Available online on 15.07.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research



Open Access Research Article

# *In-Vitro* Evaluation of Drug Release and Antioxidant Activity of Aloe Loaded Chitosan Nanoparticles

**Kesharwani Shivangi *, Gupta Durgesh Kumari, Gupta Mahesh Kumar**

Oriental College of Pharmacy and Research, Oriental University, Indore, India

## ABSTRACT

Antioxidants play significant responsibility during the treatment of cancer and it's adverse effects, because in the treatment of cancer by using chemotherapy, free radicals are generated and these free radicals accumulated in the body and cause damage, side effect (such as neurodegenerated disease, atherosclerosis, depression, leukemia, skin disease, diabetes etc.) and even death due to the biological consequence. So, antioxidant inhibits the generation of new and destroys previously present free radicals. A recent study was aimed for novel approach of herbal nanoparticles to get improved efficacy of the drug with fewer side effects. *In-vitro* Evaluation of Drug Release and Antioxidant Activity of Aloe Loaded Chitosan Nanoparticles were includes in this research. Aloe has good antioxidant property, but in the form of nanoparticles formed with the help of chitosan polymer, it's enhancing their stability, *in-vitro* release property. Chitosan is one of the best polymer for nanomedicine formulation because of their low toxicity and high compatibility. Six formulations of aloe loaded chitosan nanopaticles were prepared and evaluated as antioxidant study, *in-vitro* release and stability studies. Results have shown that the F6 was most stable, good release and high antioxidant property. So, it found that F6 was good formulation among all batches and these nanoparticles can be used as antioxidant agent during the treatment of cancer along with chemotherapeutic drugs for improve their effectiveness and reduce adverse effects.

**Keywords:** Cancer, Chemotherapy, Herbal, Chitosan nanoparticles, Antioxidant.

**Article Info:** Received 04 May 2019; Review Completed 12 June 2019; Accepted 19 June 2019; Available online 15 July 2019

**Cite this article as:**

Kesharwani S, Gupta DK, Gupta MK, *In-Vitro* Evaluation of Drug Release and Antioxidant Activity of Aloe Loaded Chitosan Nanoparticles, Journal of Drug Delivery and Therapeutics. 2019; 9(4):43-52

**http://dx.doi.org/10.22270/jddt.v9i4.2979**

***Address for Correspondence:**

Shivangi Kesharwani, PG Research Scholar, Department of Pharmaceutics, Oriental College of Pharmacy and Research, Oriental University, Indore, India

## INTRODUCTION

Nanoparticles are modern implement in pharmaceutical and biomedical fields. This system used as a physical approach to alter and improve the pharmacokinetics and pharmacodynamic properties of various types of drugs. The advantage of nanoparticles are used to protect the drugs molecules in the systemic circulation, targeting of the drug to the desire site and to deliver the drug at a controlled and sustained rate to the site of action.[1]

Herbal medicines have been used all over the world from last many years, especially in India. The herbal treatment helps to increase the therapeutic value by reducing the toxicity and side effects of drugs at the same time it also increases the bioavailability.[2,3]

Great advancement has been made in the uses of plant therapeutics, on development of novel herbal formulation like polymeric nanoparticles, nanocapsules, liposomes, phytosomes, nanoemulsions, microsphere, transferosomes and ethosomes etc. these formulations have reported to have various advantages over the traditional formulations such as improved solubility & bioavailability, reduced toxicity, controlled drug delivery, protections of plant actives from degradation. Also these having the drug targeting properties with improved selectivity, drug delivery and effectiveness with dose reduction which not only increase the safety but also patient compliance.[4]

Antioxidants are substances which help and protect the cells from the harm caused by unstable atoms known as free radicals. Antioxidants works by three mechanisms, curing the formations of latest free radicals, destroy free radicals to avoid oxidative chain reactions and repairing the damage caused to biomolecules by free radicals.[5] Antioxidant intake seems to influence the effectiveness of antitumor therapy and its adverse effects. Free radicals are generated in the treatment of cancer by using chemotherapy. These free radicals accumulated in the body and cause DNA damage of healthy cells, also causes some side effects such as neurodegenerated disease, atherosclerosis, depression, leukemia, skin disease, diabetes etc. and even death due to

the biological consequence. So the antioxidants are play important role in the treatment and prevention of cancer and it's adverse effects because off their effect on the cell cycle regulation, inflammation, inhibition of tumor cell proliferation and invasiveness, the inhibition of apoptosis and the stimulation of the detoxifying enzyme activity.[6]

Chitosan is novel pharmaceutical polymer for many formulations such us nanoparticles, because of it's ideal properties like biodegradable, biocompatible, permeation enhancer, pH sensitiveness, safe, increases absorption of drug cross the epithelial membrane, mucoadhesive, controlled release action, administered by various routes. Chitosan has chemical functional groups that can be be modified to achieve specific goal. Chitosan shows low toxicity in both *in-vitro* and in-vivo model. [7,8]

**ALOE**

**Family**: Liliaceae

**Botanical names**: *Aloe barbadensis, Aloe indica, Aloe Barbados, Aloe vera.*

**Use as anticancer:** Aloe has been used ancient period of times for its curative and therapeutic properties. Among constituents of aloe, aloe-emodin is reported for it's anticancer properties. Based on antioxidant property, several researchers have identified that aloe-emodin has been effectively analyze against neuroecletrodermal cancer, leukemia, merkel cell carcinoma, and lung squamous cell carcinoma. Aloe vera may be an valuable anti-neoplastic agent to slow down cancer cell growth and enlarge the therapeutic efficacy of another anticancer drugs like cispolatin. [9-14]

## MATERIALS AND METHODS

### Preparation of aloe loaded chitosan nanoparticles

1. Preparation of plant extract
2. Preparation of chitosan nanoparticles
3. Preparation of plant extract chitosan nanoparticles

### Preparation of chitosan nanoparticles

Chitosan (1%) was dissolved in acetic acid and adjust the pH at 4.6 to 4.8 by the help of NaOH. Then above solution was filter with millipore (0.45 membranes). 1.5%w w/v Tripolyphosphate (TPP) was dissolved in deionized water. After that TPP solution was added drop-wise (0.3ml/min) into chitosan solution with magnetic stirring for 60 min at 25$^0$C.After completing all the process, chitosan nanoparticles were form spontaneously The purification of nanoparticles were take place by centrifugation of resulting solution at 9000 rpm for 30 min for removing the excess unraective chitosan. For librating of NaOH from final product, distilled water was used to rinsing chitosan nanoparticle.[15]

