human cancer cell lines showed variations in responsiveness to D-GNPs, with significant activity against breast, lung, and prostate cancer cell lines. However, no significant difference was found in the percent cell viability of cervical, liver, and pancreatic cancer cell lines between DOX and D-GNPs. The results of the in vitro anticancer assay of D-GNPs against human cancer cell lines supports their potential for in vivo applications in cancer treatments.