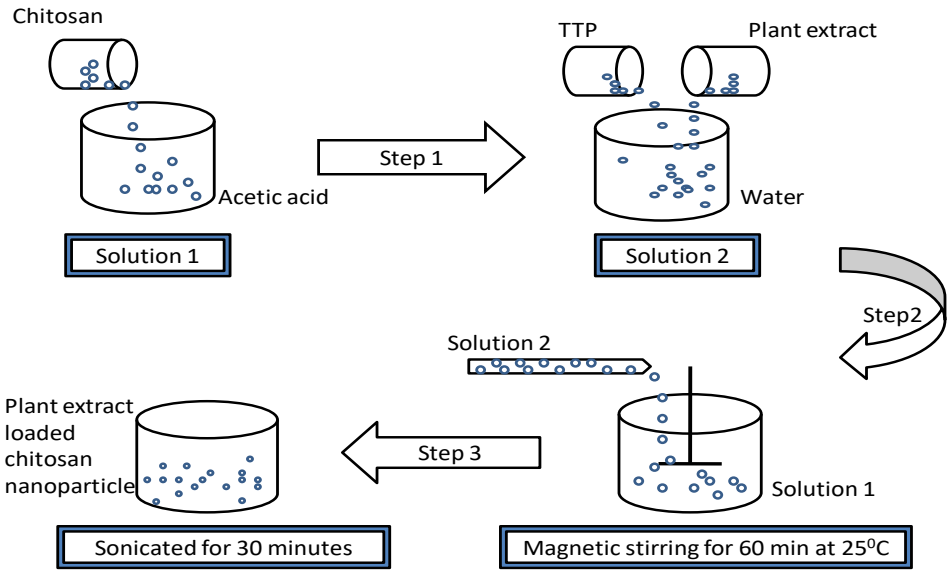


Fig 1 Preparation of plant extract loaded chitosan nanoparticles

### Preparation of plant extract

Fresh aloe vera leaves were collected and washed 2-3 times with distilled $H_2O$ to eliminate the foreign particles, subsequently dehydrate the aloe leaves by sun light. Weighed 10 grams dried aloe vera leaves, crumpled and chopped with glass rod in 500 ml glass beaker, added 200 ml sterilized distilled water in 250 ml beaker, boiled for 20 minutes, until the color of the aqueous solution changes from watery to light yellow, cool the extract at room temperature then filtered through what-man filter paper. After complete filtration, centrifuged the aloe extract at 3000 rpm for 30 min, then take the supernatant by the help of micropipette and collect in another tube. Store the aloe vera extract at -15$^0$C temperatures for further use within one week.[16]

### Preparation of plant extract loaded chitosan nanoparticles

The plant extract was dissolved directly in chitosan solution with six various concentrations (3%, 6%, 9%, 12%, 15% and 18%). To this TPP solution was added in drop and stirred at 1000 rpm on a stirrer at room temperature. The resulting mixture sonicated for 20 minutes .The resulting suspension was subsequently centrifuged at 15000 rpm for 10 minutes .the pellets obtained were resuspended in deionised water by sonication, centrifuged and dried at room temperature (about 25$^0$C). [17]

**Table 1 Formula of different batches of aloe extract loaded chitosan nanoparticle.**

| S.No. | Name of Ingredient | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| 1 | Aloe extract (% w/v) | 3 | 6 | 9 | 12 | 15 | 18 |
| 2 | Chitosan (% w/v) | 1 | 1 | 1 | 1 | 1 | 1 |
| 3 | TPP (% w/v) | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| 4 | Acetic acid (% w/v) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |

**Characterization of aloe loaded chitosan nanoparticles**

**Entrapment efficiency**

The entrapement efficiency of plant extracted chitosan nanoparticle was resolute by measuring the concentration of free drug in the dispersal medium. The free drug was resoluted by adding 2ml of the nano particle as a suspension to 8ml water for dissolving the free drug .then this suspension was centrifuged for 90 min at 15,000 rpm. The entrapment efficiency was deliberated by subtracting initial drug from the free drug. [15,18]

The percentage of encapsulation efficiency was calculated by following formula:

Encapsulation efficiency (%) = (Total amount – Free amount /Total amount) x 100

***In-vitro* drug release study**

The *in-vitro* drug release study was approved in phosphate buffer pH 6.8 in dialysis bad technique using dialysis membrane of 12,000-14,000 molecular weight. The membrane was washed with warm double distilled water (70;c) for 1 h and afterwards rinsed thrice with water to eliminate the glycerin 5ml of suspension was placed contained by the dialysis bag, joined at both ends and dipped in a receptor compartment of dissolution medium. The medium be stirred at 100 rpm using a magnetic bead at temperature at 37±0.2. 2ml aliquot were reserved at specific time intervals and replaced by an equal volume of a new/fresh dissolution medium. The samples were analyzed UV spectroscopy at 450nm. The concentration of drug in test sample were precise by UV spectrophotometer and deliberate of the calibration curve.[19]

**Zeta potential**

The zeta potential is the words most advance system of DLS and ELS. It was measured in duplicate with laser velocimetry Doppler at 25$^0$C. The nanoparticles were concentrated at 0.5 mg/ml with deionized distilled water. The nanoparticulate suspension was added to the sample dispersion holder which was stirred to minimize the particle aggregation by inter particle aggregation. The analysis was performed thrice and average hydro dynamic particle size was expressed as the value of z average size ± SD. The width of size distribution was indicated by the poly dispersion index. Distilled water was used as the dispersion medium.[20]

**Scanning electron microscopy**

The surface morphology of the herbal chitosan nanoparticles was governed by scanning electron microscopy (SEM). There were large number of nanoparticles with an approximately spherical shape and they were well separated from each other. SEM images of freeze-dried herbal chitosan nanoparticles , which prepared by ionic gelation method , was taken. The freeze- dried samples, even with some aggregation of the nanoparticles due to diffusion at some stage in freeze-drying. Exhibited nanoscale particles with diameters less than 1 mm, which is in harmony with previous studies.[21]

**Transmission electron microscopy**

The particle size and morphology of herbal chitosal nanoparticles were observed by transmission electron microscopy (TEM) using a tecnai F20 super twin TMP with field emission source as well as resolution of 0.1nm at 200KV along with 1.0 maximum magnification TEM MX camera GATAN US 1000 XP-P .herbal chitosal nanoparticles for TEM dimensions be suspended in ethanol and ultrasonically dispersed. In this study, approximately 500 particles for ethanolic extract or 100 particles for aqueous extracts were measured from quite a lot of images using images analysis software (image). The histogram founded was fitted using a gauss distribution functions.[22,23]

**Measurement of pH**

The pH of herbal chitosan nanoparticle were determined by using digital pH meter.10 ml of suspension of herbal chitosan nanoparticle was checked after calibration the pH of each formulation was completed in triplicate and average values was calculated.[24]

**Antioxidant activitie study**

Antioxidant activities of plant crude extract and its nanoparticles were evaluated by using different assays and compared with antioxidant activity of standard compounds, BTH and ascorbic acid.

- Ferric reducing antioxidant power assay
- DPPH scavenging assay.

**Ferric reducing antioxidant power assay**

Ferric reducing antioxidant power assay (FRAP) is based on detecting the capacity of sample to reduce ferric ions, which is measured as an absorbance change of ferrous TPTZ (2, 4, 6, tri(2-pyridyl)1, 3, 5- triazine) complex. The FRAP reagent was prepared by mixing 25ml of acetic buffer (pH-3.6), 2.5ml TPTZ solution (10ml TPTZ in 40ml HCL) and 2.5ml of $FeCl^3$ (20ml in water) solution. For each sample, different dilution of aloe extract loaded chitosan nanoparticles replace with freshly mixed FRAP reagent. Samples were incubated at room temperature for 30 min. After incubation 2.5ml of 10% trichloro acetic acid was added to each reaction mixture and centrifuged at 3000 rpm for 10 min. 1.5ml of the supernatant was mixed with 1.5ml of distilled water and 0.1ml $FeCl^3$ (0.1%) the absorbance at 700 nm was measured.[25]

**DPPH scavenging assay**

The free radical scavenging activity of the conjugated herbal chitosan nanoparticales and the base components used (chitosan , plant extract_nanoparticles) were measured in vitro against the stable 2,2, diphenyl-1-picryl hydrazyl (DPPH) according to the method with slight modifications. The stock solution was prepared by dissolving 4mg DPPH with 100 ml methanol and stored in dark at 20 c until required. the herbal extract (aloe)loaded chitosan nanoparticles with different concentration (10-100 /ml)were prepared in methanol. Ascorbic acid was used as standard . 1ml of the methanolic plant extract was mixed with 1 ml of DPPH solution and the reaction mixture was shaken well and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517nm

where lower absorbance indicated higher free radical scavenging activity.

The scavenging activity against DPPH was calculated by using the following equation:

Scavenging free radical activity(10%) = [(Ac – As)/Ac] X 100

Where,

Ac – Absorbance of control (DPPH solution without sample)

As – Absorbance of control solution in the presence of the sample (aloe extract and standard)[26]

**Stability studies**

The stability studies were approved according to international conference on harmonization (ICH) guidelines. Short term accelerated stability of herbal chitosan nanoparticles containing the drugs. The samples were occupied in borosilicate sealed glass vials. The vials were stored at room temperature 25 20c /50% RH and 60 20C/70 5%RH, over a period of 3 months instability compartment at 0,1,2,and 3 months to analysis the drug content and any change physical appearance.[27]

## RESULT AND DISCUSSION

**Entrapment efficiency**

Entrapment efficiency of aloe extract loaded chitosan nanoparticle carried out at λ max-254nm. The entrapment efficiency of the prepared formulation F1 to F6 was found to be 30% to 92% repectively. The maximum entrapment efficiency was shown by F6 formulation (fig 2).

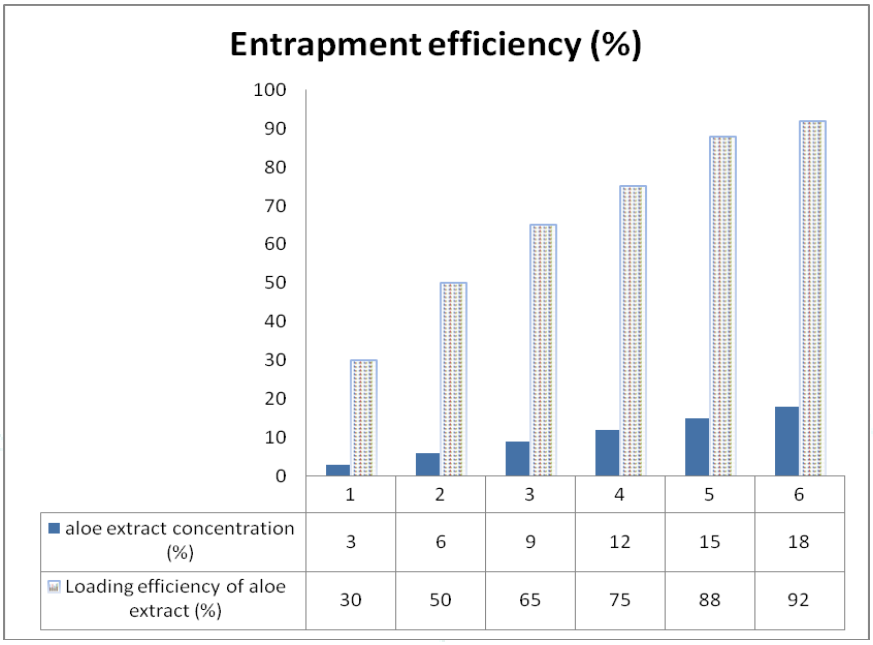


**Fig 2 Entrapment efficiency (%) of herbal extract (aloe) loaded chitosan nanoparticles**

***In- vitro* drug release study**

The percentage cumulative drug release of chitosan nanoparticle loaded with aloe extract of all formulation were noted . The maximum drug release showed by F6 that was 92% (fig 3 and Table 2).

**Table 2 *In-vitro* drug release of of aloe loaded chitosan nanoparticles**

| % Drug release | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| 0 | 10 | 15 | 18 | 20 | 25 | 30 |
| 10 | 20 | 22 | 23 | 25 | 29 | 33 |
| 20 | 18 | 25 | 24 | 27 | 30 | 39 |
| 30 | 25 | 29 | 30 | 30 | 39 | 50 |
| 40 | 30 | 42 | 45 | 49 | 52 | 60 |
| 50 | 28 | 45 | 49 | 52 | 58 | 65 |
| 60 | 45 | 58 | 60 | 63 | 65 | 70 |
| 70 | 50 | 60 | 65 | 70 | 75 | 78 |
| 80 | 52 | 63 | 72 | 76 | 80 | 89 |
| 90 | 55 | 65 | 75 | 79 | 85 | 92 |
| 100 | 55 | 65 | 75 | 79 | 85 | 92 |

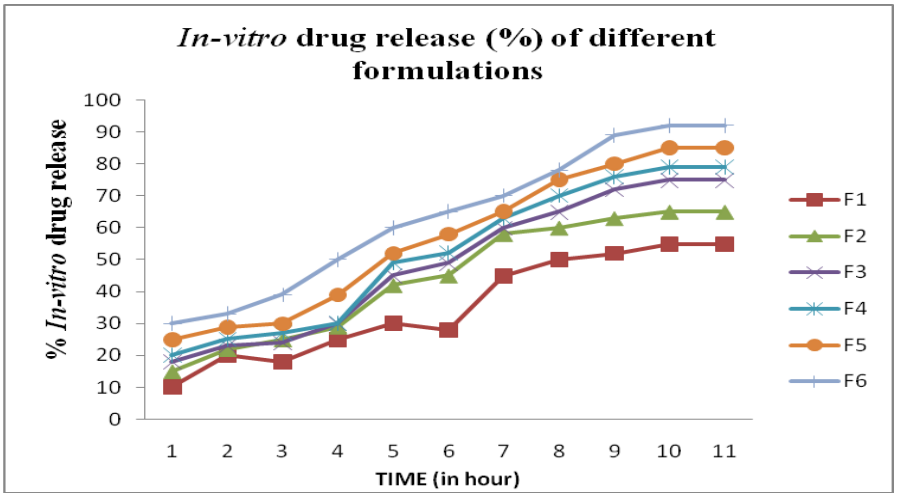


**Fig 3 *In-vitro* release study of different formulation**

### Zeta potential of formulation

The average size and zeta potential study of herbal extract (aloe) loaded chitosan nanoparticles was carried out by using zetasizer. The maximum zeta potential of chitosan nanoparticle was found to be 37.2mV. The zeta potential was found to increase with the particles surface charge also will increase. The results have also shows that the zeta potential was found to get increase with increase in particle surface charge. The size of unloaded chitosan nanoparticles (0%) were 129 nm and aloe loaded chitosan nanoharticles at different concentration such as 3%, 6%, 9%, 12%, 15% and 18% were 129, 117, 106, 95, 80, 71, and 60 nm in that order (show Table 3). The size of particles were decreases with increasing aloe extract concentration in chitosan nanoparticles due to interaction between polymers (chitosan) and extract (aloe) compositions.

**Table 3 Mean size of particles and zeta potential value of different formulations.**

| S.No. | Concentration of aloe extract present in chitosan nanoparticle in % | Size of particles in nm | Zeta potential in mV |
|---|---|---|---|
| 1 | 0% aloe extract | 129 | +37.2 |
| 2 | 3% aloe extract | 117 | +36.9 |
| 3 | 6% aloe extract | 106 | +36.1 |
| 4 | 9% aloe extract | 95 | +35.7 |
| 5 | 12% aloe extract | 80 | +34.9 |
| 6 | 15% aloe extract | 71 | +33.5 |
| 7 | 18% aloe extract | 60 | +32.1 |

### Scanning electron microscopy

Surface morphology study of herbal extract (aloe) loaded nanoparticles were carried out by scanning electron microscopy (SEM), which is shown in fig 4-10.

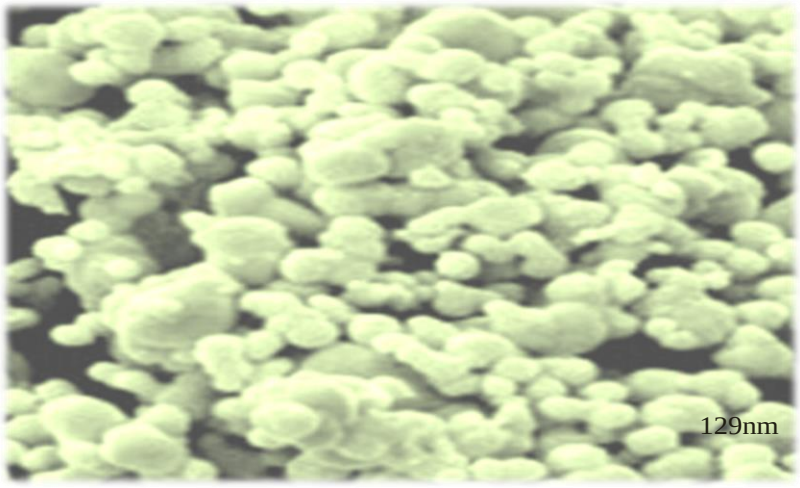


Figure 4 SEM image of aloe extract (0%) chitosan nanoparticles

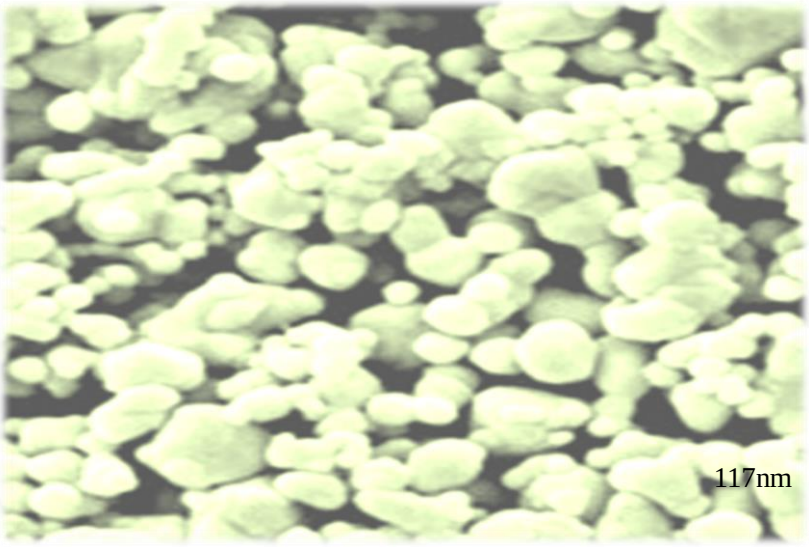


Figure 5 SEM image of aloe extract (3%)chitosan nanoparticle

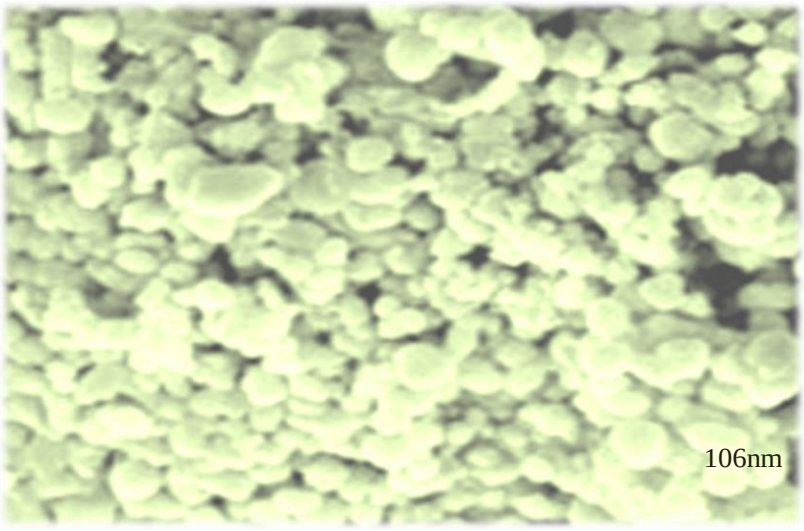


Figure 6 SEM image of aloe extract (6%) chitosan nanoparticles

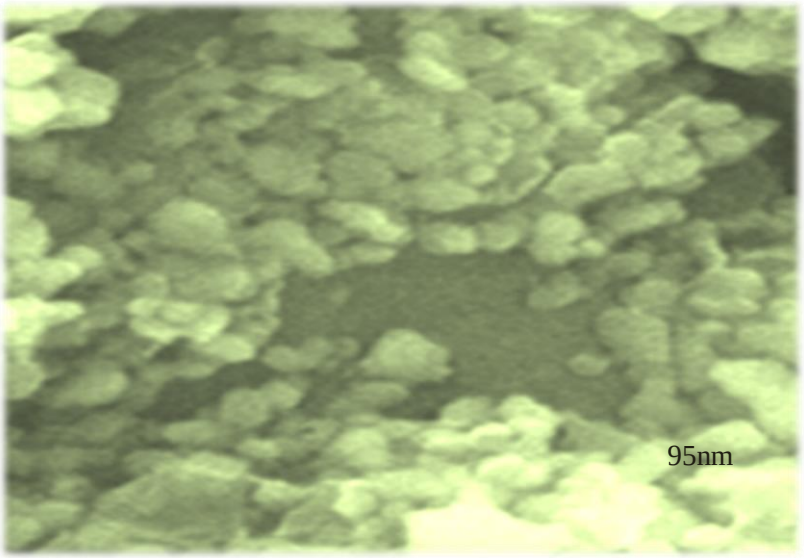


Figure 7 SEM image of aloe extract (9%) chitosan nanoparticles

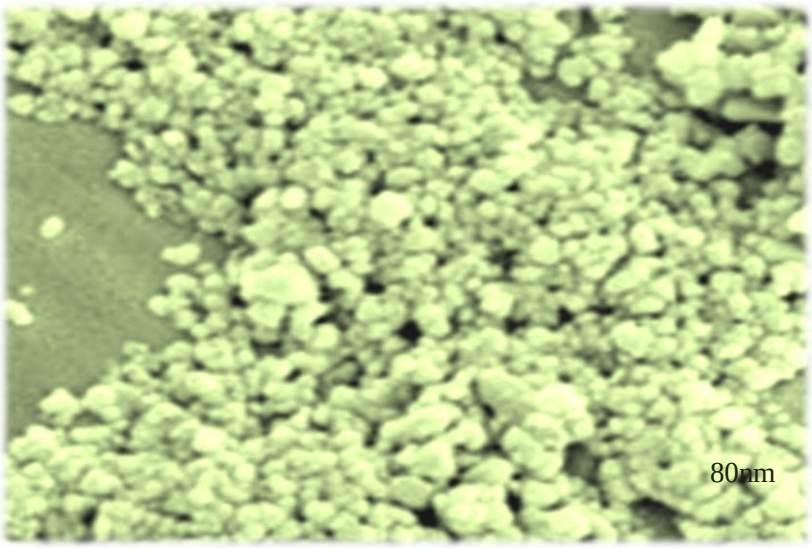


Figure 8 SEM image of aloe extract (12%) chitosan nanoparticles

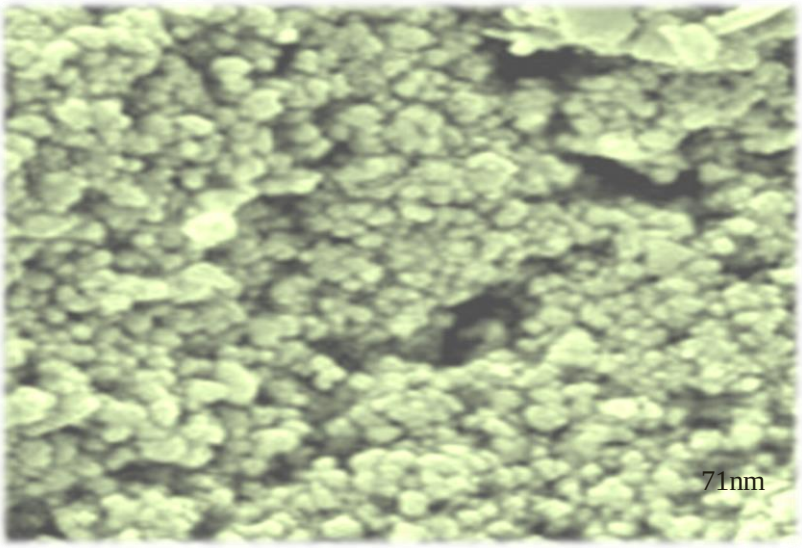


Figure 9 SEM image of aloe extract (15%) chitosan nanoparticles

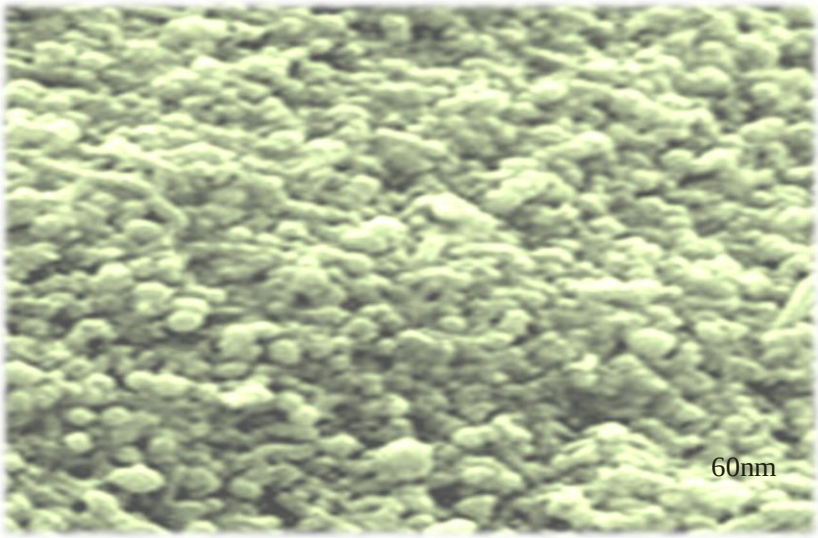


Figure 10 SEM image of aloe extract (18%) chitosan nanoparticles

**Transmission electron microscopy**

Morphological study of chitosan nanopariticles were obtained by the help of transmission electronic microscopy (TEM). The particles of different formulation of nanoparticle were sphericles with smooth sufaces. The TEM image of formulation F6 was shown in the fig 11.

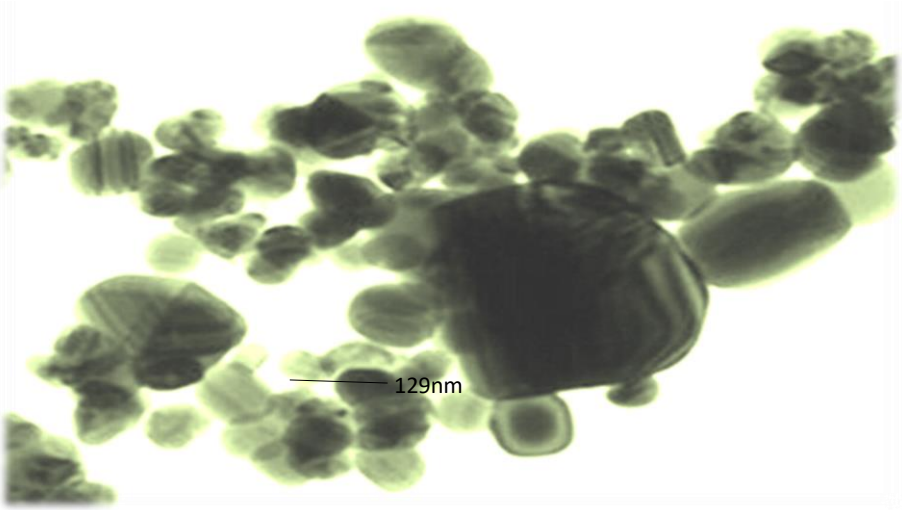


**Figure 11 TEM image of herbal extract (aloe) loaded chitosan nanoparticle (F6)**

**Antioxidant property**

**DPPH scavenging assay**

DPPH scavenging assay for ascorbic acid and aloe loaded chitosan nanoparticle shown in Table 4, 5 and fig 12.

**Table 4 DPPH scavenging assay for ascorbic acid**

| S.No. | Concentration | Absorbance of sample | Control | Control – Sample/ Control | % Inhibition |
|---|---|---|---|---|---|
| 1 | 10 | 0.1360 | 0.2560 | 0.4687521 | 46.87520 |
| 2 | 20 | 0.1290 | 0.2560 | 0.4960938 | 49.60938 |
| 3 | 30 | 0.1220 | 0.2560 | 0.5234376 | 52.34375 |
| 4 | 40 | 0.1140 | 0.2560 | 0.5546888 | 55.46875 |
| 5 | 50 | 0.1100 | 0.2560 | 0.5703126 | 57.03125 |
| 6 | 60 | 0.1030 | 0.2560 | 0.5976563 | 59.76563 |
| 7 | 70 | 0.0970 | 0.2560 | 0.6210938 | 62.10938 |
| 8 | 80 | 0.0890 | 0.2560 | 0.6523438 | 65.23438 |
| 9 | 90 | 0.0840 | 0.2560 | 0.671876 | 67.1875 |
| 10 | 100 | 0.0770 | 0.2560 | 0.699220 | 69.92189 |

**Table 5 DPPH scavenging assay for aloe loaded chitosan nanoparticle**

| S.No. | Concetration | % Inhibition of ascobic acid | % Inhibition of F1 | % Inhibition of F2 | % Inhibition of F3 | % Inhibition of F4 | % Inhibition of F5 | % Inhibition of F6 |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 49.60938 | 46.8752 | 46.99756 | 47.59643 | 47.99879 | 48.00068 | 48.96315 |
| 2 | 20 | 53.12525 | 49.60938 | 50.00695 | 51.09652 | 51.98799 | 52.00689 | 52.6981 |
| 3 | 30 | 57.42188 | 52.34375 | 52.98746 | 53.06981 | 53.6223 | 54.56255 | 55.39426 |
| 4 | 40 | 62.10938 | 55.46875 | 56.97665 | 57.00009 | 57.65896 | 58.69525 | 59.63215 |
| 5 | 50 | 67.18751 | 57.03125 | 58.68942 | 59.03169 | 59.65415 | 60.95466 | 63.26462 |
| 6 | 60 | 69.53125 | 59.76563 | 62.69579 | 64.18239 | 65.65862 | 66.66256 | 69.93125 |
| 7 | 70 | 71.48438 | 62.10938 | 64.96362 | 66.19965 | 68.89652 | 70.62942 | 73.69512 |
| 8 | 80 | 73.82813 | 65.23438 | 67.26945 | 68.99999 | 70.65526 | 72.63661 | 76.65802 |
| 9 | 90 | 80.85938 | 67.1875 | 70.62952 | 72.63249 | 77.92912 | 79.69845 | 82.65896 |
| 10 | 100 | 86.71875 | 69.92189 | 72.15656 | 76.13315 | 80.99463 | 84.69546 | 88.19563 |

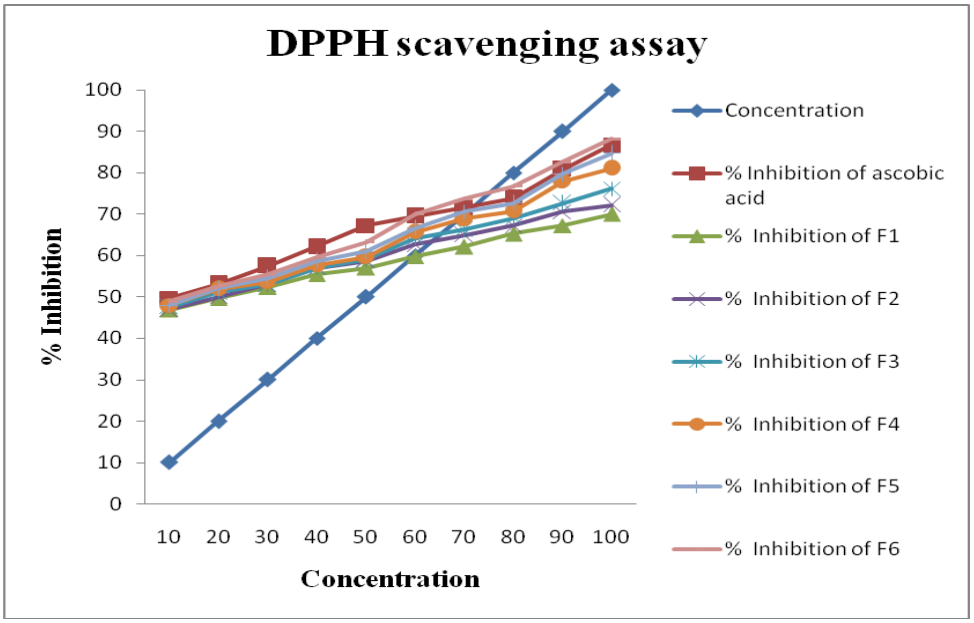


**Figure 12 DPPH scavenging assay of different formulation**

**Ferric reducing antioxidant power assay**

Ferric reducing antioxidant power assay of aloe loaded chitosan nanoparticles shown in Table 6 and fig 13.

**Table 6 Ferric reducing antioxidant power assay for aloe loaded chitosan nanoparticle**

| S.No. | Concetration | % Inhibition of ascorbic acid | % Inhibition of F1 | % Inhibition of F2 | % Inhibition of F3 | % Inhibition of F4 | % Inhibition of F5 | % Inhibition of F6 |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 29.60938 | 26.8752 | 27.00056 | 29.86542 | 32.25689 | 35.56988 | 42.45862 |
| 2 | 20 | 33.12525 | 33.60938 | 35.99856 | 36.26579 | 35.98635 | 38.0369 | 44.12654 |
| 3 | 30 | 38.42188 | 36.34375 | 37.98647 | 39.35489 | 40.63548 | 42.45695 | 48.94562 |
| 4 | 40 | 42.10938 | 38.46875 | 39.06893 | 41.89542 | 43.15963 | 45.97562 | 50.36954 |
| 5 | 50 | 47.18751 | 40.03125 | 42.48962 | 44.99865 | 47.45545 | 50.65263 | 52.56385 |
| 6 | 60 | 49.53125 | 43.76563 | 46.04569 | 48.67895 | 50.52545 | 53.21552 | 55.26779 |
| 7 | 70 | 51.48438 | 46.10938 | 49.56378 | 51.99635 | 53.25968 | 55.69126 | 57.26889 |
| 8 | 80 | 53.82813 | 47.23438 | 50.39862 | 53.8963 | 55.59634 | 58.63595 | 60.36142 |
| 9 | 90 | 60.85938 | 49.1875 | 51.6543 | 56.00063 | 58.03994 | 61.23654 | 68.26386 |
| 10 | 100 | 66.71875 | 52.92189 | 55.42365 | 57 | 61.2698 | 65.32658 | 72.13634 |

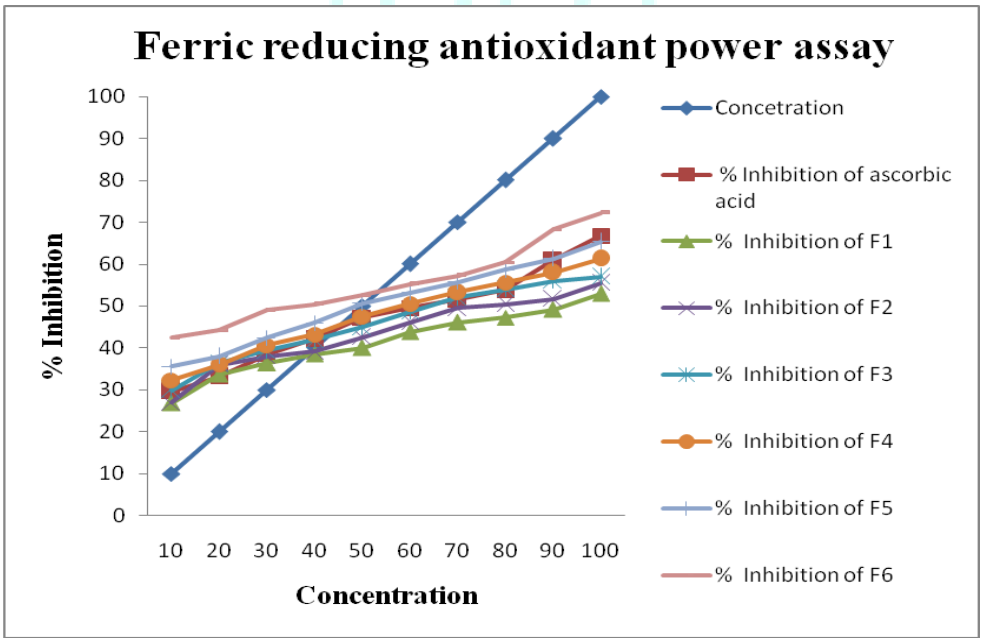


**Figure 13 Ferric reducing antioxidant power assay of different formulation**

### Stability studies

The stability and physicochemical interaction of the aloe extract loaded chitosan nanoparticles was carried out by the help of BOD incubator. The results have been shown no change in physicochemical properties. The samples of different formulation for stability study were taken at different time interval 0, 1, 2, and 3 months with different temperature such as room temperature ($25^0$C – $250^0$C) and refrigerator temperature ($2^0$C – $25^0$C) and relative humidity of 75%. These results indicated that the all formulation of aloe extract loaded chitosan nanoparicles were physically and chemically stable.

## CONCLUSION

The present research deals with *in-vitro* evaluation of drug release and antioxidant activity of aloe loaded chitosan nanoparticles. In research work, all formulations (F1-F6) were prepared and evaluated successfully. Based on experimental work, it can be concluded that F6 formulation was best in among all batches because it has show maximum antioxidant activity with high *in-vitro* drug release activity and good stability. Aloe extraxt in form of nanoparticles, increases their *in-vitro* release and stability. Finally we can say, aloe loaded chitosan nanoparticles have potent antioxidant property with maximum in vitro release study and good stability study. So these nanoparticles can be used as antioxidant agent during the treatment of cancer along with chemotherapeutic drugs for improve their effectiveness and reduce adverse effects. In further study, we can go for *in-vitro* and *in-vivo* evaluation of these nanoparticles in the treatment of cancer as a antioxidant.

## REFERENCES


1. Gadad AP, Vijay Kumar SV, Dandagi PM, Bolmol UB, Pavani Pallavi N, Nanoparticles and their Therapeutic Application in Pharmacy, International Journal of Pharmaceutical Sciences and Nanotechnology, 2014; 7(3):2509-2019.
   Patel Jitendra S, Bhatt Medha M, Patel Foram A, Dhoru Minakshi M, Patel Madhabhai M, Nanotechnology: A New Approach In Herbal Medicine, American Journal of Pharmtech Research, 2013; 3(4):275-288.
2. Mathur Manish, Vyas Govind, Role of nanoparticles for production of smart herbal drug- An overview, Indian Journal of Natural Products and Resources, 2013: 4(4):329-338.
3. Sharma Parth, Verma Surajpal, Misri Plakshi, Global Need for Novel Herbal Drug Formulations, International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(9):1535-1544.
4. Ravi Krittika, Ganapathy Dhanraj, Sherlyn Sheeba P, Antioxidants and cancer prevention - A review, Journal of Pharmacy Research, 2018; 12(1):35-38.
5. Nuria Mut-Salud, Pablo Juan Álvarez, Jose Manuel Garrido, Esther Carrasco, Antonia Aránega, and Fernando Rodríguez-Serrano, Antioxidant Intake and Antitumor Therapy: Toward Nutritional Recommendations for Optimal Results, Oxidative Medicine and Cellular Longevity, 2016; 1(1):1-19.
6. Ray Sarbani Dey, Potential aspects of chitosan as pharmaceutical excipients, Acta Poloniae Pharmaceutica - Drug Research, 2011; 68(1):619-622.
7. Yashwanth Radhakrishnan, Ganesh Gopal, Chittur C Lakshmanan and Krishna S Nandakumar, Chitosan Nanoparticles for Generating Novel Systems for Better Applications: A Review, Journal of Molecular and Genetic Medicine, 2015; 48:1-10.
8. Harlev Eli, Nevo Eviatar, Lansky Ephraim P, Ofir Rivka, Bishayee Anupam, Anticancer Potential of Aloes: Antioxidant, Antiproliferative, and Immunostimulatory Attributes, Planta Med, 2012; 78:843–852.
9. Hussain Arif, Sharma Chhavi, Khan Saniyah, Shah Kruti, Haque Shafiul, Aloe vera Inhibits Proliferation of Human Breast and Cervical Cancer Cells and Acts Synergistically with Cisplatin, Asian Pacific Journal of Cancer Prevention, 2015;16 (7):2939-2946.
10. Sampath Kumar KP, Bhowmik Debjit, Chiranjib, Biswajit, Aloe vera : A Potential Herb and its Medicinal Importance, Journal of Chemical and Pharmaceutical Research , 2010; 2(1): 21-29.
11. Rajeswari R, Umadevi M, Sharmila Rahale C, Pushpa R, Selvavenkadesh S, Sampath Kumar K P, Bhowmik Debjit, Aloe vera: The Miracle Plant Its Medicinal and Traditional Uses in India, Journal of Pharmacognosy and Phytochemistry, 2012; 1(4):118-124.
12. Sanders Brian, Ray Anna M, Goldberg Sharon, Clark Tyler, Reginald McDaniel H, Atlas Steven E, Farooqi Ashar, Konefal Janet, Lages Lucas C, Lopez Johanna, Rasul Ammar, Tiozzo Eduard, Woolger Judi M, Lewis John E, Anti-cancer effects of aloe-emodin: A systematic review, Journal of Clinical and Translational Research, 2017; 3(4):1-14.
13. Lucini Luigi, Pellizzoni Marco, Molinari Gian Pietro, Franchi Fabrizio, Aloe anthraquninones against cancer, Medical and Aromatic Plant Science and Biotechnology, 2012; 6(2):20-24.
14. Abd El-Aziz Abeer Ramadan Mohamed, Al-Othman Monira Rashed, Mahmoud Mohamed Abobakr, Shehata Shereen Mohamed and Abdelazim Nahla Shazli, Chitosan nanoparticles as a carrier for Mentha longifolia extract: synthesis, characterization and antifungal activity, 2018; 114(10):2116-2122.
15. Khan A, Shaheen A, Mahmood T, Rehman W and Yaqoob, Novel Synthesis and Characterization of Silver Nanoparticles from Leaf Aqueous Extract of Aloe Vera and their Anti-Microbial Activity, Journal of Nanoscience and Nanotechnology Applications, 2017; 1(1):1-9.
16. Praseena K and Junise V "Formulation and evaluation of chitosan nanoparticle loaded with phyllanthin" 2018; 5(04):2644-2649.
17. Salim Khan, Fahad Al-Qurainy, Mauji Ram, Sayeed Ahmad and Malik Zainul Abdin, Phyllanthin biosynthesis in Phyllanthus amarus: Schum and Thonn growing at different altitudes, Journal of Medicinal Plants Research, 2010; 4(1):41-48.
18. Hoa LTM, Chi NT, Triet NM, Nhan LNT and DM, Preparation of drug nanoparticles by emulsion evaporation method, J Phy Conf Ser.2009; 187;1-4.
19. Chitravadivu C, Manian S and Kalaichelvi K, Qualitative Analysis of Selected Medicinal Plants, Tamilnadu, India, Middle-East Journal of Scientific Research, 2009; 4 (3): 144-146.
20. Wu Y, Luo Y and Wang Q, Antioxidant and antimicrobial properties of essential oils encapsulated in zein nanoparticles prepared by liquid dispersion method, LWT. Food Sci. Technol., 2012; 48:283–290.
21. Da Silva SB, Amorim M, Pedro F, Madureira R, Ferreira D, Pintado M, Sarmento B, Natural extracts into chitosan nanocarriers for rosmarinic acid drug delivery, Pharm. Biol., 2015;53:642–652.
22. Kulani MJ, Chitosan nanoparticles functionalized with methanol medicinal plant extracts for the inhibition of the toxic effects of aflatoxin B1 and ochratoxin A, Master Thesis, University of Johannesburg, 2013, p. 97
23. Shah M, Solid Lipid Nanoparticles of a Water Soluble Drug, Ciprofloxacin Hydrochloride, Indian J Pharm Sci., 2012; 74(5):434–442.
24. Pisoschi Aurelia Magdalena, Negulescu Gheorghe Petre, Methods for Total Antioxidant Activity Determination: A Review, Biochem & Anal Biochem, 2011;1(1):1-10.
25. Kumawat Bhupendra K, Gupta Mahesh, Singh Tarachand, Singh Yogendra, Free radical scavenging effect of various extracts of leaves of Balanites aegyptiaca(L.) Delile by DPPH method, Asian Journal of Plant Science and Research, 2012; 2(3):323-329.
26. Phromsopha T, Chitosan microparticles prepared by water in oil emulsion solvent diffusion method for drug delivery, Biotechnology, 2010; 9(1): 61-66.